CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-527

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Clinical Pharmacology/Biopharmaceutics Review

PRODUCT (Generic Name):	Fingolimod
NDA:	22-527
PRODUCT (Brand Name):	Gilenia®
DOSAGE FORM:	Capsules
DOSAGE STRENGTH:	0.5 mg
INDICATION:	Relapsing Remitting Multiple Sclerosis (RRMS)
NDA TYPE:	Priority
SUBMISSION DATES:	6/15/2009, 7/24/2009, 10/5/2009, 12/21/2009, 1/20/2010, 1/28/2010, 2/3/2010, 2/8/2010, 2/9/2010, 2/15/2010, 2/16/2010, 2/18/2010, 2/22/2010, 3/2/2010, 3/4/2010, 3/9/2010, 3/10/2010, 3/11/2010, 3/12/2010, 3/15/2010, 3/16/2010, 3/18/2010, 3/19/2010, 3/23/2010, 3/29/2010, 3/30/2010, 4/1/2010, 4/2/2010
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1.0 EXECUTIVE SUMMARY

This is an original NDA 22527 (NME) submitted on December 21st, 2009 seeking for approval of Gilenia® (Fingolimod, FTY720) for the treatment of Relapsing Remitting Multiple Sclerosis (RRMS). This NDA is under the priority review classification.

Fingolimod is a sphingosine-1-phosphate (S1P) receptor modulator. Fingolimod phosphorylated to the active moiety, *S*-enantiomer fingolimod-P. Fingolimod-P is a functional antagonist of S1P receptor which reduces the peripheral lymphocyte count mediated by down-modulation of the S1P1 receptor on lymphocytes. This results in a reduced egress of lymphocytes from the lymph nodes; in particular auto-aggressive T-cells that perform a central role in the MS inflammatory disease process are prevented from recirculating to the CNS. Fingolimod-P reversibly dephosphorylated back to the inactive form fingolimod. At steady state, fingolimod and fingolimod-P are in dynamic equilibrium.

In this submission, there are 56 human study reports submitted to support the dosing and the proposed claim for fingolimod, including 31 clinical pharmacology studies. The proposed product is hard capsule, with only one proposed strength of 0.5 mg. The recommended dosing regimen is 0.5 mg once-daily administered orally.

1.1 RECOMMENDATION

The Office of Clinical Pharmacology (OCP/DCP I) has reviewed the clinical Pharmacology and Biopharmaceutics sections of the NDA 22-527. The submission is acceptable from a Clinical Pharmacology and Biopharmaceutics point of view provided the sponsor agrees with the Phase IV requirements and Agency's labeling recommendations.

Labeling recommendations outlined in the Detailed Labeling Recommendations section of the review should be conveyed to the sponsor.

PHASE IV COMMITMENTS:

The following Phase IV requirements should be conveyed to the sponsor:

1. Evaluation of the activities (toxicities) of metabolites M2 and M3. Depending on the results, studies in mild and moderated renal impaired patients might be needed.

   The levels of M2 and M3 collected in the animal toxicity studies do not cover human exposure after multiple doses. The comparable exposure after single dose in human to the steady-state exposure in animal toxicity studies doesn't assure the safety after multiple doses in human.

2. Development of the new lower strength of 0.25 mg to fit the need for dose adjustment in severe hepatic impaired patients and in patients who co-administrate with ketoconazole.

3. Pharmacogenetic determinants of fingolimod safety should be assessed. Given the demonstrated relationship between fingolimod exposure and adverse events, consider genes involved in fingolimod disposition or activation (e.g., CYP4F2, SPHK2) as candidates. Depending on the existing database, pharmacogenetic assessment may be done using existing samples (e.g., from FTY20D2302 or other studies) or in future clinical studies of fingolimod efficacy.

4. A clinical study to determine the efficacy of 0.25 mg QD is recommended.

5. *In vitro* study for evaluation of the potential of FTY720 to be an inhibitor of CYP2C8 and the potential of FTY720-P to be an inhibitor of CYP2B6.

6. *In vitro* study for evaluation of the potential of FTY720-P to be an inducer of CYP450 isoenzymes should be conducted.

7. *In vitro* DDI study showed Carbamezapine increased the metabolism of FTY720 2.3 and 1.8 folds at 10 and 50 μM, respectively. *In vivo* DDI study for Carbamezapine to be coadministered with FTY720 is needed.

8. *In vitro* study to evaluate the induction potential of statins (e.g. simvastatin, lovastatin) on CYP4F2 (± 100 folds of clinical therapeutic concentrations) is needed. Depending on the results, *in vivo* studies might be needed. (Reference: Regulation of Human Cytochrome P450 4F2 Expression by Sterol Regulatory Element-binding Protein and Lovastatin. *THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 8, pp. 5225–5236, February 23, 2007.*)

1.2 OVERALL SUMMARY OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS

The overall findings from clinical pharmacology and biopharmaceutics section are as follows:

Fingolimod is phosphorylated to the active moiety, *S*-enantiomer fingolimod-P and fingolimod-P is dephosphorylated back to the inactive form fingolimod. At steady state, fingolimod and fingolimod-P are in dynamic equilibrium.

Absorption of fingolimod is slow (Tmax is variable, median of Tmax ~12 hours) but complete as > 85 % of the radiolabelled drug was recovered in the urine. Fingolimod-P reaches Cmax at a median time of 8 hours. Fingolimod is extensively distributed to body tissues with volume of distribution (Vz) ~1200±260 L. Fingolimod is believed to be

metabolized mainly via the cytochrome P450 4F2 isoenzyme. The average apparent terminal half-life for both fingolimod and fingolimod-P is 6-9 days.

Steady-state PK:

Steady-state exposure is reached between 1 to 2 months during once-daily dosing with an estimated 11-fold accumulation of blood levels from first dose to steady state. The fingolimod blood concentration profile at steady-state shows a peak to trough fluctuation of approximately 20% while the peak to trough fluctuation for fingolimod-P is approximately 45%.

The molar ratios of fingolimod-P/fingolimod AUCτ and Cmaxss averages 0.42 and 0.49, respectively, with small CV% (12-17%) at 0.5 mg and 1.25 mg QD.

The key pharmacodynamic effect of fingolimod is a dose dependent reduction of the peripheral lymphocyte count to 30-40% of baseline values at steady-state with the doses used in clinical studies in MS, 0.5 mg and 1.25 mg. The sponsor claimed that this is linked to the clinical efficacy. This reduction is manifest within hours after the first dose administration; achieves its nadir after approximately one week of treatment and persists throughout the treatment period with small fluctuations. Lymphocyte counts start to increase immediately after treatment discontinuation and typically return to baseline values within weeks, consistent with systemic exposure of the drug.

Dose proportionality:

The pharmacokinetics of fingolimod was dose-proportional after single dose within the dose range of 0.125 to 40 mg and multiple doses at doses at 0.5 mg QD and lower. There was less than dose-proportional increase in Cmax and AUC with increasing doses higher than 0.5 mg QD.

For fingolimod-P, Cmax displays an under-proportional increase with the dose, over the range 1.25-40 mg, and AUC(0-96) rises in an apparent dose-proportional manner, over the range 5-40 mg after single dose. At steady state, the exposure is approximately dose-proportional with slightly skewed data to the underproportional from the unity line.

Dose Selection:

The proposed dosing regimen is 0.5 mg once-daily administered orally. However, based on the exposure-response, this dose has reached the therapeutic platau therefore as recommended by Division of Pharmacometrics: A lower dose, such as 0.25 mg, should be studied in a future study.

Intrinsic Factors:

Age: No age effect was noticed in the population model. No dose adjustments are therefore recommended based on age.

Gender: A slight lower concentration (10.4%) was observed in males than in females, however, the magnitude of the effect was deemed to be not clinically relevant. No dose adjustments are therefore recommended based on gender.

Weight: A gain in weight of 14 kg (from 70 to 84 kg) would be on average associated with a 6.2% decrease in steady state concentration, however, the magnitude of the effect was deemed to be not clinically relevant. No dose adjustments are therefore recommended based on weight.

Race: Based on the population PK analysis, for 70kg female who were assigned to 0.5mg, estimated mean concentrations at SS in Asian and Black population are 62% higher and 14% lower than that of Caucasian patients. However, it should be noted that there were only 14 (0.5 %) Asian subjects in the population PK report. In a separate clinical pharmacology study, Japanese and Caucasian showed comparable PK. The effect of race therefore could not be concluded. No dose adjustments are recommended based on race.

PK and PD in MS patients: Based on population analysis, the PK of fingolimod and fingolimod-P and the PD effect on lymphocyte counts following oral administration of FTY720 capsules were similar between MS patients and healthy subjects.

Hepatic impairment patients:

Moderate and severe hepatic impairments increased fingolimod AUC by 44% and 103%, respectively. The apparent elimination half-life is prolonged by 49-50% in moderate and severe hepatic impairment. Fingolimod-P Cmax and AUC(0-96) were increased by 22% and 29% in severe hepatic impaired patients.

The fingolimod dose does not need to be adjusted in mild or moderate hepatic impaired patients. The reviewer recommends decrease the dose by 50% in severe hepatic impaired patient. As there is no lower strength (0.25 mg) formulation available, use of fingolimod is not recommended in severe hepatic impaired patients.

Renal impairment patients:

Severe renal impairment increases fingolimod Cmax and AUC by 32% and 43%, respectively, and fingolimod-P Cmax and AUC by 25% and 14%, respectively. The apparent elimination half-life is unchanged for both analytes.

Exposure to the inactive metabolites is also increased with severe renal impairment, by at least 300% for M2 and by 805% for Cmax and 1356% for AUC for M3. The clinical impact of such an increase is unknown.

The use of fingolimod should be contraindicated in renal impaired patients due to uncertainty of the safety profiles of M2 and M3.

Extrinsic Factors:

Drug-drug Interactions:

Pharmacokinetic studies:

- **Cyclosporine:** Cyclosporine did not significantly change fingolimod overall exposure AUC(0-tz) or peak concentration (Cmax). The pharmacokinetics of steady-state cyclosporine were not altered during coadministration with single-dose fingolimod.
- **Ketoconazole:** Coadministration of a single 5 mg dose of FTY720 with steady-state ketoconazole 200 mg twice-daily increased both fingolimod Cmax by 1.2-fold and AUC by 1.7-fold. Fingolimod-phosphate AUC(0- tz) was increased to a similar extent (1.7-fold). Dose adjustment for fingolimod is recommended. The reviewer recommends decrease the dose of fingolimod by 50% when it is coadministerd with ketoconazole. As there is no lower strength (0.25 mg) formulation available, fingolimod is not recommended to coadminister with ketoconazole.

Pharmacodynamic studies:

- **Isoproterenol:** Isoproterenol was highly effective in reversing the negative chronotropic effect of fingolimod. Even at the time of maximal fingolimod induced negative effect (4-6 hours post-dose), heart rates >100 BPM were achieved with isoproterenol infusion. The exposure (Cmax and AUC(0-24)) to fingolimod or fingolimod-P was not altered in presence of isoproterenol.

- **Salmeterol:** Salmeterol had a mild, positive chronotropic effect on heart rate of approximately six beats per minute. The fingolimod and fingolimod-P blood concentrations and derived pharmacokinetic parameters are consistent with those from other studies.

- **Atropine:** Intravenous atropine (≤2 mg) reversed fingolimod induced negative chronotropic effect by approximately 10 BPM. Fingolimod and fingolimod-P exposure (Cmax and AUC(0-24)) were not influenced by atropine coadministered with fingolimod or administered 4 hours after fingolimod.

- **Diltiazem:** Diltiazem combined with fingolimod had no additional negative chronotropic effect than fingolimod alone.The pharmacokinetics of diltiazem (moderate CYP3A inhibitor), fingolimod, and fingolimod-P appeared not to be altered during the coadministration of the two drugs.

- **Atenolol:** Atenolol combined with fingolimod had an approximately 15% additional negative chronotropic effect (42 bpm) than fingolimod alone (51 bpm).

The pharmacokinetics of atenolol, fingolimod, and fingolimod-P appeared not to be altered during the coadministration of the two drugs.

Biopharmaceutics:

BCS Class:

Fingolimod hydrochloride is soluble in water (more than 100 mg/mL at 25° and 37°C). Fingolimod is a base with pKa of 7.82. Solubility is higher in acidic media (more than 100 mg/mL at pH 1.0) and lower at neutral or alkaline pH where absolute value could not be estimated (solubility less than 0.01 mg/mL in pH 6.8 at 25° and 37°C).

The dissolution of the 0.5 and 1.25 mg capsules was also pH-dependent [redacted] (b) (4)

Attempts were made to investigate the penetration of [14C]fingolimod through Caco-2 cell monolayers. However, due to strong unspecific binding of fingolimod to the supporting filter device (probably related to the lipophilicity of fingolimod), no reliable conclusions could be drawn from the experiment.

Due to the above described features, a BCS class could not be precisely assigned to fingolimod.

Relative Bioavailability:

All clinical studies were conducted with hard gelatin capsule formulations. Early clinical studies utilized clinical service form (CSF). Subsequent clinical trials, including all phase II and phase III studies of the MS program, used the final market image (FMI).

The relative bioavailability of 1.25 mg and 2.5 mg fingolimod FMI formulations in comparison to CSF was evaluated in healthy volunteers. The CSF and the FMI formulations are bioequivalent. Fingolimod 0.5 mg and 1.25 mg FMI formulations are compositionally proportional.

Food Effect:

The effect of food on fingolimod and fingolimod-phosphate pharmacokinetics was evaluated with high fat meal using the FMI 1.25 mg capsules in Study A2107 in healthy volunteers. Comparisons between the FMI fed and fasted indicated food has no statistically significant increase in the absorption of the FMI capsule. The mean values of AUC(0-inf), AUC(0-last) and Cmax for FMI under the fed condition were similar to those under fasted condition. However, peak plasma concentration of FTY720-P was higher in fasted state when compared to fed state. The clinical program was conducted without regard to the timing of food intake.

Fingolimod capsules can be taken without regards to food.

Analytical Assays:

The assays used to measure fingolimod and its metabolites are considered validated.

Division of Clinical Pharmacology I

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2.0 QUESTION BASED REVIEW

2.1 GENERAL ATTRIBUTES

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product as they relate to clinical pharmacology and biopharmaceutics review?

Dosage Form/Strengths: 0.5 mg capsules

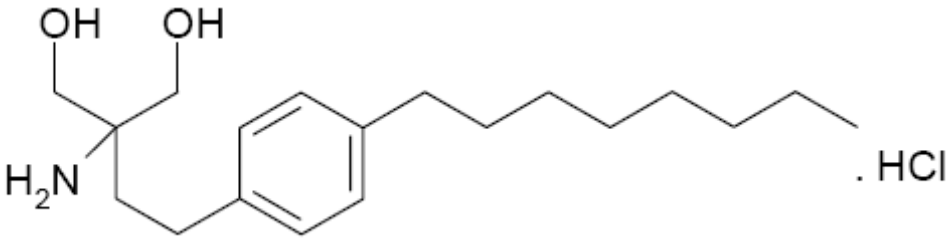
Indication: Gilenia® (Fingolimod) is indicated for relapsing remitting multiple sclerosis (RRMS).

Pharmacologic Class: Immunomodulator by functional antagonism of sphingosine-1-phosphate receptor 1 ($S1P_1$)

Chemical Name: 2-Amino-2-[2-(4-octylphenyl) ethyl]propane-1,3-diol hydrochloride. Its molecular weight is 307.47.

Molecular formula: $C_{19}H_{33}NO_2 \cdot HCl$

Chemical structure:



Physical Characteristics: Fingolimod hydrochloride (molecular weight of 343.94 (hydrochloride salt form) and 307.48 (free base)) is soluble in water (more than 100 mg/mL at 25° and 37°C). Solubility is higher in acidic media (more than 100 mg/mL at pH 1.0) and lower at neutral or alkaline pH (less than 0.01 mg/mL in pH 6.8 at 25° and 37°C). The pH of a 1% solution of fingolimod hydrochloride in water at 22°C is 4.02. The pKa in water at 22° to 25°C is estimated to be 7.82. The distribution coefficient of fingolimod hydrochloride could not be estimated in noctanol/phosphate buffer pH 6.8, because fingolimod hydrochloride is practically insoluble in phosphate buffer pH 6.8.

Formulation: Fingolimod capsules (0.5 mg and 1.25 mg FMI) are compositionally proportional. The composition of fingolimod capsules (0.5 mg and 1.25 mg FMI) is shown in the Table 1 below:

Table 1 Composition of FTY720 hard capsules

Ingredient	Amount per 0.5 mg capsule (mg)	Amount per 1.25 mg capsule (mg)	Reference to standards	Function
FTY720 HCl [1]	0.56	1.40	Novartis monograph	Drug substance
Mannitol	[redacted] (b) (4)		USP, Ph. Eur.	Diluent
Magnesium stearate [2]	[redacted] (b) (4)		NF, Ph. Eur.	Lubricant
Capsule fill weight	**48.00**	**120.00**		
Empty capsule shell, pre-printed				
Capsule shell (theoretical weight)[3]	48.00	48.00	Novartis monograph	Structure
[redacted] (b) (4)	q.s.	q.s.		
Printing ink, yellow [4]	q.s.	---		
[redacted] (b) (4)	---	q.s.		
Total capsule weight	**96.00**	**168.00**		

[1] 2-Amino-2-[2-(4-octylphenyl)ethyl]propane-1,3-diol hydrochloride. The molecular weight ratio of FTY720 HCl to FTY720 base is approximately 1.12 to 1.0

[2] [redacted] (b) (4)

[3] The composition of the capsule shells are provided in Table 1-2 and Table 1-3

[4] The qualitative composition of the inks is provided in Table 1-4.

2.1.2 What is the mechanism of action and therapeutic indication?

The sponsor proposed mechanism of action of fingolimod is described below. Under normal circumstances, T-cells selectively require S1P1 activation for emigration from the thymus, and both T- and B-cells require this receptor for egress from peripheral lymphoid organs. Fingolimod-P acts as a functional antagonist of the S1P1 receptor on lymphocytes, inducing its uncoupling/internalization. The internalization of S1P1 renders these cells unresponsive to S1P, depriving them of the obligatory signal to egress from lymphoid organs and recirculate to peripheral inflammatory tissues. Fingolimod-P may also act on other cells and, depending on the cell type, may act as an "agonist" or "functional antagonist" at S1P receptors. Fingolimod-P causes a reversible retention of a proportion of CD4 and CD8 positive T-cells and B-cells from blood and spleen into lymph nodes (LNs) and Peyer's patches; apparently without affecting many of the functional properties of these cells. The retention of CD4 and CD8 cells in peripheral LN and Peyer's patches reduces the number of these immune cells that have access to sites of MS related inflammation in the brain and therefore decreases the inflammatory component of this disease.

The proposed indication for FTY720 capsules is the treatment of relapsing remitting multiple sclerosis (RRMS).

2.1.3 What are the proposed dosages and route of administration?

Dosage and administration (Sponsor Proposed):

The recommended dose is 0.5 mg FTY720 capsule once daily by oral.

2.2 GENERAL CLINICAL PHARMACOLOGY

2.2.1 What are the clinical studies used to support dosing or claims and what are their design features?

The sponsor claimed that fingolimod is a disease modifying therapy for the treatment of patients with relapsing forms of multiple sclerosis to reduce the frequency of clinical exacerbations and to delay the accumulation of physical disability.

There are 56 human study reports submitted to support the dosing and the proposed claim, including 31 clinical pharmacology studies, which include 1 exposure-response report, 1 population PK report and 1 thorough QT study, and for efficacy and safety, 4 controlled trials, 5 cross study reports and 16 other study reports. A clinical pharmacology study (D2109) was planned to be submitted at 120 day safety update under the agreement with the Agency at the Pre-NDA meeting but not yet reveived nor reviewed at time of writing this review. The submission also contains 15 *in vitro* studies regarding protein binding (4 reports), hepatic metabolism and drug interactions (8 reports) and transporters (3 reports) and 7 bioanalytical validation and assay reports for fingolimod, its only active metabolite, fingolimod-P, and two inactive metabolites, M2 and M3. In addition, one model based report characterizing the effect of FTY720 on heart rate in healthy volunteers was provided as an analysis from more than one study.

The clinical pharmacology program has studied single fingolimod doses from 0.125 to 40 mg and multiple doses from 0.125 to 5 mg. All pivotal, Phase II and III RRMS clinical research studies have used two doses of fingolimod: 0.5 and 1.25 mg daily. Table 2 below summarized the four controlled studies conducted in RRMS patients.

Table 2: Summary of four controlled studies conducted in RRMS patients

Study No.	Study Objective, Population	No. of patients Design	Treatment Duration	Medication dose/day	Primary Efficacy Endpoint
Phase III					
D2301	Efficacy and safety in RRMS	1272 randomized, double-blind	2 years	fingolimod 1.25mg/day fingolimod 0.5mg/day Placebo	Annualized relapse rate
D2302	Efficacy and safety in RRMS	1292 randomized, double-blind, double-dummy	1 year	fingolimod 1.25mg/day fingolimod 0.5mg/day IFN β-1a i.m. 30µg once weekly	Annualized relapse rate
Phase II					
D2201	Efficacy and safety in relapsing MS	281 randomized, double-blind	6 months	fingolimod 5.0mg/day fingolimod 1.25mg/day Placebo	Total number of Gd-enhancing lesions on 6 monthly post-baseline MRI scans
D2201E1	Long-term efficacy and safety, extension of study D2201	250 Initially double-blind, then open-label	Open (interim data up to Month 60 included)	fingolimod initially 1.25 mg or 5.0 mg orally o.d., between months 15 and 24, 5.0 mg patients switched to open label 1.25 mg orally o.d.	None. MRI and clinical endpoints evaluated

All clinical studies were conducted with hard gelatin capsule formulations. Early clinical studies utilized formulations that included [redacted] (b) (4) (clinical service form, CSF). Subsequent clinical trials, including all phase II and phase III studies of the MS program, used the formulation intended for the market with yellow/white capsule shells (final market image, FMI). The CSF and the FMI formulations are bioequivalent. Absolute bioavailability of fingolimod was assessed for the FMI.

2.2.2 What are the clinical end points and how are they measured in clinical pharmacology and clinical studies?

The following variables were used in the evaluation of pharmacodynamics and effectiveness. Although the variables were similar across studies, every study did not evaluate each variable, because of the specific study design, patient population, etc.

The primary pharmacodynamic endpoints measured in clinical pharmacology studies were:

- Hematologic
    - o Peripheral lymphocyte count: absolute lymphocyte count
    - o Peripheral lymphocyte subsets: flow cytometry
- Cardiovascular

- o heart rate: pulse rate, telemetry, Holter monitoring
- o incidence of sinus pauses: Holter monitoring
- o incidence of atrioventricular block: Holter monitoring
- o blood pressure: systolic and diastolic blood pressure
- o cardiac output: Echocardiography (2D and Doppler)
- o stroke volume: Echocardiography (2D and Doppler)

• Pulmonary

- o FEV1(Forced expiratory volume in one second)
- o FEF_{25-75} (Forced mid-expiratory flow rate)
- o FVC (Forced vital capacity)
- o Exercise oximetry: subjects will be required to step at a rate of 24 cycles per minute on a 12" high step/platform. Each cycle will include stepping up and down with each foot. The test will be conducted for at most 3 minutes or onset of exhaustion. Both heart rate and oxygen saturation will be measured by pulse oximeter and recorded every minute commencing 2 minutes prior to the test and continuing through a 6 minute post test recovery period
- o D_LCO (Carbon monoxide diffusing capacity)

The primary efficacy endpoint in the pivotal RRMS trials was:

• relapse rate (ARR) of multiple sclerosis: the number of relapses per year over 24 months

The key secondary efficacy endpoints in the pivotal RRMS trials were:

• number of new/ newly enlarged T2 MRI lesions
• disability progression as measured by the Expanded Disability Status Scale (EDSS): disability progression as measured by the time to 3-month confirmed disability progression

Note that many of the pharmacodynamic endpoints measured in the clinical pharmacology studies were also measured at selected time points in the pivotal Phase II and III studies.

2.2.3 What are the characteristics of exposure/effectiveness relationships?

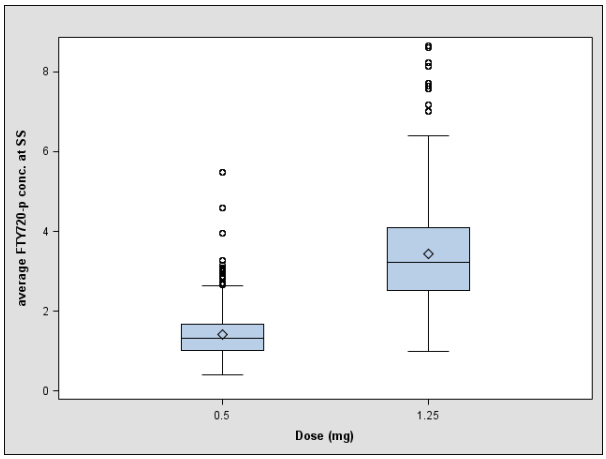
The sponsor proposes the dose of 0.5 mg QD for the treatment of RRMS. In the efficacy analyses results as well as the sponsor's exposure-response analyses across all endpoints, there was little difference shown between low (0.5mg) and high (1.25mg) doses although both doses showed superior effectiveness compared to placebo and active control.

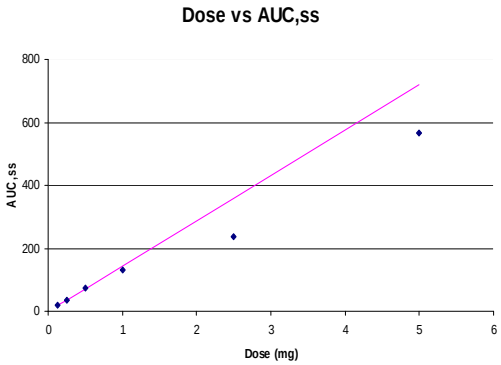
There has been safety concern with fingolimod due to the presence of S1P receptors in multiple tissues, including a transient reduction in heart rate and atrio-ventricular

conduction on treatment initiation, a dose-dependent mild increase in airway resistance, macular edema, a mild increase in blood pressure, and asymptomatic elevation in serum levels of hepatic transaminases. Hence, the reviewer performed the independent analyses to see if lower dose than 0.5mg would produce sufficient effectiveness based on primary endpoint (ARR).

First, the dose-proportionality was examined. Figure **1** displays the distribution of predicted FTY720-p concentration from two phase III studies(left panel) and the relationship between AUC at SS and dose (right panel) with dose proportionality at steady state at doses lower than 1 mg. The predicted median concentration at 0.5mg and 1.25mg are 1.25ng/mL and 3.13ng/mL, respectively. Based on this observation, the concentration at the dose of 0.25mg would be approximately a half of concentration at the dose of 0.5mg if 0.25mg would have studied in the clinical trial.

Figure 1. The distribution of predicted FTY720-p concentration at each dose (left panel) and dose-proportionality (right panel)

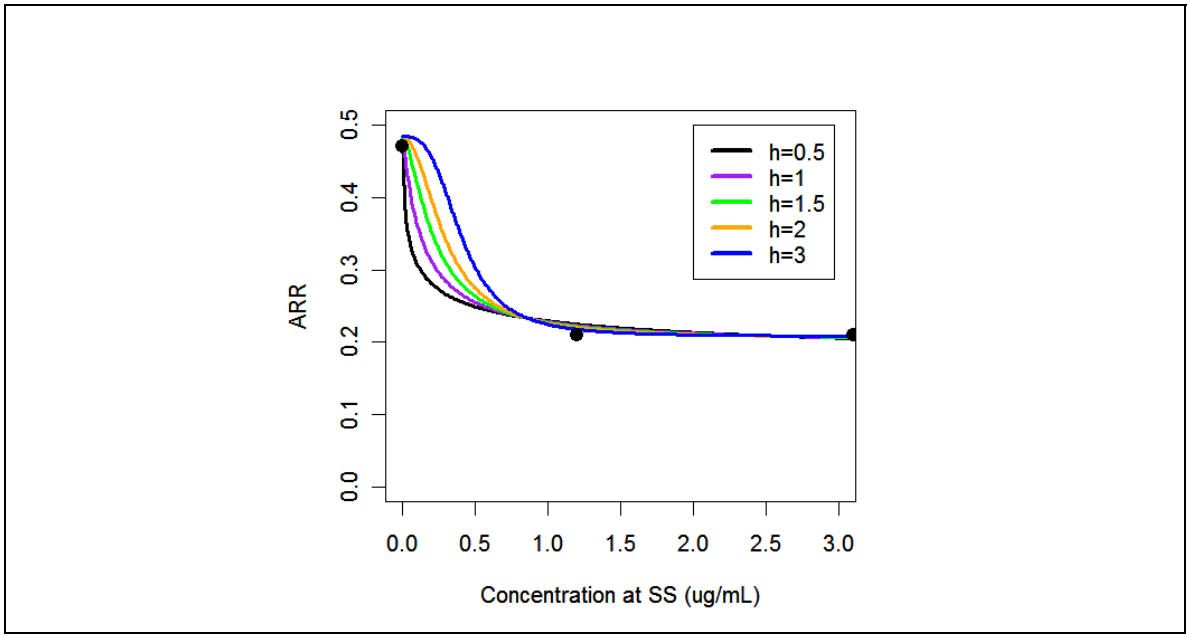




As the first attempt to quantify the relationship between ARR and FTY720-p concentration, the negative binomial model (1) for the number of confirmed relapse was fitted to pooled dataset. The reviewer tried to estimate hill-coefficient parameter in the model. However, due to the lack of data around declining part of model, hill-coefficient could not be estimated. Hence the reviewer fixed it at the range over 0 to 3 rather than estimating it. Figure **2** shows the model-predicted relationship between ARR and FTY720-p concentration based on the reviewer's model by different hill-coefficient. The black dots represent the observed mean ARR at each dose group which were marked at the median of exposure range at each dose. As shown in Figure **2**, the model describes the observed data well regardless of different values of hill-coefficient, implying that there was uncertainty on the shape of relationship, making it impossible to predict ARR at unstudied lower dose such as 0.25 mg precisely.

Hence, given the availability of lymphocyte counts data and the presumed relationship between lymphocyte counts and ARR, the reviewer used lymphocyte counts (PD biomarker) as a bridge to link ARR and FTY720-p concentration as it was expected that lymphocyte counts-concentration relationship and the ARR- lymphocyte counts relationship would be quantified more precisely.

Figure 2. The model predicted ARR and FTY720-p concentration relationship (solid black line) by different hill-coefficient values. The black dots are observed ARR at each dose groups which are marked at the median exposure at each dose.



Before fitting the model for lymphocyte counts, the reviewer examined the time-profile of lymphocyte counts, which shows that the number of lymphocyte counts appears to be at steady state from month 2 (Figure **3**). Therefore, the lymphocyte counts from month 2 were averaged for each patient to match to exposure and ARR. Hereafter, lymphocyte counts at SS refers to average lymphocyte counts at SS.
It is well known that Avonex has different mechanism of action from fingolimod. However, as shown in Figure 4, placebo and active control (Avonex) groups show very similar distribution with median of about 1.7E9/L so the patients who were assigned to Avonex were also included in the analyses.

Figure 3. The absolute number of lymphocyte counts at each month.

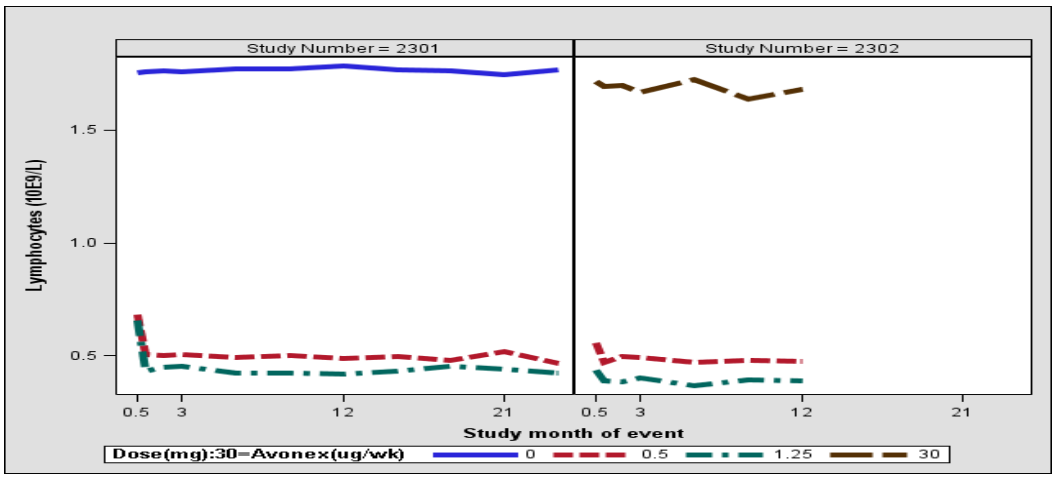
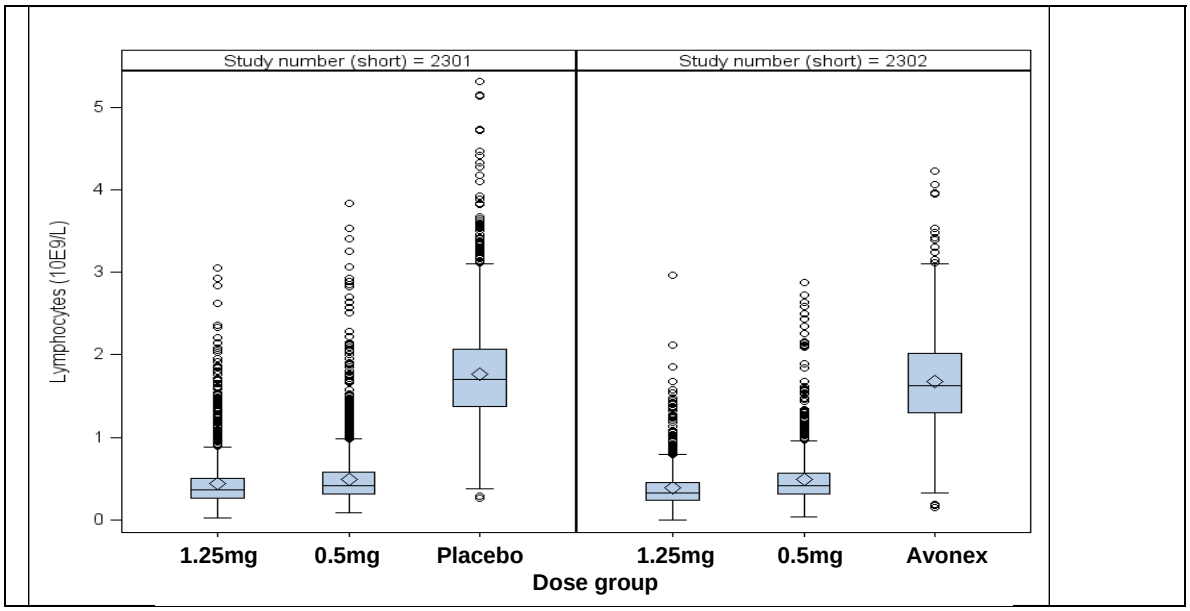
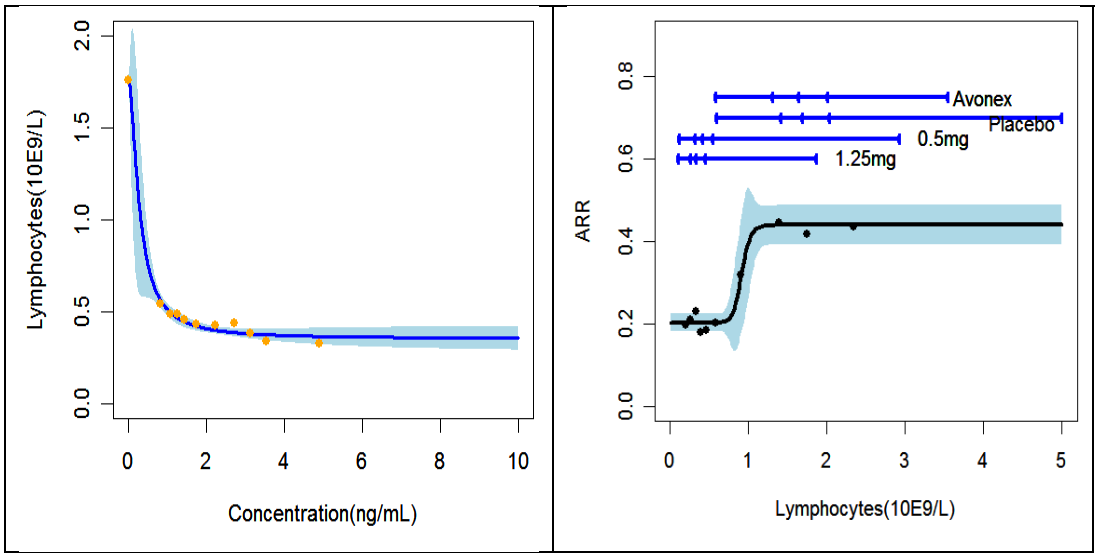


Figure 4. The distribution of absolute number of lymphocyte counts at SS at each treatment group by study.



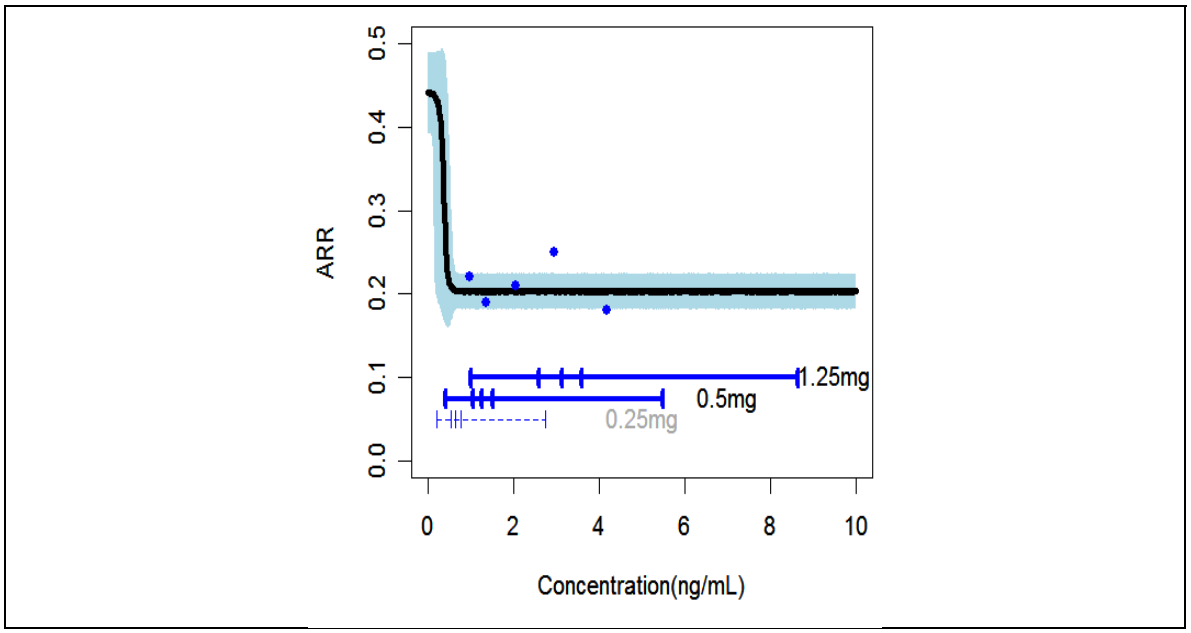
The left panel in Figure **5** displays the relationship between lymphocyte counts at steady state (SS) and FTY720-p concentration, and the right panel represents the relationship between ARR and lymphocyte counts at SS. Both models describe the observed data reasonably well. ARR-lymphocyte counts relationship suggest that mean lymphocyte reduction below 1E9/L would be necessary to get mean ARR of about 0.2 and further lower lymphocyte counts does not provide additional ARR benefit.

Figure 5. The model predicted relationship for lymphocyte counts at SS and FTY720-p concentration relationship (left) and the relationship for ARR and lymphocyte counts at SS (right) with 95% prediction interval (blue shaded area). The orange dots on the left are observed absolute number of lymphocyte at SS at decile of exposure range. The black dots on the right panel indicate the observed ARR at decile of lymphocyte counts at SS. Also blue vertical bars on the right panel shows the distribution of lymphocyte counts for each treatment group.



To link concentration with ARR, the lymphocyte counts were predicted over the observed concentration range (lymphocyte-concentration model) and then the predicted lymphocyte counts were used to predict ARR (ARR- lymphocyte model). Figure **16**6 shows the results for ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge. The shape of ARR-concentration relationship was quantified with improved precision. Based on this relationship, the model predicted average ARR of 0.26 (95%CI: 0.22-0.30) at 0.25 mg, which could be almost as effective as 0.5mg.

Figure 6. The predicted ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge with 95% confidence interval. Four dots represent observed ARR at quintile of exposure range.



In conclusion, the reviewer's analyses showed that exposure-response relationship is flat within the observed exposure range and 0.25mg would be almost as effective as 0.5mg.

2.2.4 What are the characteristics of exposure-safety relationships in clinical pharmacology studies?

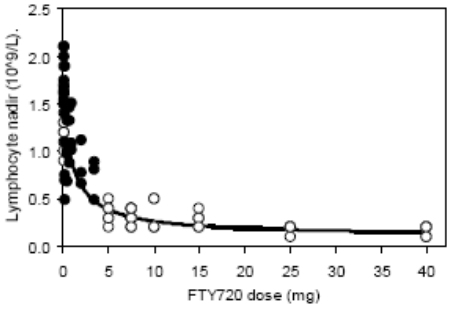
The three main dynamic effects of fingolimod measured in clinical pharmacology trials were decreased peripheral lymphocyte count, negative chronotropic effect and increased small airway resistance, all of which can occur after a single dose of fingolimod.

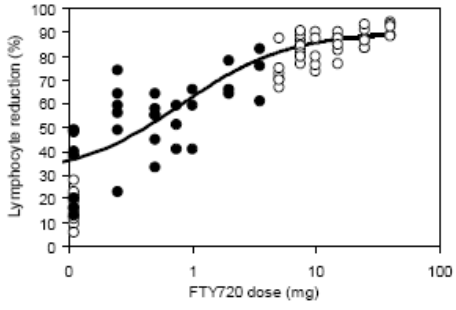
2.2.4.1 Dose-lymphocyte count relationship

For lymphocyte count, a pooled analysis was done, combinding data from both the low single dose study FTY720AB101 (0.25 mg to 3.5 mg, n=32 stable renal transplant patients) and the high single dose study FTY720A2215 (5 mg to 40 mg, n=56 healthy subjects). Because fingolimod-P, the active moiety, was not measured in study FTY720AB101, dose was used as the measure of exposure over a pooled dose range of 0.25 to 40 mg. Given the high correlation of dose to fingolimod-P systemic exposure, this pooled analysis using dose as a measure of systemic exposure is justified.

As shown in Figure 7, the relationships between single dose fingolimod and lymphocyte nadir were adequately described by an inhibitory effect Emax model. When the lymphocyte nadir was expressed as the measured values (cells x 10^9/L), the model-estimated nadir in the absence of fingolimod (placebo) was 1.39 x 10^9/L (CV 5%), the half-maximal dose was 1.4 mg (CV 24%), and the minimal nadir was 0.11 x 10^9/L (CV 35%). When the lymphocyte nadir was expressed as percent reduction from the predose count, the model-estimated reduction (that is, temporal fluctuation) in the absence of fingolimod (placebo) was 30% (CV 4%), the half-maximal dose was 0.8 mg (CV 22%), and the maximal reduction was 90% (CV 14%) as shown in the right panel of Figure 7.

Figure 7. Fingolimod dose-lymphocyte response





Fingolimod dose vs. lymphocyte nadir relationship. Shown are the individual values from renal transplant patients (FTY720AB101, *filled circles*) and healthy subjects (FTY720A2215, *open circles*) and the fit of an inhibitory effect E_{max} model to the data.

As in left panel for lymphocyte nadir expressed as percent reduction from predose count with a logarithmic dose scale (x-axis).

Source: FTY720A2215 FSR

It is concluded that single doses of fingolimod >2.5 to 5 mg have little additional effect compared to lower doses on lymphocyte count.

The trajectory of the lymphocyte counts for 24 hours after first dose of fingolimod administration (0.125 mg to 5 mg) is shown in the Figure 8 below (N=65). Lymphocyte counts decrease is apparent 4 to 8 hours after dosing.

Figure 8. The trajectory of the lymphocyte counts for the first 24 hours post dosing

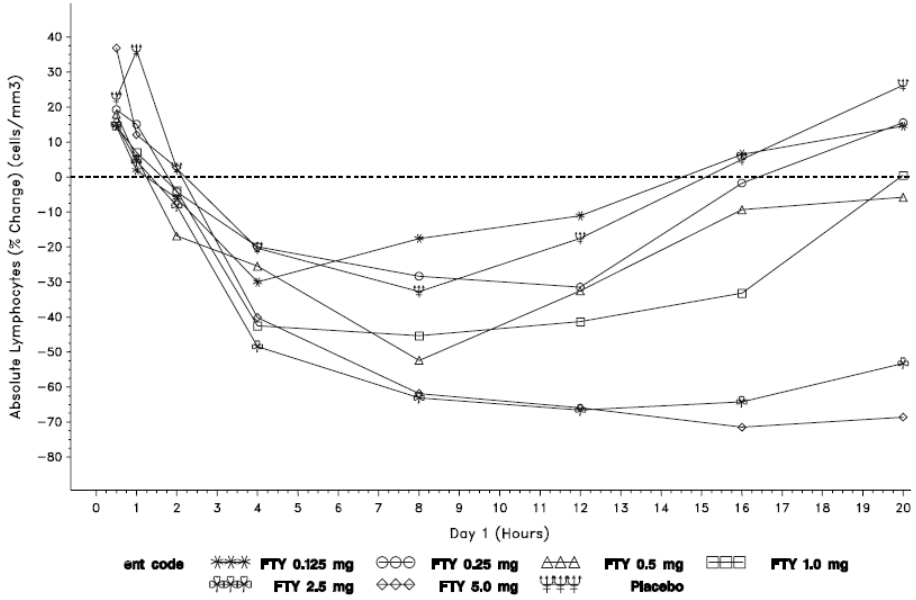
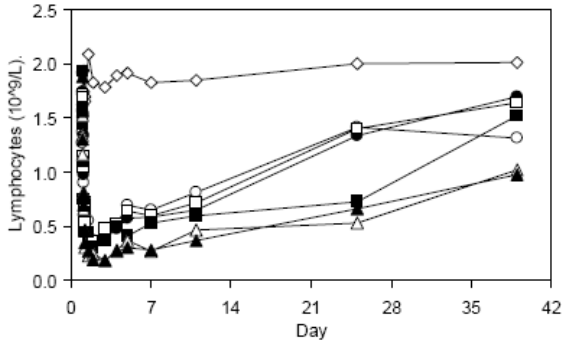


Figure 9 below shows the lymphocyte trajectory over ~40 days after single dose of fingolimod (5 mg to 40 mg) administration (N=56).

Figure 9. The trajectory of the lymphocyte counts for 40 days

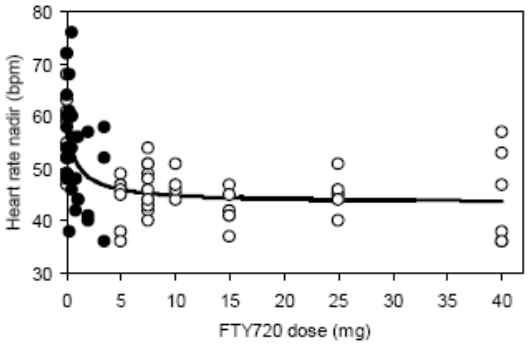


Lymphocyte trajectories after placebo (*open diamonds*) and fingolimod doses of 5mg (*open circles*), 7.5mg (*filled circles*), 10mg (*open squares*), 15mg (*filled squares*), 25mg (*open triangles*) and 40mg (*filled triangles*).

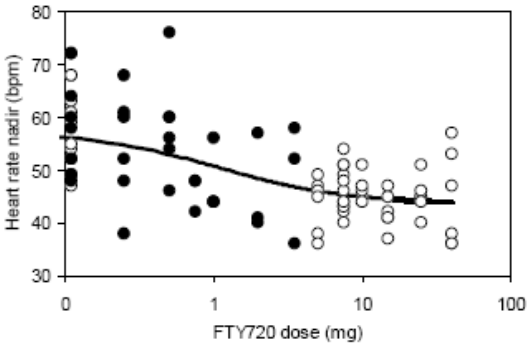
2.2.4.2 Dose-heart rate relationship

Same as lymphocyte count analysis, the negative chronotropic effect was done by pooling two same studies, FTY720AB101 and FTY720A2215,
As shown in Figure 10, the relationships between fingolimod dose and nadir heart rate from vital signs recordings (pulse) were adequately described by an inhibitory effect Emax model. The model-estimated nadir in the absence of fingolimod (placebo) was 57 BPM (CV 4%), the half-maximal dose was 1.1 mg (CV 70%), and the minimal nadir was 44 BPM (CV 4%). When a similar evaluation was performed on AUE(0-4) as the response parameter, the model estimated AUE(0-4) in the absence of fingolimod (placebo) was 245 BPM.h (CV 3%), the half maximal dose was 2.2 mg (CV 114%), and the minimal AUE(0-4) was 205 BPM.h (CV 5%).

Figure 10. Fingolimod dose-heart rate response



Fingolimod dose vs. heart rate nadir relationship. Shown are the individual values from renal transplant patients (FTY720AB101, *filled circles*) and healthy subjects (FTY720A2215, *open circles*) and the fit of an inhibitory effect E_{max} model to the data.



As in left panel with a logarithmic dose scale.

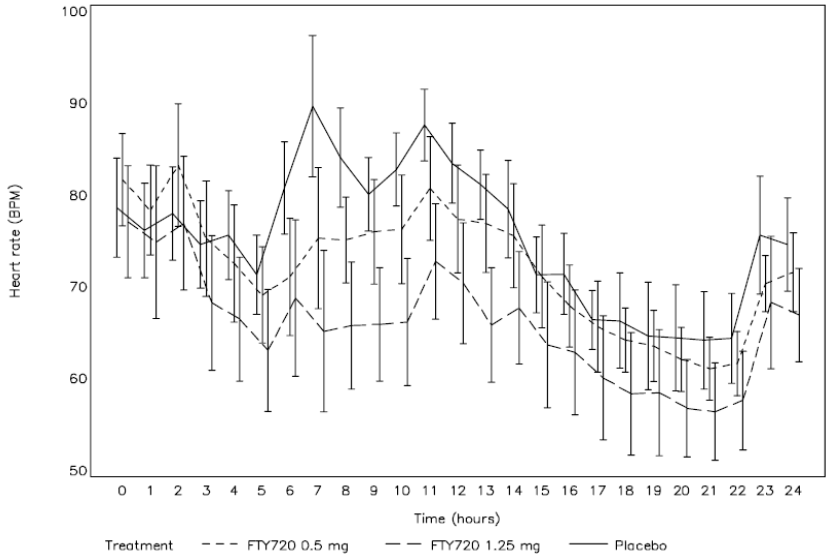
Source: FTY720A2215 FSR

In study FTY720D2105 (N=37) the effects of the clinical doses of fingolimod, 0.5 and 1.25 mg given daily for 14 days, on heart rate and heart function were measured. On day 1, single doses of fingolimod 0.5 and 1.25 mg had a mean negative chronotropic effect of approximately 7-8 and 10-15 beats per minute, respectively (see Figure 11 below). This effect is seen within 3 hours of the first dose, with a nadir of heart rate occurring approximately 4-5 hours post single dose. The negative chronotropic effect persisted but attenuated over the remainder of the 24 hours post dose. On days 7 and 14 of dosing, both doses had a similar magnitude of negative chronotropic effect, approximately 8-12 beats per minute (see Figure 11 below). Neither the 0.5 nor the 1.25 mg dose had an effect on cardiac output or stroke volume. It was not possible to detect a clear effect of either of these two doses on blood pressure.

Hourly heart rate on day 1 is shown in the Figure 11 below:

Figure 11. Hourly heart rate on day 1 (mean plus minus 95 % CI)

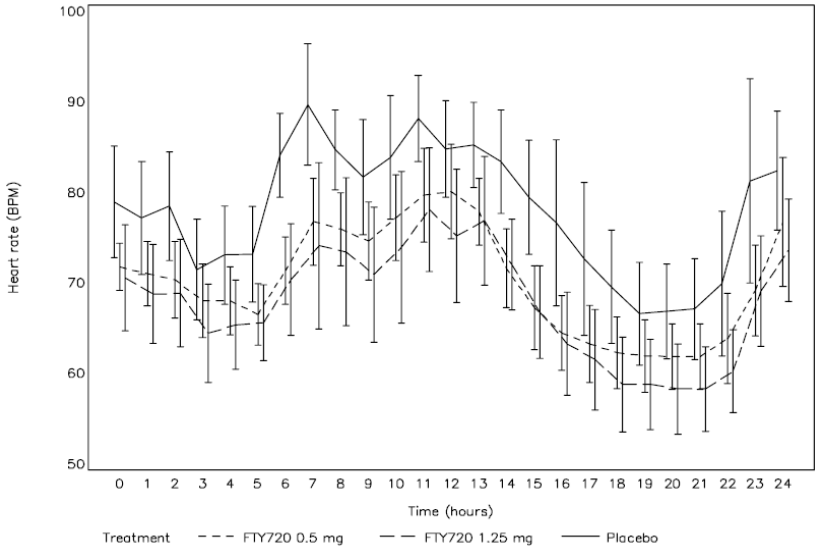
Figure 11-2 **Day 1: Hourly average heart rate (mean plus minus 95% CI) post placebo dose**



Note: Time =0 hours is approximately 8-9 AM.
Source: Post-text Figure 14.2-2.2

Hourly heart rate on day 14 is shown in the Figure 12 below:

Figure 12. Hourly heart rate on day 14 (mean plus minus 95 % CI)



Note: Time =0 hours is approximately 8-9 AM.
Source: Post-text Figure 14.2-2.2

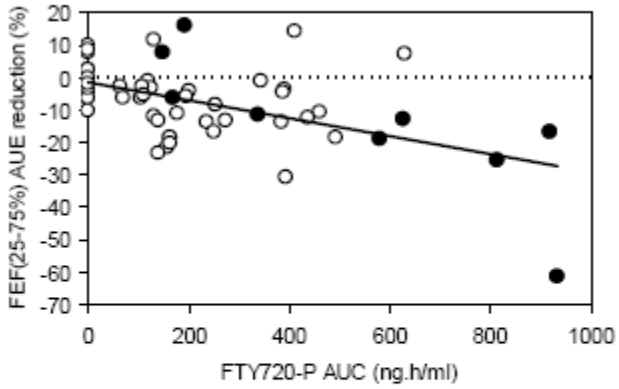
It is concluded that there is a plateau of fingolimod effect on heart rate. Single doses of fingolimod >1 to 2 mg compared to lower doses have little additional negative chronotropic effect.

2.2.4.3 Exposure – Pulmonary function relationship

For small airway resistance, it is not possible to detect an effect of fingolimod at doses <5mg. Therefore only the high single dose (5 to 40 mg) study FTY720A2215 data was used. Because FTY720A2215 (n=56 healthy subjects) was a later study in the fingolimod development program, an assay for fingolimod-P was by that time available and was done in this study. Therefore for small airway resistance, exposure was fingolimod-P whole blood concentration and response was FEF_{25-75}. The fingolimod doses used in the single high dose study FTY720A2215 ranged from 5 to 40 mg.

Given the large differences in FEF_{25-75} at baseline in study FTY720A2215 and the fact that each subject did not have a 4-day trajectory in the absence of drug for comparison, the following approach was taken to assess an exposure versus response relationship capturing both the acute and recovery phases of the change in mid-expiratory flow. The area under the effect curve over the 4-day observation period of 0 to 78 hours (AUE(0-78)) was calculated based on the FEF_{25-75} values expressed as fraction of baseline for each subject. The mean AUE(0-78) for placebo-treated subjects was 77 L/sec x h. Each subject's percent reduction from this mean value [(77 – AUE) / 77 x 100] was then plotted versus the corresponding fingolimod-P AUC(0-72) as shown in Figure 13. Attempts to fit an inhibitory effect Emax model to the data did not yield a more informative description than a simple linear regression: percent reduction in FEF_{25-75} AUE(0-78) = −0.021 x AUC(0-72) − 2.06 (r^2 = 0.272).

Figure 13. Fingolimod exposure-pulmonary function response



Linear regression relating the percent decrease in mid-expiratory flow area under the effect curve AUE(0-78) versus fingolimod-P $AUC_{(0-72)b}$. *Filled circles* designate subjects reporting chest tightness or discomfort.

In study FTY720D2105 the effects of the clinical doses of fingolimod, 0.5 and 1.25 mg given daily for 14 days, on pulmonary function were measured. Over the entire course of the study, neither dose had an effect on FEV1, FEF_{25-75}, FVC, or exercise oximetry.

Doses of 0.5 and 1.25 mg, corresponding to a maximal fingolimod-P AUCτ of approximately 28 and 48 ng*hr/mL, had no effect on FEV1 or FEF_{25-75}. Over a fingolimod single dose range of 5 to 40 mg, there is a linear increase in the dynamic effect of fingolimod on airway resistance. This is uniquely different from the plateau of effect seen with lymphocyte count and heart rate.

2.2.5 Is there any significant exposure-response relationship? And does the relationship support the proposed dose (0.5mg QD)?

Yes, there is a significant relationship between exposure (FTY720-p average concentration at steady state (ng/mL)) and all efficacy endpoints including aggregate annualized relapse rate (ARR) and MRI lesion count when the placebo group was included in the analysis. However, the relationship is flat without placebo within the observed exposure range.

Two phase III studies (CFTY720D2301, CFTY720D2302) were included in the sponsor's exposure-response analyses for both efficacy and safety; CFTY720D2301 was a 24-month double blind, multicenter, parallel-group study comparing the efficacy and safety of 0.5 mg and 1.25mg fingolimod QD versus placebo; CFTY720D2302 was a 12-month double-blind, randomized, multicenter, parallel-group study comparing the efficacy and safety of 0.5 mg and 1.25 mg fingolimod QD versus interferon β-1a i.m. (Avonex®) administered i.m. once weekly in patients with relapsing-remitting multiple sclerosis.

Primary endpoint was the ARR which was computed as total number of confirmed relapses for all the patients divided by the total number of days on the study for all patients, multiplied by 365.25. Fingolimod at both dose groups (0.5mg and 1.25mg) showed superior effectiveness (study 2301: 18%, 16%, study 2302: 26%, 20%) compared to placebo (40%) and active control (interferon β-1a, 33%) in the efficacy analysis with little difference between two dose groups. The similar results were shown in other secondary endpoints such as disability-related endpoint (EDSS score) or MRI measures of inflammation (MRI lesion count).

The sponsor conducted exposure-response analyses to characterize the relationship between FTY720-p concentrations at steady state and key efficacy (MRI lesion count, relapses) and safety endpoints (lymphocyte reduction, liver enzyme, FEV1 and HR), focusing on estimating potency parameter at each dose group to justify the proposed dose of 0.5mg QD. The sponsor's exposure-response (E-R) analyses using lymphocyte counts, new T2 lesions and ARR showed that there was significant exposure-response relationship across all three endpoints and the dose of 0.5mg would achieve about 80-88% of maximum response.

Due to the presence of S1P receptors in multiple tissues, fingolimod manifests a number of other biological effects in addition to the reduction in circulating lymphocytes. These include a transient reduction in heart rate and atrio-ventricular conduction on treatment

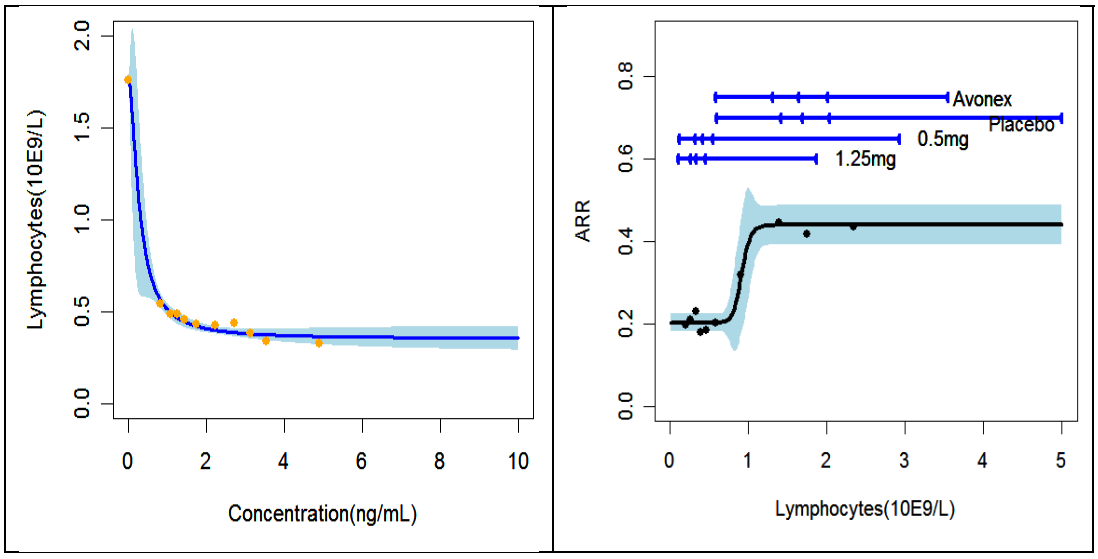
initiation, a dose-dependent mild increase in airway resistance, macular edema, a mild increase in blood pressure, and asymptomatic elevation in serum levels of hepatic transaminases. The sponsor's exposure-safety analyses showed that most safety endpoints including FEV1, heart rate (HR) and infection rate were not related to FTY720-p concentration within the studied exposure range but liver enzyme level such as AST and ALT slightly increases with increasing concentration especially in male population.

In addition to the sponsor's E-R analyses, the reviewer performed independent analyses to examine whether the sponsor's proposed dose is appropriate or not. Given the benefit and risk profile of fingolimod the reviewer evaluated whether lower doses than 0.5 mg can provide acceptable effectiveness based on the primary endpoint (ARR). The reviewer's analysis indicates that 0.25 mg may provide comparable effectiveness as 0.5 mg.

First the reviewer tried to quantify the relationship between ARR and FTY720-p concentration directly. However, due to the lack of data at lower exposure range, there was uncertainty on the shape of relationship, making it impossible to predict ARR at unstudied lower dose such as 0.25 mg precisely (refer to section 4. reviewer's analysis). Hence, given the availability of lymphocyte counts data and the presumed relationship between lymphocyte counts and ARR, the reviewer used lymphocyte counts (PD biomarker) as a bridge to link ARR and FTY720-p concentration as it was expected that lymphocyte counts-concentration relationship and the ARR- lymphocyte counts relationship would be quantified more precisely.

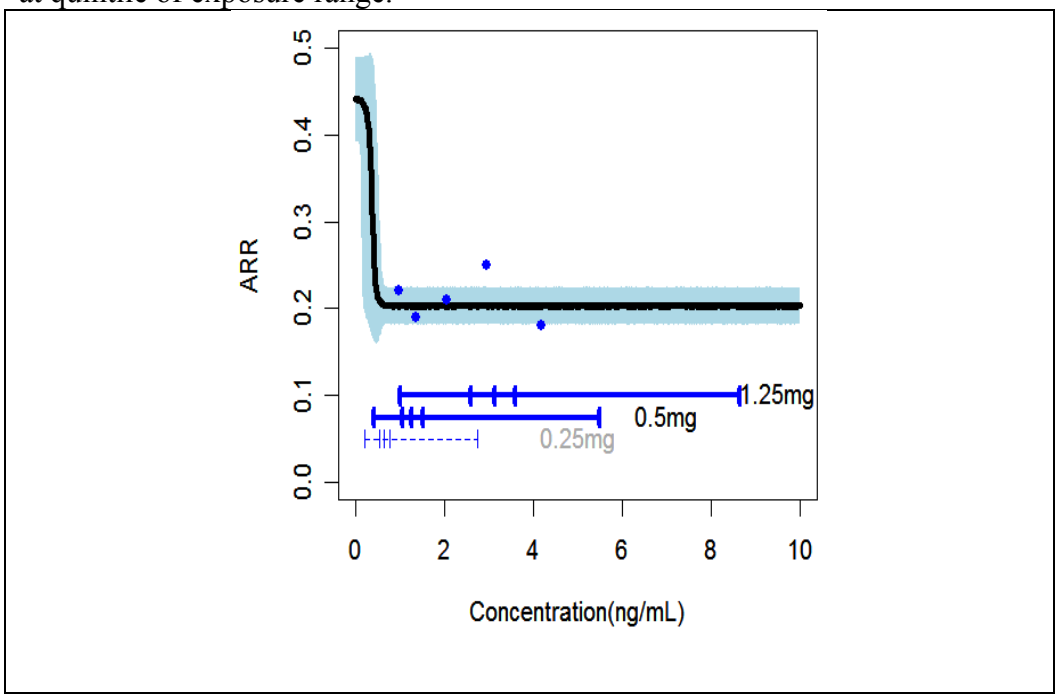
The left panel in Figure 14 displays the relationship between lymphocyte counts at steady state (SS) and FTY720-p concentration, and the right panel represents the relationship between ARR and lymphocyte counts at SS. Both models describe the observed data reasonably well. ARR-lymphocyte counts relationship suggest that mean lymphocyte reduction below 1E9/L would be necessary to get mean ARR of about 0.2 and further lower lymphocyte counts does not provide additional ARR benefit.

Figure 14. The model predicted relationship for lymphocyte counts at SS and FTY720-p concentration relationship (left) and the relationship for ARR and lymphocyte counts at SS (right) with 95% prediction interval (blue shaded area). The orange dots on the left are observed absolute number of lymphocyte at SS at decile of exposure range. The black dots on the right panel indicate the observed ARR at decile of lymphocyte counts at SS. Also blue vertical bars on the right panel shows the distribution of lymphocyte counts for each treatment group.



To link concentration with ARR, the lymphocyte counts were predicted over the observed concentration range (lymphocyte-concentration model) and then the predicted lymphocyte counts were used to predict ARR (ARR- lymphocyte model). Figure 15 shows the results for ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge. The shape of ARR-concentration relationship was quantified with improved precision. Based on this relationship, the model predicted average ARR of 0.26 (95%CI: 0.22-0.30) at 0.25 mg, which could be almost as effective as 0.5mg.

Figure 15. The predicted ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge with 95% confidence interval. Four dots represent observed ARR at quintile of exposure range.



Recommendations

The Division of Pharmacometrics has reviewed the submission (NDA 22527) and has the following recommendation:

- A lower dose than 0.5mg QD should be studied if the current safety profile at 0.5 mg QD is not acceptable.

2.2.6 Does this drug prolong QT or QTc interval?

The QT study conducted at 1.25 mg and 2.5 dose of FTY720 has been reviewed and analyzed by IRT group in 2008. The summary of the review is provided below.

This study failed to exclude a 10 ms prolongation of the QT interval for both doses of FTY720 (1.25 and 2.5 mg). At 6 hours post-dosing on Day 7, the maximum mean ΔΔQTcI for both 1.25-and 2.5-mg doses was 10 ms with an upper one-sided 95% CI of ~14 ms (see Table 3).

Table 3. The Point Estimates and the 90 % CIs Corresponding to the Largest Upper Bounds for FTY720(1.25 mg and 2.5 mg) and the Largest Lower Bound for Moxifloxacin (FDA Analysis)

Treatment	Time (hour)	ΔΔQTcI (ms)	90% CI (ms)
FTY720 1.25 mg	6	10.0	13.6
FTY720 2.50 mg	6	10.5	14.0
Moxifloxacin 400 mg*	6	10.5	5.7

* Multiple endpoint adjustment is not applied. The largest lower bound after Bonferroni adjustment for 3 timepoints is 4.3 ms. Note: The sponsor specified times 1.5, 3, and 6 as the times to be tested for Moxifloxacin. At 8 hours the estimated ΔΔQTc was 11.0 ms and the unadjusted lower bound of the 90% C.I. was 7.5 ms.

We do not have confidence in the accuracy of the estimated effect of administering FTY720 on the QTc interval for the following reasons:

1. The positive control, a single oral dose of 400 mg moxifloxacin, failed to have the expected effect on ΔΔQTcI (change from baseline and placebo corrected); the largest ΔΔQTcI for moxifloxacin was about 10.5 ms and occurred at 6 and 8 hours post-dose. This profile is not likely since the Tmax of moxifloxacin observed in this study was 3 hours. This is especially relevant, since the largest ΔΔQTcI for FTY720 was of the same magnitude and occurred at the same time points as that observed for moxifloxacin.
2. Despite a 2-fold increase in the exposure to FTY720 plasma concentrations, there was no dose-response relationship for QT prolongation. There was also not a concentration-QTc relationship for FTY720 and its metabolite FTY720-P. This does

not, however, rule out the existence of a positive exposure-response relationship because of the small range of steady-state concentrations observed on Day.

We recommend baseline and periodic on-therapy ECGs are collected for safety assessments in clinical trials irrespective of the results of the TQT study because bradycardia and conduction defects have been noted in the clinical program (although there have been no cases of Mobitz II or 3rd degree blocks). According to the guidance to investigators in the current IB, vitals signs (including BP, HR and ECG) are being monitored pre-dosing and following a 6-hour observation period after administration of FTY720.

The sponsor had followed the Agency's recommendation in their pivotal trials.

2.2.7 Are the active moieties in the blood (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters?

Yes. The analytical methods for fingolimod and its metabolites, fingolimod-P, M2 and M3, are acceptable. Concentrations of fingolimod and fingolimod-P in whole blood were determined using specific LC-MS methods with lower limit of quantification (LLOQ) of 0.08 ng/mL for fingolimod and 0.1 ng/mL for fingolimod-P (1-1.5 ng/mL in early studies). Blood concentrations of the metabolites M2 and M3 were determined using a LC-MS method with a LLOQ set at 0.1 ng/mL for both analytes. Urine fingolimod together with metabolite M2 and M3 concentrations were determined using a LC-MS/MS method with a LLOQ set at 1 ng/mL for all three analytes. Fingolimod has been shown to be stable for at least 6 months in whole blood when stored below -18°C. Fingolimod-P is stable in whole blood for at least 17 months when stored below -70°C. M2 and M3 are stable for at least 13 weeks in whole blood when stored below -18°C. Fingolimod, M2 and M3 are stable for at least 7.5 months in urine when stored below -18°C.

A summary of all methods used is given in the analytical section 2.6 of this review.

2.2.8 What are the general ADME characterstics of fingolimod?

The key ADME characteristics of fingolimod are summarized below:

Absorption:

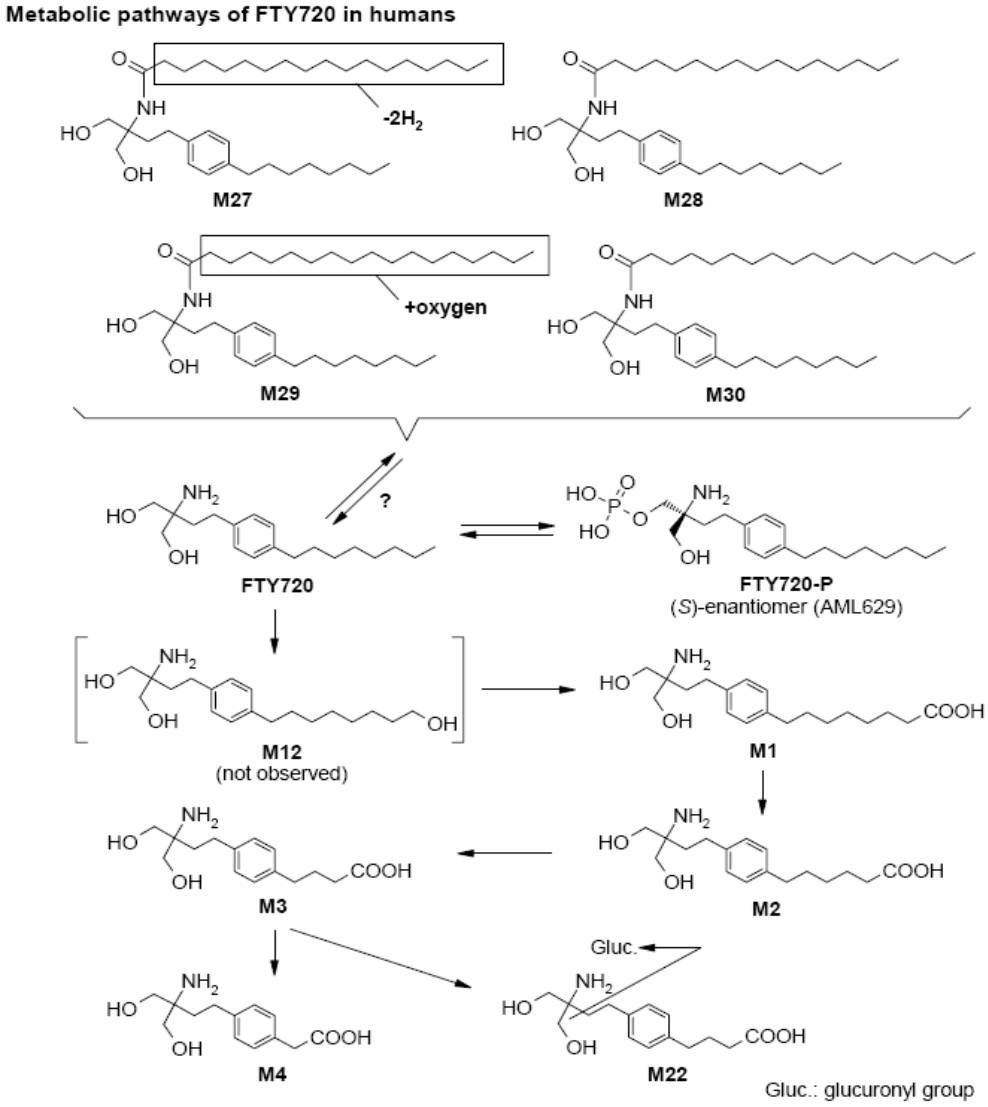
Fingolimod absorption is slow (tmax of 12-16 hours) and extensive (≥85%, based on the amount of radioactivity excreted in urine and the amount of metabolites in feces extrapolated to infinity). The absolute oral bioavailability is high (94%).

Distribution:

Fingolimod highly distributes in red blood cells, with the fraction in blood cells of 86%, but fingolimod-P has a smaller uptake in blood cells of <17%. Fingolimod and fingolimod-P are highly protein bound (>99.7%) (mainly to albumin) . Fingolimod and fingolimod-P protein binding is not altered by renal or hepatic impairment. Fingolimod is extensively distributed to body tissues with volume of distribution (Vz) of about 1200±260 L.

Metabolism:

The biotransformation of fingolimod in humans occurs by three main pathways: (i) by reversible stereoselective phosphorylation to the (*S*)-enantiomer of fingolimod-P (AML629, the active moiety), (ii) by oxidative biotransformation mainly via the cytochrome P450 4F2 isoenzyme and subsequent fatty acid-like degradation to inactive metabolites, (iii) by formation of nonpolar ceramide analogs of fingolimod. Fingolimod-P can be converted back to fingolimod and the nonpolar ceramide metabolites of fingolimod may also be converted back to fingolimod. Therefore, it is assumed that fingolimod, fingolimod-P and the nonpolar ceramide analogs of fingolimod are in dynamic equilibrium at steady-state. Following single oral administration of [14C]fingolimod, the major fingolimod-related components in blood, as judged from their contribution to the AUC(0-816) of total radiolabeled components, are fingolimod itself (23.3%), fingolimod-P (10.3%), M3 (8.3%), M29 (8.9%) and M30 (7.3%).

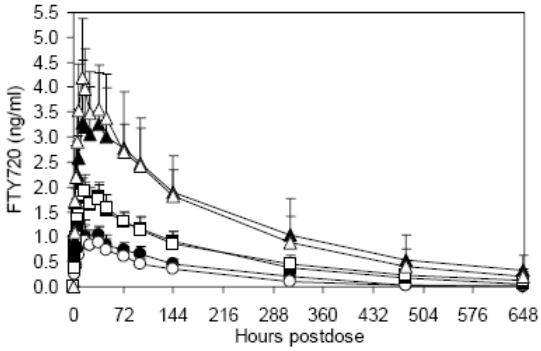


Elimination:

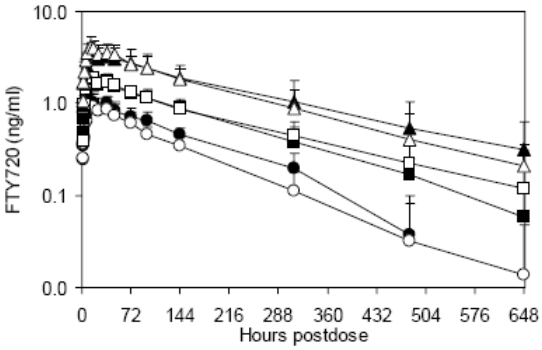
Fingolimod blood clearance is low, 6.3±2.3 L/h, and the average apparent terminal half-life (t1/2) is long, 6-9 days. Blood levels of fingolimod-P decline in parallel with fingolimod in the terminal phase yielding similar half-lives for both. After an oral administration, about 81% of the dose is slowly excreted in the urine as inactive metabolites. Fingolimod and fingolimod-P are not excreted intact in urine but are the major components in the feces with amounts representing less than 2.5% of the dose each. After 34 days, the recovery of the administered dose is 89%.

The concentration-time profiles of fingolimod after single dose of 1.25 mg, 2.5 mg and 5 mg of fingolimod administration in healthy Caucasian and Japanese (FTY720A2304, n=69) are shown in the Figure 16 below:

Figure 16. Fingolimod single-dose profiles



Mean FTY concentration profiles at 1.25mg (*circles*), 2.5mg (*squares*), 5mg (*triangles*) in white subjects (*open symbols*) and Asian subjects (*filled symbols*).



As in left panel on a logarithmic concentration scale. Individual and group plots are in Appendix 4 Figure 1.

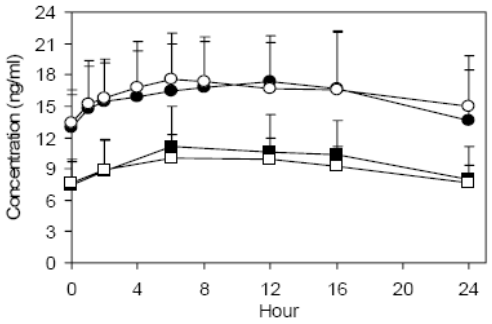
Steady-state PK:

Fingolimod and fingolimod-P pharmacokinetics after multiple once-daily doses are consistent with those after single dose and are time-independent. Steady-state exposure is reached between 1 to 2 months during once-daily dosing with an estimated 11-fold accumulation of blood levels from first dose to steady state. The fingolimod blood concentration profile at steady-state shows a peak to trough fluctuation of approximately 20%. Tmax is variable, occurring at a median time of 12 hours. Fingolimod-P reaches Cmax at a median time of 8 hours, and the peak to trough fluctuation is approximately 45%.

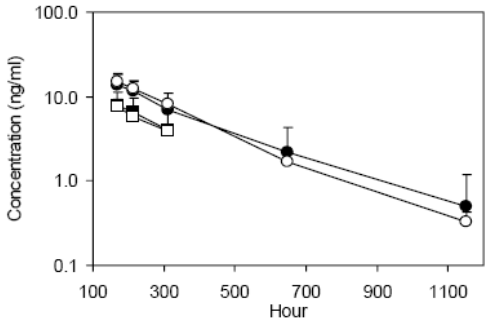
The molar ratios of fingolimod-P/fingolimod AUCτ and Cmaxss averages 0.42 and 0.49, respectively, with small CV% (12-17%) at 0.5 mg and 1.25 mg QD.

The concentration-time profiles of fingolimod and figolimod-P after multiple doses of 5 mg QD of fingolimod administration in healthy Caucasian and Japanese are shown in the Figure 17 below:

Figure 17. fingolimod and fingolimod-P multiple-dose profiles



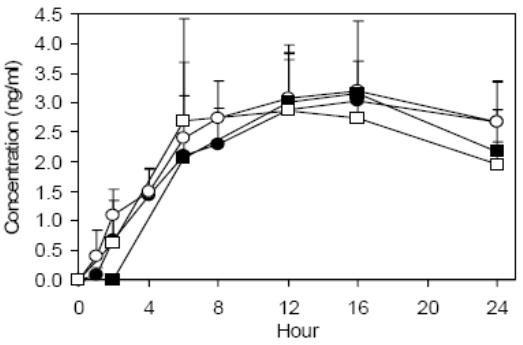
Mean concentration profiles over the 24-hour dose interval on day 7 in Asian subjects (*filled symbols*) and white subjects (*open symbols*). Shown are FTY720 (*circles*) and FTY720-phosphate (*squares*). *Bars* represent 95% confidence intervals.



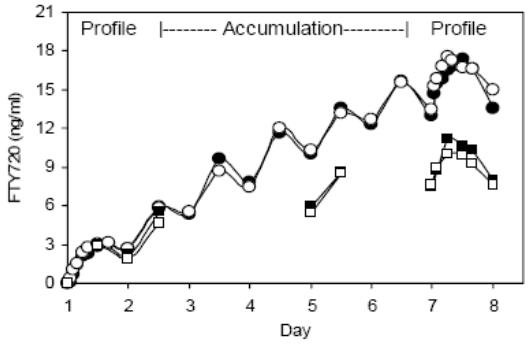
As in left panel during the washout after day 7. the assay quantification limit for FTY720 was 0.08 ng/ml and for FTY720-phosphate was 1 ng/ml.

The concentration-time profiles of fingolimod and figolimod-P on day 1 and the accumulation over 8 days after multiple doses of 5 mg QD of fingolimod administration in healthy Caucasian and Japanese are shown in the Figure 18 below:

Figure 18. Fingolimod accumulation profiles



Mean concentration profiles over the 24-hour dose interval on day 1 in Asian subjects (*filled symbols*) and white subjects (*open symbols*). Shown are FTY720 (*circles*) and FTY720-phosphate (*squares*). *Bars* represent 95% confidence intervals.

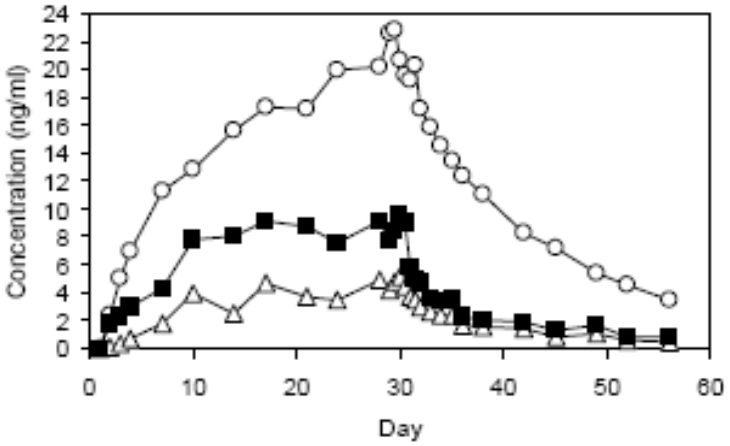


Mean concentration profiles on days 1 and 7 with the mean predose C0 and peak C12 concentrations on days 2 through 6. Symbols as defined in left panel,

PK of M2 and M3

The blood concentration-time profiles of finfolimod, M2 and M3 over 56 days with administration of 5 mg fingolimod QD for 28 days are shown in the Figure 19 below (FTY720AB102, n=69):

Figure 19. Fingolimod, M2 and M3 PK profiles



Mean morning concentrations of FTY720 (*open circles*), metabolite 3 (*filled squares*), and metabolite 2 (*open triangles*) during treatment with 5 mg/day FTY720 in 3 patients from day 1 to 28. Plots at all dose levels are in Post-text Figure 3.

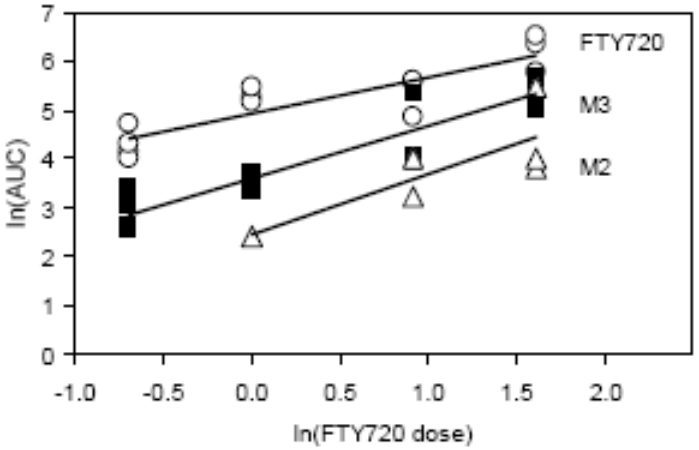
Based on the steady-state data, M2/FTY720 and M3/FTY720 AUCratio, were about 20 and 50 percent of the corresponding exposure to FTY720, respectively. The terminal decline in M2 and M3 blood concentrations paralleled that of FTY720 yielding a similar half-life (see Table 4 below). The M2 and M3 AUCτ,ss on day 28 rose with dose over the range 0.5 to 5 mg/day in parallel with the dose-AUC relationship of FTY720 (see Figure 20 below).

Table 4. Fingolimod metabolites PK parameters

Parameter/analyte	0.5 mg/day	1 mg/day	2.5 mg/day	5 mg/day
N	4	3	3	3
$AUC_{\tau,b}$ (ng.h/ml)				
FTY720	77 ± 26	204 ± 31	219 ± 79	522 ± 184
M2	--	--	40 ± 21	113 ± 110
M3	20 ± 8	35 ± 7	106 ± 87	237 ± 74
$AUC_{\tau,b}$-ratio				
M2/FTY720	--	--	0.15 ± 0.08	0.21 ± 0.18
M3/FTY720	0.28 ± 0.13	0.17 ± 0.04	0.47 ± 0.29	0.52 ± 0.30
$t_{1/2}$ (days)				
FTY720	7.9 ± 2.7	10.8 ± 0.4	7.6 ± 1.2	9.5 ± 2.7
M2	--	--	12.4 ± 7.0	7.3 ± 4.0
M3	--	12.2 ± 4.7	10.0 ± 2.6	8.1 ± 3.1

Data are mean ± sd. Data source: Post-text Table 6, Table 7.

Figure 20. Relationship of fingolimod, M2 and M3 AUC versue fingolimod dose



Dose versus AUC relationships for FTY720 and metabolites 2 and 3 on day 28. Shown are the linear regression lines.

Fate of drug as seen in mass balance studies:

Following single oral administration of [14C]fingolimod to healthy subjects (Study FTY720A2217), fingolimod was slowly and well absorbed with a fraction of the dose absorbed estimated to be greater than 85% (based on the amount of radioactivity excreted in urine and the amount of metabolites in feces extrapolated to infinity). Fingolimod was extensively metabolized as judged from the recovery in excreta. The biotransformation of fingolimod occurred essentially by three pathways (See Section 2.2.8 Metabolism). Fingolimod-P was eliminated mainly by dephosphorylation back to fingolimod, followed by oxidative metabolism of the latter.

The major fingolimod-related components in blood, as judged from their contribution to the AUC(0-816) of total radiolabeled components, were fingolimod itself (23.3%), fingolimod-P (10.3%), M3 (8.3%), M29 (8.9%) and M30 (7.3%). In terms of Cmax, Fingolimod, fingolimod-P and M3 were the major components while the concentrations of M29 and M30 were low at all time points analyzed. M2, M27 and M28 were minor metabolites in blood. The AUC values of the metabolites, relative to that of fingolimod, amounted to 36% for M3, 44% for fingolimod-P, 53% for M29 and 41% for M30. The AUC values of M2, M27 and M28 were below 10% of the AUC of fingolimod. M29 and M30 showed half-lives around 400 hours, similar to that of the total radiolabeled components and longer than those of fingolimod and fingolimod-P (137 hours on average and 166 hours, respectively).

The radioactivity was excreted very slowly (recovery of 62% of dose on average after 10 days, reaching 89% of dose by Day 34), predominantly in the urine (81% based on extrapolation to infinity) and to a lesser extent in the feces (11% based on extrapolation to infinity). Metabolite M3 accounted for a major proportion of the radioactivity excreted in urine (about 70% of total urinary radioactivity; 36.6% of dose) with smaller contributions of the metabolites M2, M4 and M22. Fingolimod and fingolimod-P were not detected in urine but were the major components in the feces with 2.4 ± 0.5% and 1.7

± 0.4% of dose, respectively, accompanied by smaller amounts of the metabolites M1, M2 and M3.

Table 5. Balance of excretion of radioactivity in urine and feces

Collection time period	Excretion (% of dose) (mean ± SD, n=4)		
	Urine	Feces	Total
0-72 h	22.3 ± 5.3	5.4 ± 1.1	27.7 ± 5.3
0-240 h	52.3 ± 6.3	9.9 ± 1.7	62.1 ± 7.6
288-312 h	2.0 ± 0.5	n.s.	-
456-480 h	1.0 ± 0.4	n.s.	-
792-816 h	0.3 ± 0.2	n.s.	-
Estimated excretion in the period of 0 to 816 h	78 ± 3	11 ± 2	89 ± 3
Estimated excretion in the period of 0 to infinity (extrapolated)	81 ± 3	11 ± 2	92 ± 2
n.s.: not sampled			

2.2.9 What is the variability in the PK data?

The inter- and intra-subject variability of fingolimod and fingolimod-P pharmacokinetics after single dose administration was determined from the bioequivalence study FTY720A2309 and from the food effect study FTY720D2107. The cross-over design of these two studies allowed splitting the variability of the pharmacokinetic parameters into their inter- and intra subject components. Specifically the inter- and intra-subject variability were derived from the variance components estimated from a linear mixed effect model adjusted for the sequence, period and treatment as fixed effects, and for the subject as a random effect. Following single oral administration, the inter-subject variability of AUC and Cmax are 31% and 15%, respectively, for fingolimod, and 33% and 16%, respectively, for fingolimod-P. The intrasubject variability of AUC and Cmax are 16% and 10%, respectively, for fingolimod, and 12% and 14%, respectively, for fingolimod-P.

Table 6. Inter- and intra-subject variability (%) of Cmax and AUC for fingolimod and fingolimod-P after single doses

		Inter-subject		Intra-subject	
Study	Analyte	$C_{max,b}$	AUC_b	$C_{max,b}$	AUC_b
FTY720A2309	Fingolimod	16%	31%	11%	16%
FTY720D2107	Fingolimod	14%	31%	10%	17%
FTY720A2309	Fingolimod-P	17%	-	14%	-
FTY720D2107	Fingolimod-P	15%	33%	-	12%

Source: Values derived from the inter- and intra-subject variability of the respective linear mixed effect model in [Study FTY720A2309 Appendix 6] and [Study FTY720D2107 Appendix 6].

At steady-state, the variability of fingolimod and fingolimod-P pharmacokinetics was determined from study FTY720D2101 where healthy subjects received a loading dose regimen such that escalating doses of fingolimod were administered over a 4 day period in order to achieve pharmacokinetic steady state concentrations at the end of this time period. As the exposure parameters were determined only once per subject, precise estimates of intra and inter-subject variability could not be calculated as described above. Variability was expressed as the coefficient of variation (CV%). The variability of AUCτ,ss and Cmax,ss, as measured by CV%, were 29% and 28%, respectively, for fingolimod, and 27% and 24%, respectively, for fingolimod-P.

2.2.10 Based on the pharmacokinetic parameters, what is the degree of linearity or nonlinearity in the dose-concentration relationship?

The pharmacokinetics of fingolimod was dose-proportional after single dose in the dose range of 0.125 to 40 mg and multiple doses at doses at 0.5 mg QD and lower. There was less than dose-proportional increase in Cmax and AUC with increasing doses higher than 0.5 mg QD. But T1/2 does not change.

For fingolimod-P, fingolimod-P Cmax displays an under-proportional increase with the dose, over the range 1.25-40 mg, and AUC(0-96) rises in an apparent dose-proportional manner, over the range 5-40 mg after single dose. At steady state, the exposure is approximately dose-proportional with slightly skewed data from the unity line.

The above description is different from the sponsor's conclusion for dose proportionality at steady state which is shown below.

Spnsor's conclusion: At steady state, using once daily administration, there is no major deviation from dose proportionality for the fingolimod and fingolimod-P blood exposure.

Single dose:

Fingolimod: the estimate and 90% confidence interval (CI) for the exponent parameter of the statistical power model showed that in various studies with different dose ranges, fingolimod PK parameter (Cmax and AUC) appear to be dose proportional.

FTY720AB101: 0.25-3.5 mg.
FTY720A2304: 1.25-5 mg.
FTY720A2215: 5-40 mg.

Table 7. Estimate and 90% CI of the exponent (slope) of the power model for fingolimod

Study	AUC_b	$C_{max,b}$
Study FTY720AB101	1.11 (1.01- 1.21)	1.08 (1.01- 1.15)
Study FTY720A2304	1.16 (0.99- 1.32)	0.98 (0.86- 1.11)
Study FTY720A2215	1.05 (0.91- 1.19)	1.04 (0.96- 1.12)

Source: [FTY720A2215 Appendix 6]. Results derived from the PK parameters listed in [FTY720A2304 Appendix 4] and [FTY720AB101 Appendix 4]

In addition, in study FTY720AB102, the dose-normalized Cmax and AUC also showed dose proportionality over the dose range of 0.125-5 mg (see Table 8 below).

Table 8. Fingolimod PK: first dose

Parameter	0.125 mg	0.25 mg	0.5 mg	1 mg	2.5 mg	5 mg
N	9	8	12	10	10	7
t_{max} (h)	12 (6-24)	12 (8-24)	14 (6-24)	20 (6-25)	12 (4-23)	12 (4-16)
$C_{max,b}$ (ng/ml)	0.08 ± 0.01	0.17 ± 0.03	0.35 ± 0.09	0.65 ± 0.17	1.37 ± 0.33	3.02 ± 0.58
$C_{max,b}$/dose (ng/ml/mg)	0.60 ± 0.10	0.66 ± 0.12	0.70 ± 0.18	0.65 ± 0.17	0.55 ± 0.13	0.60 ± 0.12
$AUC_{\tau,b}$ (ng.h/ml)	1.2 ± 0.1	3.0 ± 0.7	6.1 ± 1.7	11.5 ± 3.8	23.0 ± 7.5	54.4 ± 10.8
$AUC_{\tau,b}$/dose (ng.h/ml/mg)	9.2 ± 0.9	11.9 ± 2.7	12.2 ± 3.5	11.5 ± 3.8	9.2 ± 3.0	10.9 ± 2.2

Values are mean ± sd except for tmax which is median (range).

Data source: Post-text Table 1, Table 2.

Fingolimod-P: the estimate and 90% CI for the exponent parameter of the statistical power model showed that there is no clear deviations from dose-proportionality for AUC(0-96), however, either at higher doses in study FTY720A2215 or data unavailable for study 2304. There is a clear under-proportional increase in fingolimod-P Cmax with the dose in both studies. A dose increased by a factor 2 results in a Cmax increased by a factor 1.60.

Table 9. Estimate and 90% CI of the exponent (slope) of the power model for fingolimod-P

Study	$AUC_{(0-96)b}$	$C_{max,b}$
[FTY720A2304]	NA	0.68 (0.57- 0.79)
[FTY720A2215]	0.99 (0.87- 1.12)	0.75 (0.64- 0.86)

Source: $AUC_{(0-96)b}$: Results derived from partial exposure computed from raw concentration data of the evaluable subjects for the pharmacokinetic analysis in [Study FTY720A2215 Appendix 5].

$C_{max,b}$: [FTY720A2215 Appendix 6]. Results derived from the PK parameters listed in [FTY720A2304 Appendix 4].

Multiple doses:

Fingolimod: the sponsor claimed that in study FTY720D2101, fingolimod and fingolimod-P AUCτ and Cmax,ss were twice greater for the 2.5 mg dose (n=61) than for

the 1.25 mg dose (n=52) suggesting no major deviation from dose proportionality at steady state. (see Table 10 below)

Table 10. Fingolimod and fingolimod-P PK on day 7 after a four-day loading dose regimen and a three-day once-daily maintenance dose administration in healthy subjects

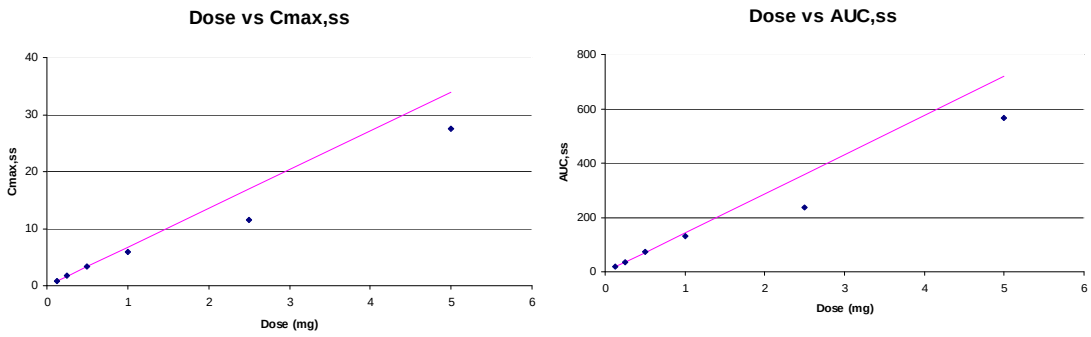
PK parameters	Fingolimod		Fingolimod-P	
	1.25 mg	2.5 mg	1.25 mg	2.5 mg
N	52	61	52	61
$C_0^{ss}{}_{,b}$ (ng/mL)	5.09 ± 1.46	10.1 ± 2.93	2.66 ± 0.781	5.18 ± 1.45
t_{max} (h)	8.00	8.00	6.00	6.00
	(0.75- 16.0)	(3.00- 24.0)	(6.00- 8.08)	(5.92- 8.02)
$C_{max}{}^{ss}{}_{,b}$ (ng/mL)	6.73 ± 1.82	13.4 ± 3.87	4.11 ± 0.951	7.94 ± 1.79
$AUC_{\tau,b}$(ng/mL.h)	140 ± 39.1	280 ± 81.7	75.1 ± 20.0	147 ± 38.2
PTF (%)	28 ± 10	28 ± 11	48 ± 14	47 ± 13
R	7.0 ± 1.5	6.8 ± 2.1	5.0 ± 1.0	5.2 ± 1.3 [+]

Values are mean ± SD except for t_{max} which is median (range). Subscript *b* is for measurement in blood; [+]: n=60 Source: [Study FTY720D2101]

However, in study FTY720B102 which covers the clinically relevant doses (0.125, 0.25, 0.5, 1, 2.5 and 5 mg once daily for 28 days), statistical power model showed some deviations from dose proportionality for fingolimod PK parameters. The estimate (90% CI) of the exponent (slope) of the power model were 0.89 (0.80-0.98), 0.89 (0.81-0.96) and 0.88 (0.80-0.96) for C0,ss, Cmax,ss and AUCτ, respectively, although the sponsor still claimed that fingolimod C0,ss, Cmax,ss and AUCτ rose in an apparent dose-proportional manner over the investigated dose range.

The following figure 21 show effect of dose on fingolimod Cmax,ss and AUC,ss:

Figure 21. Effect of dose on fingolimod Cmax,ss and AUC,ss



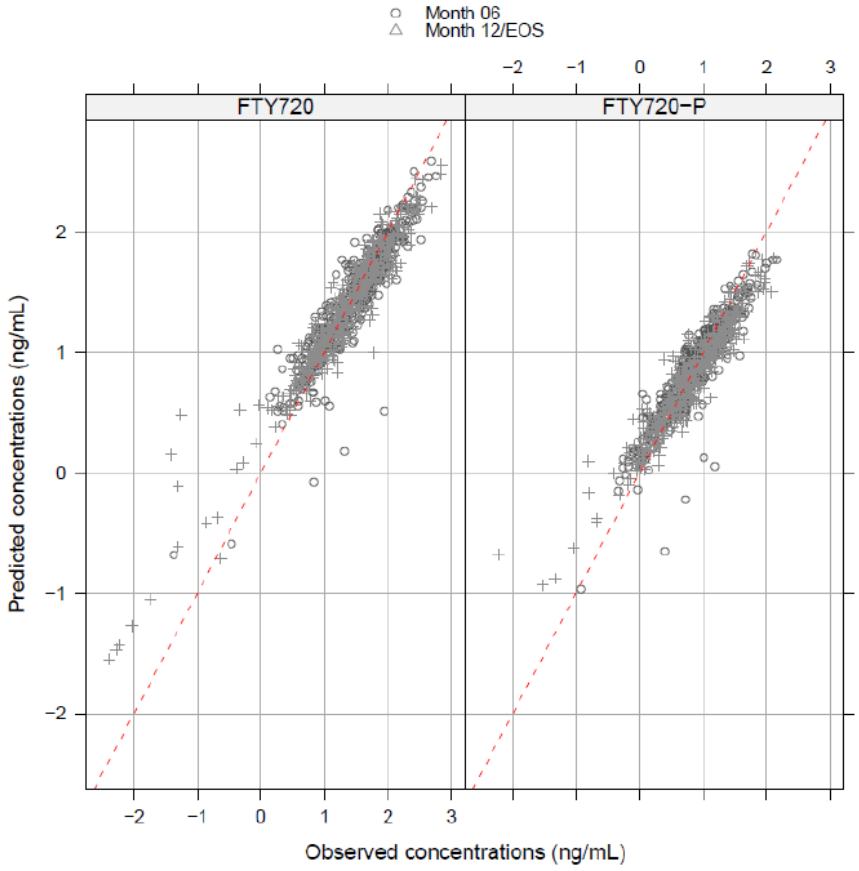
The under-proportionality was also evident starting at 1 mg by dose-normalized parameters shown in the table 11 below.

Table 11. Dose normalized AUC,ss and Cmax,ss on multiple dosing (FMI capsules)

Parameter	0.125 mg	0.25 mg	0.5 mg	1 mg	2.5 mg	5 mg
N	9	8	9	9	9	5
$C_{max,b}^{ss}$/dose (ng.h/ml/mg)	6.7 ± 1.4	6.7 ± 2.3	6.9 ± 1.9	6.0 ± 2.6	4.6 ± 2.2	5.5 ± 1.7
$AUC_{\tau,b}^{ss}$/dose (ng.h/ml/mg)	140 ± 30	142 ± 51	147 ± 39	130 ± 62	95 ± 47	114 ± 32

Fingolimod-P: The sponsor claimed that there is no major deviation from dose proportionality for fingolimod-P based on the exposure-response report FTY720D2302. However, although the predicted line went through the majority of the observed data, the data tend to be skewed from the predicted line as shown in the Figure 22 below.

Figure 22. Predicted vs. observed concentration values from the dose-proportionality model



2.3 INTRINSIC FACTORS

2.3.1 What intrinsic factors influence exposure and/or response and what is the impact of any differences in exposure on the pharmacodynamics? Based on what is known about exposure response relationships and their variability, is dosage adjustment needed for any of the subgroups?

The intrinsic factors have been discussed below:

2.3.1.1 PK in MS patients:

The comparison between healthy subjects and patients showed that fingolimod and fingolimod-P pharmacokinetics is comparable among multiple sclerosis patients, stable renal transplant on Neoral® cyclosporine based immunosuppression and healthy subjects.

Fingolimod was originally developed in the indication of renal transplantation therefore the first in human single ascending dose study FTY720AB101 and the multiple ascending dose study over 28 days (Study FTY720AB102) were both conducted in stable renal transplant patients with characterization of the pharmacokinetic properties of fingolimod (fingolimod-P was not measured in these early studies). No dedicated clinical pharmacology studies have been conducted in patients with multiple sclerosis but in the two clinical studies conducted in patients (Study FTY720D2201 and Study FTY720D2302), blood samples were collected for pharmacokinetic purposes. The results demonstrated in the Table 12 below showed comparable PK betweem MS patients and heakthy subjects.

Table 12. Mean (SD) pedose concentrations (ng/mL) in the first phase III pivotal study (CFTY720D2302) and on day 28

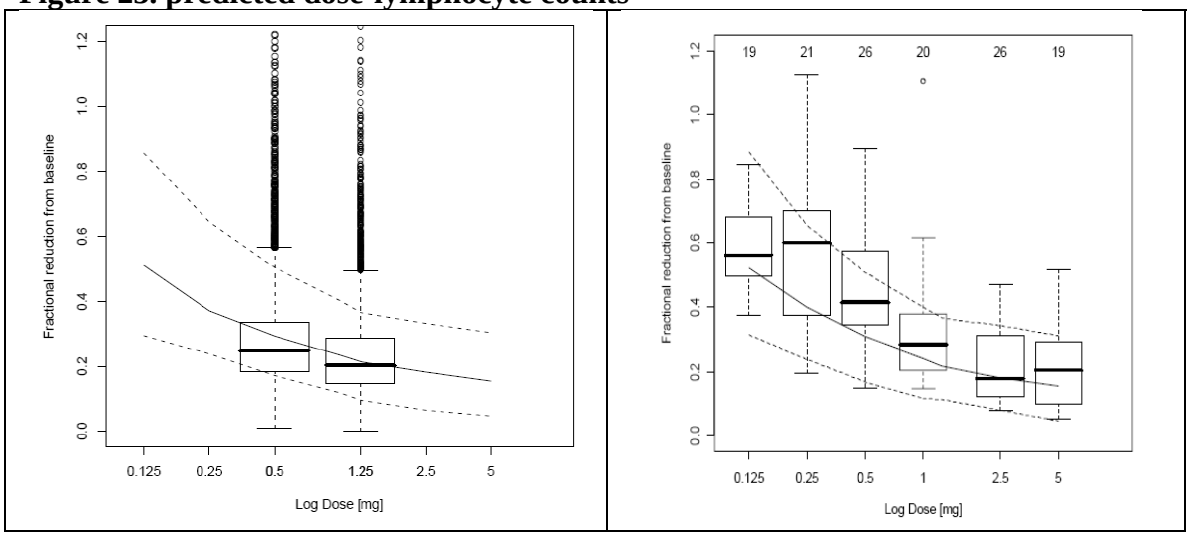
FTY720 Dose	Study		FTY720	FTY720-P
0.5 mg/d	CFTY720D2302	N	281	255
		Mean ± SD	2.31 ± 1.35	1.28 ± 0.706
1.25 mg/d	CFTY720D2302	N	267	239
		Mean ± SD	5.35 ± 2.93	2.97 ± 1.46
0.5 mg/d	CFTY720D2113	N	28	28
		Mean ± SD	3.21 ± 1.01	1.39 ± 0.529
1.25 mg/d	CFTY720D2113	N	27	27
		Mean ± SD	7.55 ± 2.74	3.19 ± 1.26

2.3.1.2 PD in MS patients:

There are no MS patients in clinical pharmacology studies. Based on the population analysis, the predicted dose response in lymphocytes was found to be similar between RRMS patients, stable renal transplant patients (study CFTY720A B102), and healthy volunteers (pooled phase 1 data).

Below shows the figure of predicted dose-response (lymphocyte counts). Left panel :The boxplots characterize the distribution of the lymphocyte responses at each dose level of the phase 3 studies. The solid line is the predicted median dose-response based on a simulation of 500 healthy volunteers at each dose level following 90 days treatment and the dashed lines are the 5th and 95th percentiles; Right panel: The box plots characterize the distribution of the lymphocyte responses in each cohort in study FTY270A-B102. The numbers at the top are the sizes of the respective cohorts. The solid line is the predicted median dose-response based on a simulation of 500 healthy volunteers at each dose level; the dashed lines are the 5th and 95th percentiles

Figure 23. predicted dose-lymphocyte counts



Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 35-36.

2.3.1.3 Effect of age:

There are no elderly enrolled in the clinical pharmacology studies while PK in adolescents (11-16 years old, stable renal transplant, n=7) was compared to that of adults. Data below shows comparable PK between adolescents and adults.

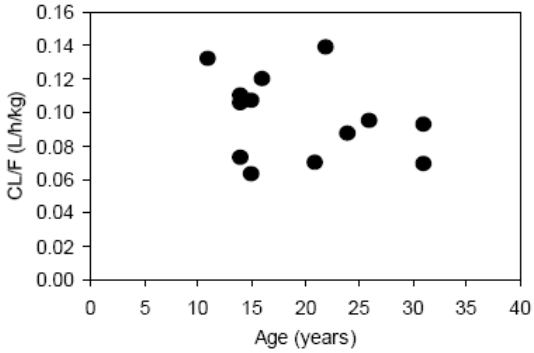
Table 13. PK parameters of adolescents versus adults:

Pharmacokinetic parameters

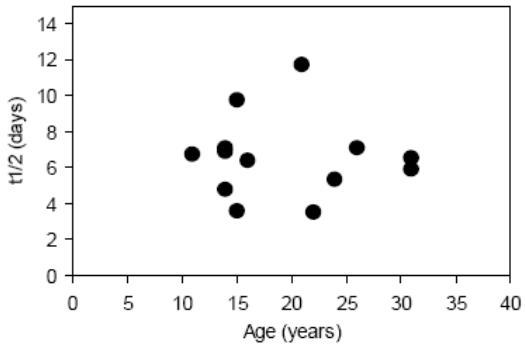
Parameter	Fingolimod		Fingolimod-phosphate	
	Adolescents	Adults	Adolescents	Adults
tmax (h)	8 (4-24)	12 (12-16)	6 (4-24)	12 (6-12)
Cmax (ng/mL)	3.6 ± 0.6	4.4 ± 0.9	3.2 ± 1.4	3.6 ± 0.8
AUC(0-tlast) (ng.h/mL)	675 ± 194	794 ± 250	100 ± 69	142 ± 72
AUC(0-inf) (ng.h/mL)	731 ± 240	861 ± 302	--	--
CL/F (L/h/kg)	0.10 ± 0.02	0.09 ± 0.03	--	--
Vz/F (L/kg)	22 ± 6	20 ± 5	--	--
t1/2 (days)	6.5 ± 1.9	6.7 ± 2.8	--	--

Data are mean ± sd except for tmax which is median (range)
Adult data (n = 6) are from study 2304 in which a fixed 5-mg dose was given averaging 0.07 ± 0.02 mg/kg.

Figure 24. Scatter plots demonstraiting age (~10 to 32 years old) versus Cl/F and t1/2:



Scatter plot of age versus weight-normalized apparent clearance of fingolimod.

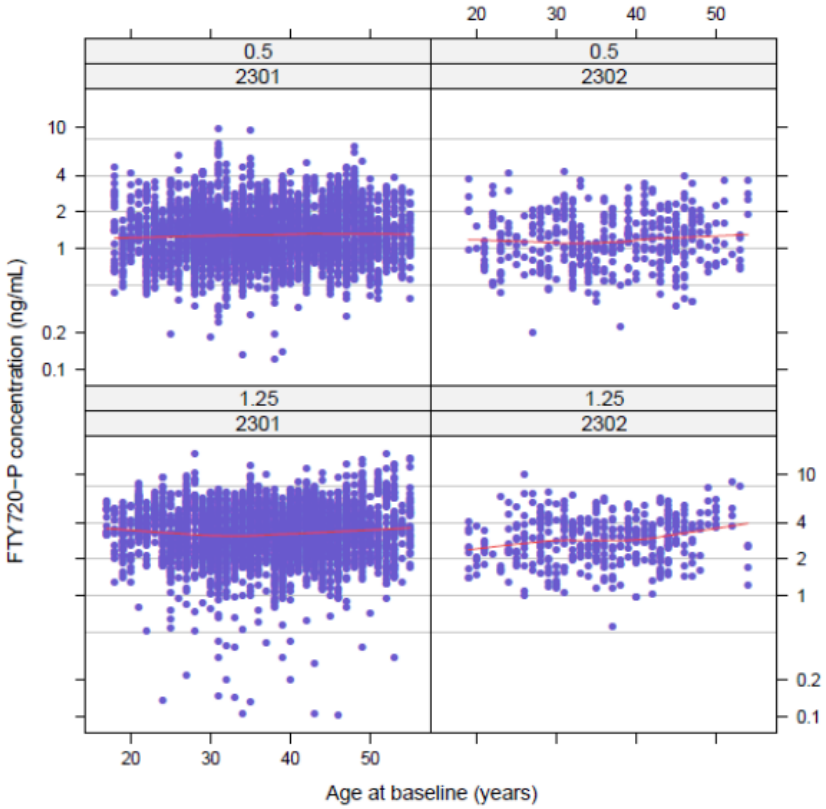


As in left panel for fingolimod half-life.

The PK is comparable between the adults and the adolescents.

In addition, no age effect was identified as a significant factor when examining in the population analysis. Additional plots demonstrating the relationship of FTY-P concentrations versus age in two pivotal trials are shown below.

Figure 25. Relationship of fingolimod-P concentrations versue age

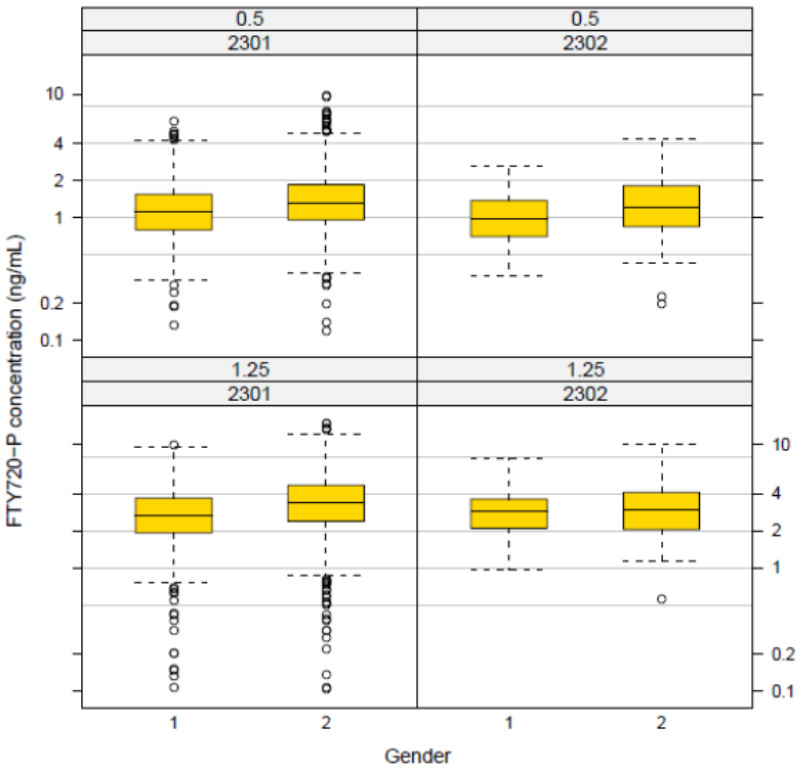


Dosage adjustment:
No dose adjustments are therefore recommended based on age.

2.3.1.4 Effect of Gender:

There are no formal clinical pharmacology studies conducted to explore gender effect on fingolimod PK. Based on the population analysis, a slight lower concentration (10.4%) was observed in males than in females, however, the magnitude of the effect was deemed to be not clinically relevant. Plots demonstrationg the relationship of FTY-P concentrations versus gender in two pivotal trials are shown below.

Figure 26. Relationship of fingolimod-P concentrations versue gender

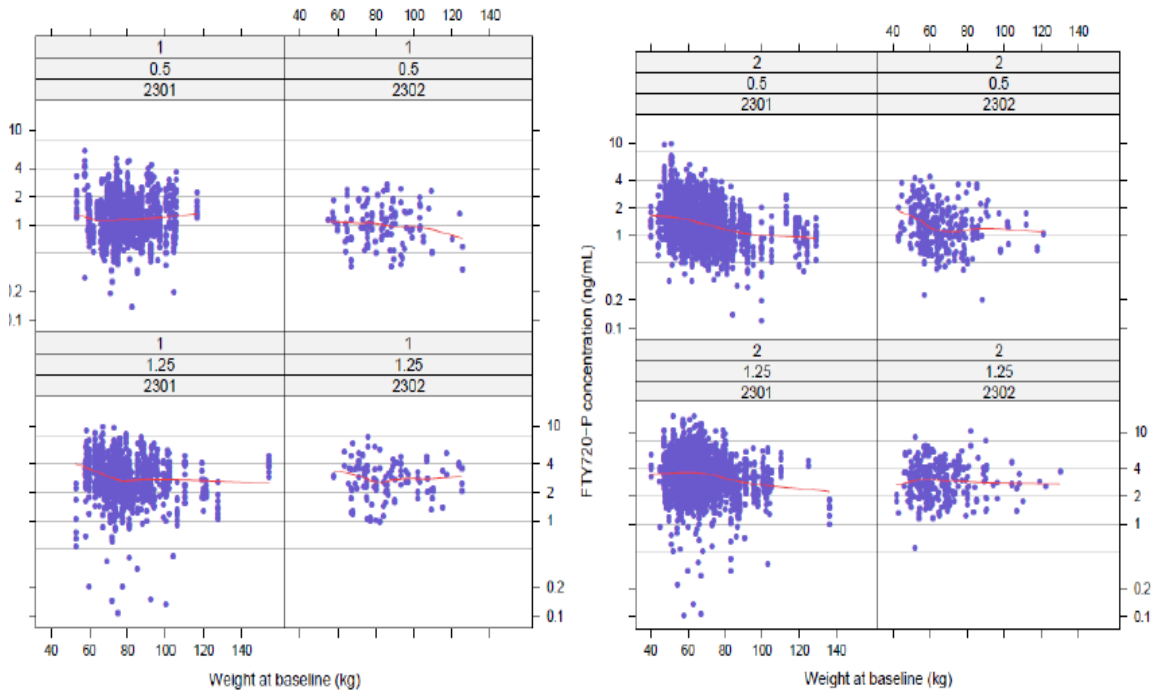


Dosage adjustment:
No dosage adjustment is recommended based on gender.

2.3.1.5 Effect of Weight:

A gain in weight of 14 kg (from 70 to 84 kg) would be on average associated with a 6.2% decrease in concentration, however, the magnitude of the effect was deemed to be not clinically relevant. Plots demonstrationg the relationship of FTY-P concentrations versus weight in two pivotal trials are shown below (left : Male, right : Female).

Figure 27. Relationship of fingolimod-P concentrations versue weight

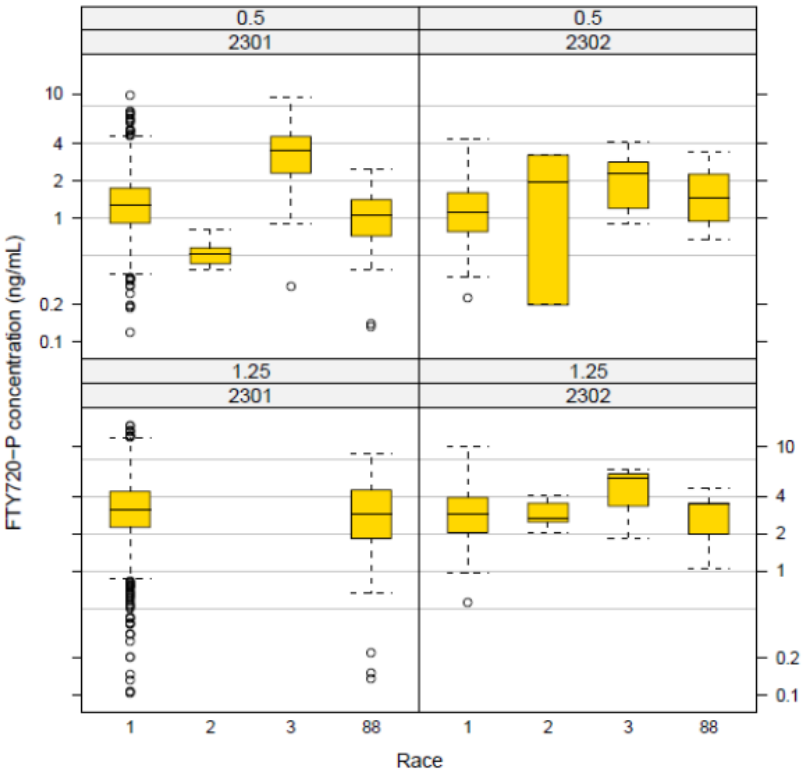


Dosage adjustment:
No dosage adjustment is recommended based on weight.

2.3.1.6 Effect of Race:

Based on three population PK evaluations, the results showed that for 70kg female who were assigned to 0.5mg, estimated mean concentrations at SS in Asian and Black population are 62% higher and 14% lower than Caucasian patients. However, it should be noted that there were only 14 (0.5 %) Asian subjects in the population PK report therefore the results might not be reliable. One clinical pharmacology study (FTY720A2304) showed comparable PK between Japanese and Caucasian (see Table 14 below). The effect of race therefore could not be concluded. Plots demonstrationg the relationship of FTY-P concentrations versus race in two pivotal trials are shown below

Figure 28. Relationship of fingolimod-P concentrations versue race



Note: 1=Caucasian, 2=Black, 3=Asian, 88=Other

Table 14. Multiple-dose pharmacokinetics: FTY720 and FTY720-phosphate

Parameter	FTY720		FTY720-phosphate	
	Asian (n=6)	White (n=6)	Asian (n=6)	White (n=6)
Day 1:				
t_{max} (h)	14 (6-16)	14 (12-16)	14 (6-16)	12 (6-16)
$C_{max,b}$ (ng/ml)	3.1 ± 0.8	3.3 ± 0.5	3.7 ± 1.1	3.3 ± 0.9
$AUC_{\tau,b}$ (ng.h/ml)	54 ± 12	59 ± 11	52 ± 14	53 ± 14
Day 7:				
R	7.0 ± 0.7	6.6 ± 0.4	4.6 ± 1.1	4.2 ± 0.6
$C0_b$ (ng/ml)	13.0 ± 3.0	13.4 ± 3.5	7.4 ± 2.3	7.6 ± 2.0
t_{max} (h)	12 (6-16)	7 (6-16)	9 (6-16)	6 (0-12)
$C_{max,b}$ (ng/ml)	18.2 ± 4.8	17.9 ± 3.4	11.3 ± 3.5	10.9 ± 1.8
$AUC_{\tau,b}$ (ng.h/ml)	382 ± 106	390 ± 73	236 ± 76	219 ± 42
$C_{avg,b}$ (ng/ml)	15.9 ± 4.4	16.3 ± 3.0	9.8 ± 3.2	9.1 ± 1.7
PTF (%)	32 ± 8	28 ± 6	40 ± 10	34 ± 23
$t_{1/2}$ (days)	7.9 ± 2.0	7.4 ± 0.8	6.0 ± 2.4	7.1 ± 2.0

Values are median (range) for tmax and mean ± sd for all others.
Data source: Appendix 4 Table 2 and Table 4.

Dosage adjustment:
No dosage adjustment is recommended based on race.

2.3.1.7 Effect of Hepatic Impairment:

A single oral dose of fingolimod was administered to mild (1 mg, n=8), moderate (1 mg, n=8) and severe (5 mg, n=6) hepatic impaired subjects and matched healthy subjects (n =22). The classification of the degree of hepatic impairment was based on the Child-Pugh score. Child-Pugh scores of ≥ 5, ≥ 7 and ≥ 10 were utilized for inclusion of mild (Child-Pugh class A), moderate (Child-Pugh class B) and severe (Child-Pugh class C) hepatic impaired patients, respectively.

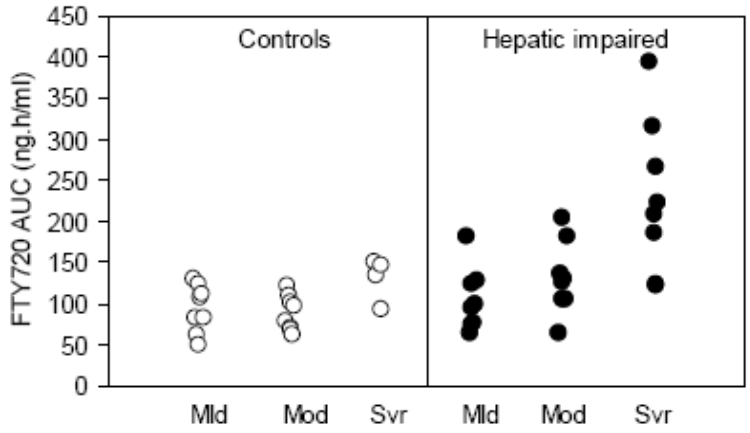
Mild, moderate and severe hepatic impairments have no influence on fingolimod Cmax but fingolimod AUC is increased by 12%, 44% and 103%, respectively. The apparent elimination half-life is unchanged in mild hepatic impairment but is prolonged by 49-50% in moderate and severe hepatic impairment. Fingolimod-P was measured in severe hepatic impairment only, and Cmax and AUC(0-96) were increased by 22% and 29%.

Table 15. Fingolimod PK parameters in healthy, mild moderate and severe hepatic impaired subjects:

Parameter	Controls	Hepatic impaired		
	Healthy (n = 22)	Mild (n = 8)	Moderate (n = 8)	Severe (n = 6)
t_{max} (h)	12 (8 - 48)	12 (6 – 36)	24 (8 – 48)	36 (36 – 96)
$C_{max,b}$ (ng/ml)	0.65 ± 0.15	0.65 ± 0.12	0.57 ± 0.10	0.75 ± 0.22
AUC_b (ng.h/ml)	101 ± 29	105 ± 39	131 ± 45	265 ± 78
$t_{1/2}$ (days)	5.1 ± 2.2	4.9 ± 1.7	6.7 ± 2.5	10.7 ± 1.8
CL_b/F (L/h)	10.8 ± 3.6	10.6 ± 3.4	8.5 ± 3.4	4.0 ± 1.1
$V_{z,b}/F$ (L)	1772 ± 594	1667 ± 348	1794 ± 351	1494 ± 437
Lymphocyte recovery (10^9/L/day)	0.046 ± 0.018	0.031 ± 0.032	0.028 ± 0.025	0.011 ± 0.005

Values are mean ± sd except for time parameters which are median (range).
Cmax and AUC are scaled to a 1-mg dose.

Figure 29. Fingolimod AUC in healthy, mild moderate and severe hepatic impaired subjects:



M2 and M3 blood concentrations were measured in mild and moderate hepatic patient. (see Table below). However, the elimination half-life of fingolimod-P, M2 and M3 were not determined.

Table 16. Metabolite PK parameters

Parameter	Mild impairment		Moderate impairment	
	Controls	Impaired	Controls	Impaired
Metabolite 2:				
Subjects with blood concs	5	4	3	1
Blood conc range (ng/ml)	0.10 – 0.17	0.10 – 0.21	0.10 – 0.13	0.11 – 0.15
Urine Ae(0-96) (mcg)	18.8 ± 11.5	18.5 ± 21.8	17.9 ± 7.1	6.6 ± 4.9
Urine Ae(0-inf) (mcg)	30.4 ± 21.3	25.8 ± 27.3	31.2 ± 13.6	17.2 ± 22.4
Metabolite 3:				
Subjects with blood concs	7	8	7	6
$C_{max,b}$ (ng/ml)	0.28 ± 0.18	0.42 ± 0.33	0.20 ± 0.11	0.24 ± 0.25
t_{max} (h)	8 (6 – 36)	12 (6 – 24)	8 (6 – 48)	10 (6 – 12)
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	20 ± 24	27 ± 31	7 ± 7	7 ± 10
Metabolite/parent AUC_b-ratio	0.24 ± 0.28	0.39 ± 0.48	0.09 ± 0.09	0.05 ± 0.07
Urine Ae(0-96) (mcg)	84 ± 36	60 ± 52	83 ± 32	29 ± 15
Urine Ae(0-inf) (mcg)	157 ± 66	117 ± 61	177 ± 62	93 ± 63

Data are mean ± sd except for time parameters which are median (range).

Source data in Appendix 4, Tables 4 - 8 and Appendix 6, Table 1.

The reviewer's proposal for dosage adjustment:

The fingolimod dose does not need to be adjusted in mild or moderate hepatic impaired patients. The reviewer recommends decrease the dose by 50% in severe hepatic impaired patient. However, as there is no lower strength (0.25 mg) formulation available, use of fingolimod is not recommended in severe hepatic impaired patients.

2.3.1.8 Effect of Renal Impairment:

The effect of renal impairment on the pharmacokinetics of fingolimod, fingolimod-P, and inactive metabolites M2 and M3, was studied in nine subjects with severe renal impairment (CLcr<30 mL/min) compared to nine demographically-matched healthy subjects after administration of a single 1.25 mg oral dose of fingolimod (Study FTY720D2108). No study in mild and moderate renal impaired patients has been conducted.

Severe renal impairment increases fingolimod Cmax and AUC by 32% and 43%, respectively, and fingolimod-P Cmax and AUC by 25% and 14%, respectively. The apparent elimination half-life is unchanged for both analytes. Exposure to the inactive metabolites is also increased with severe renal impairment, by at least 300% for M2 and by 805% for Cmax and 1356% for AUC for M3. The apparent elimination half-life for M3, is slightly greater than in healthy controls.

Table 17. Goemetric mean ratio of fingolomod Cmax and AUC (impaired/normal):

	Adjusted geo-mean*		Geo-mean ratio*		
Parameter (Unit)	**Impaired**	**Control**	**Estimate**	**Lower 90% CL**	**Upper 90% CL**
$C_{max,b}$ (ng/mL)	0.844	0.639	1.32	1.06	1.65
$AUC_{(0-tz),b}$ (h*ng/mL)	91.226	61.576	1.48	0.94	2.33
AUC_b (h*ng/mL)	109.440	76.723	1.43	0.94	2.18
$AUC_{(0-72),b}$ (h*ng/mL)	45.638	34.379	1.33	1.04	1.69

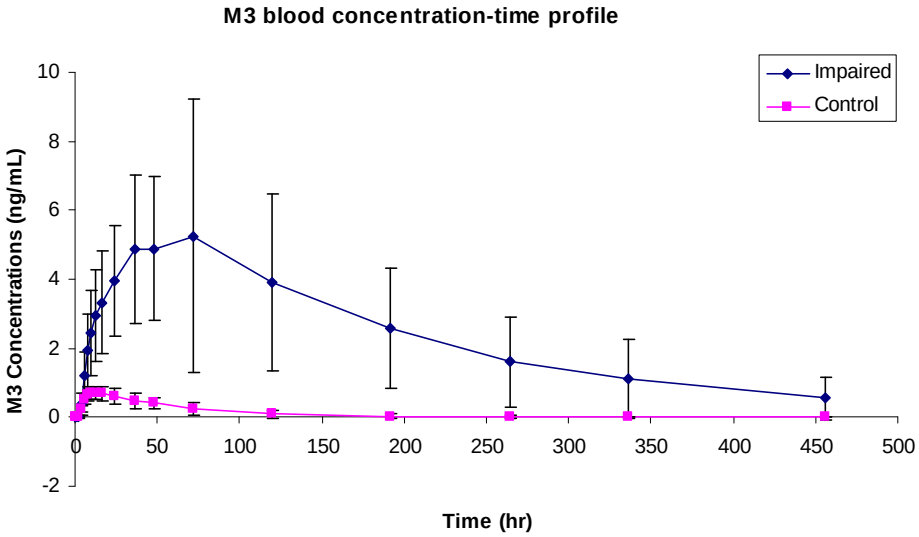
* back-transformed from log scale; geo-mean=geometric mean.
Source: Post-text Table 14.2-1.1

Table 18. Goemetric mean ratio of M3 Cmax and AUC (impaired/normal):

	Adjusted geo-mean*		Geo-mean ratio*		
Parameter (Unit)	**Impaired**	**Control**	**Estimate**	**Lower 90% CL**	**Upper 90% CL**
$C_{max,b}$ (ng/mL)	5.082	0.631	8.05	5.53	11.72
$AUC_{(0-tz),b}$ (h*ng/mL)	905.959	40.928	22.14	13.43	36.49
AUC_b (h*ng/mL)	1002.995	73.979	13.56	7.95	23.14
$AUC_{(0-72),b}$ (h*ng/mL)	267.001	29.060	9.19	6.50	12.99

* back-transformed from log scale; geo-mean=geometric mean.
Source: Post-text Table 14.2-1.1

Figure 30. The concentration-time profile of M3 is shown below:



The reviewer's proposal for dosage adjustment:
Fingolimod is contraindicated in renal impaired patients due to uncertainty of the safety profiles of M2 and M3.

2.4 EXTRINSIC FACTORS

2.4.1 Are fingolimod and fingolimod-P a substrate, inhibitor or inducer of CYP enzymes?

Substrate:
To identify the human cytochrome P450 isozymes involved in the biotransformation of fingolimod an *in vitro* exploratory study dmpk99202 was conducted. In this study definitive conclusion was not obtained and sponsor conducted two confirmatory studies dmpk0301153 and dmpk0400708 to evaluate the role CYP450 enzymes in biotransformation of fingolimod.

Fingolimod is metabolized at 5 and 100 μM substrate concentration by recombinant human CYP4F2 (>80%), 2D6 (<10%), 2E1 (<10%), 3A4, 4F3B and 4F12 (smaller contribution) with measurable turnover. Slight metabolic activity was also observed in incubations with CYP1A1 and 2J2. Ketoconazole inhibited the biotransformation of fingolimod by recombinant CYP4F2 and 4F12 with IC50 of 1.6 and 0.6 μM, respectively.

It was further identified that CYP4F2 has a major role in the metabolism of fingolimod and CYP3A has comparatively less involvement in the metabolism.

Inhibitor:
The inhibitory potential of fingolimod and fingolimod-phosphate towards the metabolism of CYP-specific substrates was determined in human liver microsomes pooled from adult males and females. Fingolimod up to 100 μmol/L concentration showed that fingolimod was not a direct or time dependent inhibitors of CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2El, CYP4A9/11, CYP2B6 and/or CYP3A4/5. Fingolimod-phosphate concentration at 10 μM concentration was not an inhibitor of CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6.

Fingolimod's inhibitory potential of CYP2C8 was not evaluated and fingolimod-phosphate's inhibitory potential of CYP2B6 was also not evaluated.

Inducer:
Cultured human hepatocytes from 3 donors were utilized for this study. The potential for fingolimod to induce human hepatocytes at 0.01 μM, 0.1 μM, and 1 μM fingolimod had no effect on CYP3A, CYP1A2, CYP4F2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or ABCB1 (Pgp) activities.

Fingolimod-P's induction potential of CYP450 was not evaluated.

2.4.2 Is there an *in vitro* evidence of increased metabolism after induction of cytochrome P450s in human hepatocytes?

To evaluate the effect of induction on metabolism of fingolimod several enzyme inducers including rifampin (RIF), phenytoin (PHYT), phenobarbital (PB) and carbamezepine (CARB) were evaluated in a study dmpk0500655. The results indicated that the inducers, RIF, PHYT, PB, and CARB have the potential to increase the hepatic oxidative metabolic clearance of FTY720 in vivo. RIF, PHYT, PB, and CARB treatment of human hepatocytes for 72h increased [^{14}C]fingolimod metabolism up to 2.5- to 4-fold and it is likely this increase was due to induction of CYP4F activity.

We are recommending a PMR for *in vivo* DDI study using carbamezapine based on the discussion with the medical officer regarding its clinical relevance.

2.4.3 Are fingolimod and fingolimod-P an inhibitor of efflux transport (MDR1, MXR, MRP2, BSEP) processes or uptake transporters (OATP1B1, OATP1B3, OATP2B1, NTCP)?

In vitro studies indicated that fingolimod and figolimod-phosphate did not inhibit the efflux transport by MXR, BSEP or MRP2. However, figolimod showed inhibition on MDR1-mediated transport at concentrations above 10 μM which is approximately 400 times the highest plasma concentration attained with 1 mg dose. But, AML629 (fingolimod-P) did not inhibit MDR1 mediated transport. Therefore, clinically significant interactions with drugs that are substrates of these transporters are unlikely.

2.4.4 Is there an in vitro basis to suspect drug-drug interaction?

The in vitro findings related to the potential drug-drug interaction are listed below:

- In vitro studies have shown that fingolimod is largely metabolized by CYP4F2 (>80%), and minor contribution by CYP2D6 (<10%), CYP2E1 (<10%), and even smaller contributions by CYP3A4, CYP4F3B and CYP4F12. Inhibitors or inducers of the major isozymes may influence the exposure of fingolimod. The drug-drug interaction study with ketoconazole, an inhibitor of CYP 3A and 4F2, was conducted (see Section2.4.6.1).
- Fingolimod is not an inhibitor or an inducer of CYP450 isoenzymes at therapeutic levels. Exposure of drugs that are substrates of CYP450 isoenzymes is not likely to be afftected in the presence of fingolimod.
- Several enzyme inducers including rifampin, phenytoin, phenobarbital and carbamezepine increased [^{14}C]fingolimod metabolism up to 2.5- to 4-fold. Non-specific enzymes might decreas blood levels fingolimod. An *in vivo* study with carbamezapine is recommended.
- Fingolimod is largely bound to plasma proteins (>99%). The potential for drug interactions based upon protein binding have not been studied with fingolimod.

2.4.5 What extrinsic factors (such as herbal products, diet, smoking and alcohol) influence exposure and or response and what is the impact of any differences in exposure on pharmacodynamics?

The effects of extrinsic factors like herbal products and smoking have not been conducted.

2.4.6 Are there any in-vivo drug-drug interaction studies that indicate the exposure alone and/or exposure response relationships are different when drugs are coadministered? If yes, is there a need for dosage adjustment?

Six clinical drug interaction studies were conducted including two PK and four PD studies. The two pharmacokinetic studies include one with cyclosporine (a CYP3A substrate and potent inhibitor of the transporters PgP, MRP2 and OATP-C) and one with ketoconazole (a CYP4F and strong CYP3A inhibitor) and four PD studies were designed to assess PD endpoints but also included PK measurements.

In addition, the effect of fingolimod on cyclosporine steady state PK was assessed in stable renal transplant patients (Study FTY720AB102) and a screen for drug-drug interactions on fingolimod and fingolimod-P was a component of the population pharmacokinetic evaluation of the transplantation phase III studies (CYP3A and CYP2D6 inhibitors, and other comedications).

2.4.6.1 Pharmacokinetic studies:

Cyclosporine

Fingolimod was developed as an immunomodulator for prophylaxis of acute rejection after organ transplantation. It was intended to be used in multidrug immunosuppressive regimens with agents such as cyclosporine. In order to provide administration guidance, this study was conducted to assess whether coadministration of fingolimod with cyclosporine alters the disposition of either agent "A two-period, randomized, crossover study to evaluate the effect of Neoral on the bioavailability of FTY720 in psoriatic patients" was conducted.

Cyclosporine did not modify fingolimod overall exposure AUC(0-tz) or peak concentration (Cmax). The pharmacokinetics of steady-state cyclosporine were not altered during coadministration with single-dose fingolimod.

Following table represents PK profile of for fingolimod administered alone or in the presence of cyclosporine.

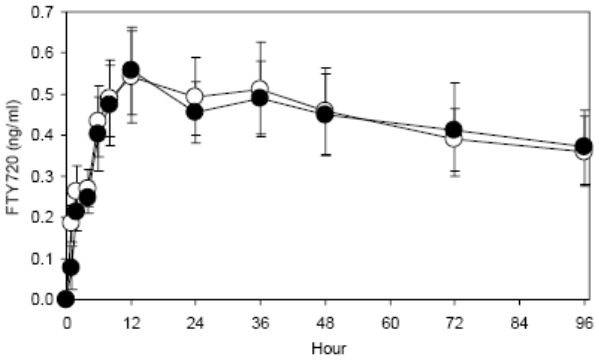
Table 19. Fingolimod pharmacokinetic parameters

Parameter	Alone	Coadministration
Peak exposure:		
$C_{max,b}$ (ng/ml)	0.58 ± 0.19	0.57 ± 0.17
t_{max} (h)	18 (8 – 48)	12 (12 – 72)
$C_{ap,b}$ (ng/ml)	0.52 ± 0.17	0.52 ± 0.16
t_{ap} (h)	24 (10 – 36)	24 (8 – 42)
$t_{ap,dur}$ (h)	30 (24 – 88)	38 (4 – 84)
Early exposure:		
AUC(0-t_z) (ng·h/ml)	41 ± 13	41 ± 13
Total exposure:		
AUC_b (ng·h/ml)	110 ± 49	138 ± 75
CL_b/F (L/h)	10.8 ± 4.8	10.1 ± 6.4
$V_{z,b}/F$ (L)	1845 ± 736	1931 ± 629
$t_{1/2}$ (days)	6.0 ± 4.8	7.0 ± 3.2

Data are mean ± sd except for time parameters which are median (range).

Following figure represents PK profile of for fingolimod administered alone or in the presence of cyclosporine.

Figure 31. Fingolimod PK profiles



Mean FTY720 concentration profiles after administration alone (*open circles*) and coadministration with cyclosporine (*filled circles*). *Bars* represent 95% confidence intervals. Individual subject

Ketoconazole

In vitro studies indicated that ketoconazole, a CYP4F inhibitor and a strong CYP3A inhibitor, resulted in 70% inhibition of fingolimod's metabolism. In order to evaluate the clinically relevant effects of co-administration of fingolimod with CYP4F and CYP3A4 inhibitor ketoconazole, the sponsor conducted a drug-drug interaction study "An open-label, two-period, single-sequence, crossover study to evaluate the influence of ketoconazole on the pharmacokinetics of FTY720 in healthy volunteers was conducted.

Study Design:

In period 1 (days 1-35) subjects received a single 5 mg dose of FTY720 on day 1 with pharmacokinetic blood sampling and clinical assessments up to day 35. In period 2 (days 36-73) subjects received ketoconazole 200 mg twice-daily for 9 days (days 36-44) and a single 5 mg dose of FTY720 coadministered on the fourth day of ketoconazole treatment (day 39).

Coadministration of a single 5 mg dose of FTY720 with steady-state ketoconazole 200 mg twice-daily increased both fingolimod peak exposure, Cmax by 1.2-fold and overall exposure AUC by 1.7-fold. Fingolimod-phosphate AUC(0- tz) was increased to a similar extent (1.7-fold) as the parent fingolimod but Cmax was unaffected by ketoconazole. Dose adjustment for fingolimod is recommended. The reviewer recommends decrease the dose of fingolimod by 50% when it is coadministerd with ketoconazole. As there is no lower strength (0.25 mg) formulation available, fingolimod is not recommend to coadminister with ketoconazole.

Following table represents pharmacokinetic profiles of fingolimod and fingolimod-phosphate when fingolimod was administered alone and in the presence of ketoconazole.

Table 20. Fingolimod and fingolimod-phosphate pharmacokinetics

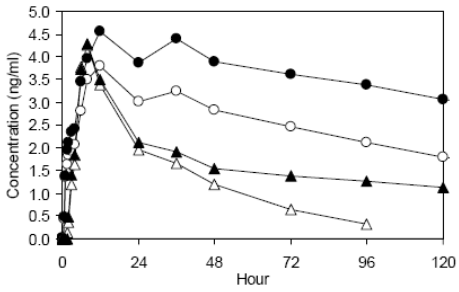
Parameter	Fingolimod alone	Fingolimod with ketoconazole	Ratio of geometric means (90%CI)
Fingolimod:			
t_{max} (h)	12 (8-36)	12 (3-48)	--
$C_{max,b}$ (ng/ml)	3.9 ± 0.7	4.8 ± 1.1	1.22 (1.15 – 1.30)
AUC_b (ng.h/ml)	665 ± 202	1124 ± 293	1.71 (1.53 – 1.91)
$t_{1/2}$ (days)	5.1 ± 1.6	5.8 ± 1.6	1.15 (1.06 – 1.26)
Fingolimod-phosphate:			
t_{max} (h)	8 (6-12)	8 (6-12)	--
$C_{max,b}$ (ng/ml)	4.5 ± 1.3	4.4 ± 1.1	0.99 (0.92 – 1.06)
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	128 ± 49	217 ± 99	1.67 (1.50 – 1.85)

Values are mean ± sd except for temporal parameters which are median (range).

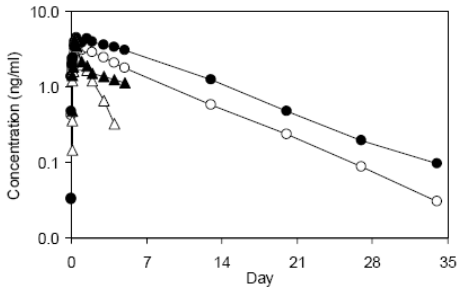
b denotes parameter from blood concentrations.

Following figure represents pharmacokinetic profiles of fingolimod and fingolimod-phosphate when fingolimod was administered alone and in the presence of ketoconazole.

Figure 32. Fingolimod and fingolimod-phosphate profiles



Mean concentration-time profiles to day 5 postdose for fingolimod alone (*open circles*) and with ketoconazole (*filled circles*) and for fingolimod-phosphate alone (*open triangles*) and with ketoconazole (*filled triangles*).



As in left panel with concentrations on a logarithmic scale plotted over the full 35-day assessment periods.

Individual plots in Appendix 4, Figure 1.

2.4.6.2 Pharmacodynamic studies:

Isoproterenol

The effect of isoproterenol (≤5 µg/min) i.v. on reversing the negative chronotropic effect of fingolimod (5 mg) was evaluated. Isoproterenol was highly effective in reversing the negative chronotropic effect of fingolimod. Even at the time of maximal fingolimod induced negative effect (4-6 hours post-dose), heart rates >100 BPM were achieved with isoproterenol infusion. The exposure (Cmax and AUC(0-24)) to fingolimod or fingolimod-P was not altered in presence of isoproterenol.

Salmeterol

The effect of inhaled salmeterol 250 µg on reversing the negative chronotropic effect of fingolimod (1.25 mg) was evaluated. Salmeterol had a mild, positive chronotropic effect on heart rate of approximately six beats per minute. Salmeterol was active in significantly reversing the negative chronotropic effect of fingolimod. After 3 hours of salmeterol treatment, the negative heart rate effect of fingolimod treatment was almost completely reversed, with the heart rate returning to a level measured pre-fingolimod dose. While blood sampling for pharmacokinetic assessment took place over a 12-hour time frame only, the fingolimod and fingolimod-P blood concentrations and derived pharmacokinetic parameters are consistent with those from other studies.

Atropine

The effect of either prophylactic or therapeutic atropine in reversing the negative chronotropic effect of fingolimod (5 mg) was evaluated. Intravenous atropine (≤2 mg) reversed fingolimod induced negative chronotropic effect by approximately 10 BPM. Fingolimod and fingolimod-P exposure (Cmax and AUC(0-24)) were not influenced by atropine coadministered with fingolimod or administered 4 hours after fingolimod.

Diltiazem

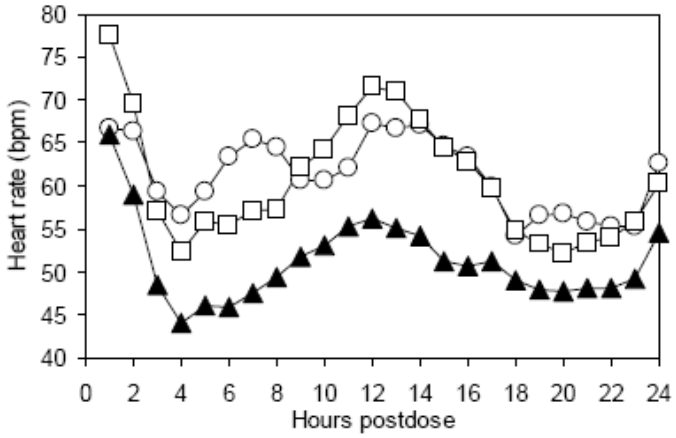
The drug drug interaction which measured the PK and PD (heart rate) of single-dose fingolimod 5 mg when given concomitantly with steady-state calcium channel blocker (diltiazem 240 mg extended-release once-daily) treatment was evaluated. Diltiazem combined with fingolimod had no additional negative chronotropic effect than fingolimod alone. The pharmacokinetics of diltiazem (moderate CYP3A inhibitor),

fingolimod, and fingolimod-P appeared not to be altered during the coadministration of the two drugs.

Atenolol

The drug drug interaction which measured the PK and PD (heart rate) of single-dose fingolimod 5 mg when given concomitantly with steady-state β-blocker (atenolol 50 mg once-daily) was evaluated. Atenolol combined with fingolimod had an approximately 15% (42 bpm) additional negative chronotropic effect than fingolimod alone (51 bpm) (see figure below). The pharmacokinetics of atenolol, fingolimod, and fingolimod-P appeared not to be altered during the coadministration of the two drugs.

Figure 33. Mean heart rate trajectories after coadministration of fingolimod and atenolol



Mean heart rate trajectories from telemetry after atenolol alone (*open circles*), FTY720 alone (*open squares*), and FTY720 + atenolol (*filled triangles*).

Sponsor's proposal in the labeling: Use caution when co-administered with beta-blocker.

The clinical pharmacology reviewer will work with the medical officer and safety team for the action(s) to take when coadministered with beta-blocker and will provide appropriate language in the label.

2.4.6.3 Population PK analysis:

Due to their potential risk of interaction with FTY720-P concentration, the effect of the following concomitant medication on the FTY720-P average concentration was investigated: fluoxetine and paroxetine (CYP2D6 inhibitor), itraconazole, ketoconazole and clarithromycin (CYP3A4 inhibitor), carbamazepine, oral contraceptive (CYP3A4 inducer) and corticosteroids (CYP3A4 substrate). Besides, due to their high frequency of use in the studied population, the effect of the most frequent concomitant medication on FTY720-P average concentration was also checked. The most frequent concomitant medications include: baclofen, gabapentin, oxybutin, amantadine, amitriptyline,

pregabalin and modafinil. For all these concomitant treatments, no unexpected effect was observed on FTY720-P concentration.

In PopPK analyses for DDI, actual concentration level from comedication was not used. Instead, they compared concentration level of FTY720-p with/without comedication. The table below includes actual sample size and results.

Table 21. Change in FTY720-p geometric mean concentration with concomitant treatment relative to without concomitant treatment. Nsamples* refers to the number of samples with concomitant treatment and FTY720 0.5 mg / FTY720 1.25 mg. The number of samples without concomitant treatment was approximately ranging between 3000 and 4000, in all cases.

Concomitant treatment	Effect	$N_{samples}$*	Relative change (0.5 mg)	Relative change (1.25 mg)
Fluoxetine and Paroxetine	Minor	141 / 105	-11.6%	-4.3%
Itra- and keta-conazole	? (N small)	16 / 3	-13.7%	+20.0%
Carbamazepine	As expected	59 / 49	-27.4%	-28.2%
Clarithromycin	? (N small)	5 / 4	+41.0%	+16.3%
Oral corticosteroids	? (N small)	11 / 2	-8.8%	-47.5%
IV corticosteroids	Minor	28 / 19	-15.4%	-10.0%
Oral contraceptive	Minor	666 / 594	+15.9%	+15.8%
Baclofen	Null	103 / 164	-9.7%	+9.8%
Gabapentin	Null	67 / 85	+4.5%	-16.0%
Oxybutin	Null	72 / 91	+2.9%	-4.8%
Amantadine	Null	91 / 118	-17.8%	+6.9%
Amitriptyline	Null	52 / 90	-6.4%	-13.4%
Pregabalin	Minor	26 / 44	-20.2%	-30.0%
Modafinil	Minor	82 / 22	-20.2%	-35.5%

Source: the sponsor's report : Modeling of the exposure-response relationship in patientswith relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 30.

2.5 GENERAL BIOPHARMACEUTICS

2.5.1 Based on the BCS principles, in what class is this drug and formulation? What solubility, permeability and dissolution data support this classification?

Fingolimod hydrochloride is soluble in water (more than 100 mg/mL at 25° and 37°C). Fingolimod is a base with pKa of 7.82. Solubility is higher in acidic media (more than 100 mg/mL at pH 1.0) and lower at neutral or alkaline pH where absolute value could not be estimated (solubility less than 0.01 mg/mL in pH 6.8 at 25° and 37°C).

The dissolution of the 0.5 and 1.25 mg capsules was also pH-dependent [redacted] (b) (4)

Attempts were made to investigate the penetration of [14C]fingolimod through Caco-2 cell monolayers. However, due to strong unspecific binding of fingolimod to the supporting filter device (probably related to the lipophilicity of fingolimod), no reliable conclusions could be drawn from the experiment. However, in humans, the fraction of the dose absorbed was greater than 85% of dose (based on the amount of radioactivity excreted in urine and the amount of metabolites in feces extrapolated to infinity). The apparent absolute bioavailability, as measured in an absolute bioavailability study, was estimated to be 94%. Finally, high fat food had no influence on fingolimod disposition. These data indicate that the systemic availability of fingolimod after oral administration is high, and relatively independent from physiological (pH) factors.

Due to the above described features, a BCS class could not be precisely assigned to fingolimod.

2.5.2 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

The relative bioavailability of 1.25 mg and 2.5 mg fingolimod Final Market Image (FMI) formulations in comparison to Clinical Service Formulations (CSF) was evaluated in healthy volunteers. The proposed commercial drug product was bioequivalent to clinical service formulation with respect to Cmax and AUC at 2.5 mg strength. For 1.25 mg strength, both Cmax and AUC0-t of FMI were bioequivalent to CSF. The 90% CI of total exposure ratio is 0.9-1.29, which is not thought to be clinical meaningful. The data are shown in the Table below.

Table 22. Pharmacokinetic Parameter Ratios of Point Estimates and 90% Confidence Intervals (N=25)

Treatment Comparisons	Parameter	1.25 mg capsules	2.5 mg capsules
Final Market Image Vs Clinical Service Formulation	Cmax, (ng/ml)	1.09 (1.03 – 1.17)	1.02 (0.97 – 1.08)
	AUC(0-tz)(ng.h/ml)	1.08 (0.97 – 1.20)	1.00 (0.94 – 1.07)
	AUC (ng.h/ml)	1.08 (0.90 – 1.29)	1.02 (0.93 – 1.11)

2.5.3 What is the absolute bioavailability of the proposed to-be-marketed formulation?

Sponsor conducted an absolute bioavailability study in healthy volunteers titled “A randomized, open-label, crossover study to measure the absolute bioavailability, safety, and tolerability of FTY720 in healthy volunteers”.

Fingolimod has a high bioavailability from the capsule formulation; however, due to the reversible interconversion between fingolimod and fingolimod-phosphate in vivo, a definitive value for fingolimod absolute bioavailability could not be derived. A conventional evaluation of the pharmacokinetic data yielded an overall average oral/intravenous AUC-ratio of 94 percent. Of the eleven subjects, three had a higher AUC

after oral versus intravenous administration yielding AUC-ratios greater than 100 percent.

In general, a four-way crossover study is necessary to determine the absolute bioavailability of a compound that undergoes reversible metabolism. In such a study the parent and metabolite are administered separately by oral and intravenous routes. Intravenous administration of fingolimod-phosphate to humans is not feasible based on a single-dose toxicity study of intravenous fingolimod-phosphate in rats. Therefore, accurate absolute bioavailability could not be obtained from this study.

2.5.4 What is the effect of food on the bioavailability of the drug from the dosage form? What dosing recommendations need to be made regarding the administration of fingolimod in relation to meals or meal types?

The effect of food on fingolimod and fingolimod-phosphate pharmacokinetics was evaluated with high fat meal using the FMI 1.25 mg capsules in Study A2107 in healthy volunteers. Comparisons between the FMI fed and fasted indicated food has no statistically significant increase in the absorption of the FMI capsule. The mean values of AUC(0-inf), AUC(0-last) and Cmax for FMI under the fed condition were similar to those under fasted condition. However, peak plasma concentration of FTY720-P was higher in fasted state when compared to fed state as shown in the Tables below:

From Study A2107 (FMI formulation):

Table 23. Geometric mean and 90% confidence intervals for the fingolimod (FTY720) parameter ratios (fed/fasted) - Pharmacokinetic Analysis Population

	Adjusted geo-mean		Geo-mean ratio		
Parameter (Unit)	Fed	Fasted	Estimate	Lower 90% CL	Upper 90% CL
$C_{max,b}$ (ng/mL)	1.264	1.378	0.92	0.88	0.96
$AUC_{last,b}$ (ng/mL.h)	225.887	230.104	0.98	0.91	1.06
$AUC_{0-\infty,b}$ (ng/mL.h)	252.171	255.459	0.99	0.92	1.06

Table 24. Geometric mean and 90% confidence intervals for the FTY720-P PK parameter ratios (fed/fasted) - Pharmacokinetic Analysis Population

	Adjusted geo-mean		Geo-mean ratio		
Parameter (Unit)	Fed	Fasted	Estimate	Lower 90% CL	Upper 90% CL
$C_{max,b}$ (ng/mL)	1.019	1.537	0.66	0.62	0.71
$AUC_{last,b}$ (ng/mL.h)	87.724	93.295	0.94	0.87	1.01
$AUC_{0-\infty,b}$ (ng/mL.h)	119.461	121.793	0.98	0.92	1.04

Food effect on figolimod was also determined in another study A0106 on clinical formulation showing similar results to study with final maket image. However, in this study fingolimod-phosphate pharmacokinetics were not determined.

Fingolimod capsules can be taken without regards to food.

2.5.5 What data support the bioequivalence of different strengths of to-be-marketed formulation of fingolimod?

Bioequivalence of two different strengths 1.25 and 2.5 mg of to-be-marketed formulations of fingolimod was demonstrated in study A2309. Comparisons between the 2 x 1.25 mg and 2.5 mg dosage groups had no statistically significant changes in pharmacokinetics of fingolimod. The mean values of AUC(0-inf), AUC(0-last) and Cmax for FMI were similar with similar dose using different strength formulations.

Table 25. PK parameters, geometric mean and 90% confidence intervals for the fingolimod (FTY720) parameter ratios

Parameter	Fingolimod		
	1.25 mg	2.5 mg	Ratio (90%CI)
$C_{max,b}$ (ng/ml)	1.9	2.0	1.05 (1.00, 1.10)
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	269	276	1.02 (0.96, 1.09)
AUC_b (ng.h/ml)	294	297	1.01 (0.95, 1.08)

Values are geometric means. Source: Appendix 6.

2.5.6 How do the dissolution conditions and specifications assure in vivo performance and quality of the product?

The dissolution method and acceptance criteria are summarized in the table below.

Table 26. Diddolusion method and acceptance criteria for fingolimod hard gelatin capsules

Apparatus type:	USP Apparatus 1 (basket)
Media:	0.1N HCl with 0.2% (w/v) SLS
Volume:	500 mL
Temperature:	37 ± 0.5°C
Speed of rotation:	100 rpm
Sampling time:	30 minutes
Analysis:	Q = (b) (4)

The fingolimod drug molecule is known to adsorb onto various materials, including the dissolution testing equipment. Therefore, in order to achieve full recovery of fingolimod

from the dissolution medium at pH 1 and to fulfill analytical method validation recovery acceptance criteria (95.0% to 105.0%), the anionic surfactant sodium laurel sulfate (SLS) (0.2%) was used. Dissolution for each batch of the 0.5 mg and 1.25 mg dosage strengths was investigated at 3 pH values (1, 4.5 and 6.8). Data from these experiments showed a pH dependency of dissolution [redacted] (b) (4)

2.6 ANALYTICAL

2.6.1 What bioanalytical method is used to assess concentrations of active moieties and is the validation complete and acceptable?

The assay validations for fingolimod and its metabolites, fingolimod-P, M2 and M3, are acceptable. Concentrations of fingolimod and fingolimod-P in whole blood were determined using specific LC-MS methods with lower limit of quantification (LLOQ) of 0.08 ng/mL for fingolimod and 0.1 ng/mL for fingolimod-P (1-1.5 ng/mL in early studies). Blood concentrations of the metabolites M2 and M3 were determined using a LC-MS method with a LLOQ set at 0.1 ng/mL for both analytes. Urine fingolimod together with metabolite M2 and M3 concentrations were determined using a LC-MS/MS method with a LLOQ set at 1 ng/mL for all three analytes. Fingolimod has been shown to be stable for at least 6 months in whole blood when stored below -18°C. Fingolimod-P is stable in whole blood for at least 17 months when stored below -70°C. M2 and M3 are stable for at least 13 weeks in whole blood when stored below -18°C. Fingolimod, M2 and M3 are stable for at least 7.5 months in urine when stored below -18°C.

Table 27. Summary of all analytical methods

Report number	Biological fluid	Analyte	Method	LLOQ	Between-run precision (% CV)	Between-run accuracy (% bias)
DMPK(CH) R98-1236	0.5 mL blood	Fingolimod	LC/MS/MS	0.08 (0.0635) (ng/mL)	< 8.6 %	< 2.8 %
DMPK R0500263	0.1 mL blood	Fingolimod-P	LC/MS/MS	1.5 (0.1) (ng/mL)	< 11.2 %	< 7.3 %
DMPK (CH) R00-0474	0.2-0.4 mL blood	M2	LC/MS/MS	0.1 (µg/mL)	< 11.1 %	< 2.1 %
DMPK (CH) R00-0474	0.2-0.4 mL blood	M3	LC/MS/MS	0.1 (ng/mL)	< 10.0 %	< 3.2 %
DMPK (CH) R00-0474	0.75 mL urine	Fingolimod	LC/MS/MS	0.1 (ng/mL)	< 10.6 %	< 6.3%
DMPK (CH) R00-0474	0.75 mL urine	M2	LC/MS/MS	0.1 (ng/mL)	< 11.1 %	< 2.7 %
DMPK (CH) R00-0474	0.75 mL urine	M3	LC/MS/MS	0.1 (ng/mL)	< 11.0 %	< 4.7%

Adequate concentrations of Quality Controls were used in these assay validations.

3.0 DETAILED LABELING RECOMMENDATION

The reviewer's labeling recommendations are shown by track changes to the sponsor proposed label. These labeling changes should be incorporated in the revised label:

23 page(s) of Draft Labeling have been Withheld in Full immediately following this page as B4 (CCI/TS)

4.0 APPENDIX

4.1 APPENDIX I

INDIVIDUAL STUDY REVIEW

4.1-1. BIOPHARMACEUTICS STUDIES
4.1-1.1 Bioavailability

FTY720A0106: A two-period, randomized, crossover study to evaluate the effect of food on the bioavailability of FTY720 in healthy volunteers

Forteen healthy subjects received FTY720 1 mg capsules under fasted and fed conditions with four weeks of wash-out period.

RESULTS

Neither the peak nor the total exposure to FTY720 was altered when administered after a high-fat meal as tabulated below.

Following table provides primary pharmacokinetic parameters, ratios of point estimates and 90% confidence intervals.

Food effect anlaysis: Fingolimod 1 mg capsules under fasted conditions (A) vs Fingolimod 1 mg capsules under fed conditions (B)

Parameter	Fasting	Fed	Point estimate (90%CI)
t_{max} (h)	28 (12 - 36)	36 (12 - 36)	--
$C_{max,b}$ (ng/ml)	0.65 ± 0.17	0.64 ± 0.13	1.00 (0.86 - 1.17)
AUC(0-t_z) (ng·h/ml)	72 ± 17	72 ± 14	1.02 (0.88 - 1.18)
AUC_b (ng·h/ml)	149 ± 65	139 ± 43	0.98 (0.86 - 1.11)

Values are mean ± standard deviation except for tmax which is median (range).

Reviewer's Comment:

Food effect analyzed in this study is not on the final commercial formulation. Another food effect study was conducted using final commercial formulation with clinical service formulation as a reference.

CONCLUSIONS

- A high-fat meal had no effect on FTY720 peak or total exposure.
- Lymphocyte counts decreased from baseline thereafter increased back toward prestudy values as observed in other studies.
- The heart rate-vs-time curve was decreased by 10 percent over the first day postdose and then recovered to prestudy values by day 3 to 5 postdose. Circadian rhythm in supine heart rate was preserved, which is similar to the findings observed in other studies.

FTY720A0108: A randomized, open-label, crossover study to measure the absolute bioavailability, safety, and tolerability of FTY720 in healthy volunteers

Objectives:

The primary objective was to determine the absolute bioavailability (oral/intravenous AUC-ratio) of fingolimod in healthy subjects. The secondary objectives were to compare the cardiac effects and the lymphocyte effects of fingolimod between oral and intravenous administration and to document the safety and tolerability of fingolimod by both routes of administration.

Study Design	This was a randomized, two-period, crossover study planned for 12 healthy subjects. On day -1 of each period baseline clinical assessments were made including serial cardiac monitoring and lymphocyte counts. On day 1 of each period subjects received a single dose of FTY720 either as 1mg intravenous infusion over 2 hours or as 1.25mg capsule. Clinical and pharmacokinetic assessments including cardiac and lymphocyte monitoring were made to day 3 in-house. The subject returned for clinical and pharmacokinetic assessments up to day 28 after each administration. The two dose administrations were separated by at least 30 days.
Study Population	Healthy male and female, Age: 18-45 years 12 subjects were randomized, and 11 completed the study
Treatment Groups	Treatment A = FTY720 1.25 mg capsules under fasted conditions Treatment B = FTY720 1 mg/mL solution for i.v infusion The treatment phases were separated by washout periods 4 weeks.
Investigational Drug	FTY720 1.25mg hard gelatin capsule (final market formulation, batch US03079, code 3761319.005); FTY720 1mg/ml solution for intravenous infusion (batch Y077 1002, code 3768454.002).
Sampling: Blood	Venous blood samples of 1.2 ml were drawn into EDTA-containing vacuum tubes for analysis of fingolimod and into sodium citrate-containing tubes for analysis of fingolimod-phosphate. Blood samples were taken before the FTY720 dose and then at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 192, 240, 336, 504, and 648 hours after administering the oral dose or after starting the intravenous infusion.

<table>
<tr><td>Analysis</td><td>Fingolimod concentration was determined in plasma samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 0.08 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (µg/mL)</td><td>0.24, 5, 25 ng/mL</td><td>0.08, 0.2, 1.0, 3, 10, 20, and 30 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>4.2 to 4.6</td><td>2.3 to 6.8</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.998</td></tr>
<tr><td>Linear Range (µg/mL)</td><td colspan="2">0.08 to 30 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, µg/mL)</td><td colspan="2">0.08 ng/mL</td></tr>
</table></td></tr>
<tr><td>Feces</td><td>None</td></tr>
<tr><td>PK Evaluations</td><td>Whole blood samples for fingolimod and fingolimod-phosphate were drawn over a 28-day period after each administration. Blood concentrations of fingolimod and fingolimod-phosphate were analyzed by LC/MS/MS methods. Noncompartmental pharmacokinetic parameters were derived.
Following PK parameters were derived: AUC_{0-t}, AUC_{0-inf}, C_{max}, F, CL, Vz, tmax and $t_{1/2}$.</td></tr>
<tr><td>PD Evaluations</td><td>Peripheral blood lymphocyte counts were obtained at baseline and at visits over the study duration. Holter monitoring, standard electrocardiograms, and pulse rate were collected from day -1 to 3. Lymphocyte and heart rate response parameters included predose value, nadir value, and area under the effect-time curve (AUE).</td></tr>
<tr><td>Statistical Methods</td><td>Pharmacokinetic parameters were compared between treatments in an ANOVA model from which the oral/intravenous AUC-ratio of the geometric means and 95% confidence interval were derived.</td></tr>
</table>

RESULTS

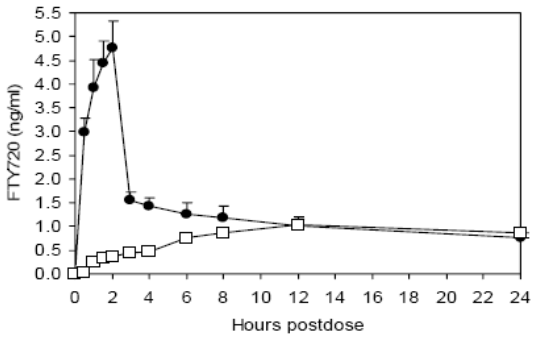
Following table represents PK parameters calculated for different treatments.

Parameter	Intravenous	Oral
Dose (mg)	1	1.25
t_{lag} (h)	--	0.5 (0 – 1)
t_{max} (h)	2 (1.5 – 2.0)	12 (8 – 36)
$C_{max,b}$ (ng/ml)	4.9 ± 0.8	1.1 ± 0.2
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	149 ± 44	174 ± 32
AUC_b (ng.h/ml)	175 ± 50	201 ± 31
AUC_b/Dose (ng.h/ml/mg)	175 ± 50	161 ± 25
CL_b (L/h)	6.3 ± 2.3	--
$V_{z,b}$ (L)	1199 ± 260	--
$t_{1/2}$ (days)	6.0 ± 1.9	6.1 ± 1.0

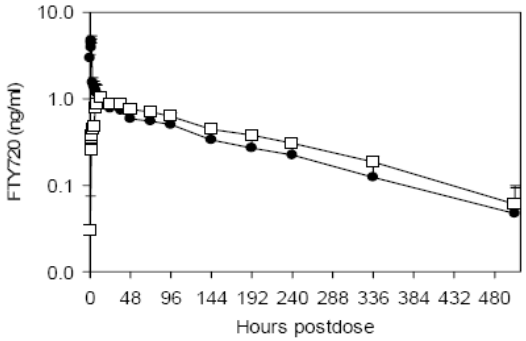
Values are mean ± sd except for temporal parameters which are median (range).

The mean fingolimod plasma concentration (±SD) vs time profiles (left: linear scale, right: log scale).

Fingolimod concentration profiles



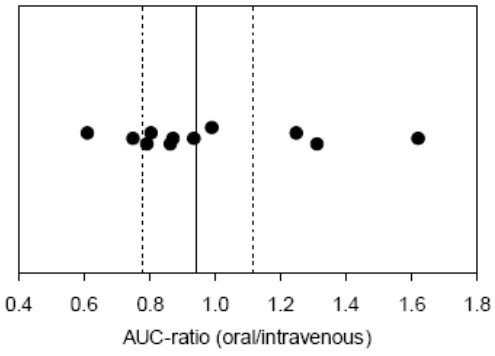
Fingolimod mean concentration-time profiles to 24 hours postdose after intravenous (*filled circles*) and oral (*open squares*) administration. *Bars* represent the 95% confidence interval.



As in left panel showing concentrations on a logarithmic scale and time to week 3 (504 hours postdose). Individual plots are in Appendix 4 Figure 1.

Fingolimod Oral/Intravenous AUC-ratios

Across all eleven subjects the oral/intravenous geometric mean AUC-ratio was 0.94 (95% confidence interval, 0.78 to 1.12) as shown in Figure below. The AUC after oral administration, when scaled to a 1-mg dose, was higher than the corresponding AUC after intravenous administration in three subjects yielding bioavailability estimates greater than 100%. The specific ratios were 1.25 (*subject 5105*), 1.31 (*subject 5108*), and 1.62 (*subject 5104*).



Individual fingolimod oral/intravenous AUC-ratios. *Vertical line* is the geometric mean ratio and *dashed lines* represent the 95% confidence interval.

After oral administration of 1.25mg FTY720, the Cmax of 1.1 ± 0.2 ng/ml occurred at 12 hours postdose and the AUC was 201 ± 31 ng.h/ml. After intravenous infusion of 1mg, Cmax at the end of the 2-hour infusion was 4.9 ± 0.8 ng/ml, AUC was 175 ± 50 ng.h/ml, CL was 6.3 ± 2.3 L/h, and Vz was 1199 ± 260 L. The oral/intravenous AUC-ratio was 0.94 (95%CI: 0.78 - 1.12). Of the eleven subjects, three had a higher AUC after oral versus intravenous administration yielding AUCratios greater than unity.

Fingolimod-phosphate could be measured in only a few subject's blood samples after oral administration, but no pharmacokinetic parameters could be derived.

Pharmacodynamic parameters lymphocyte responses and heart rate reponses obtained in the study were similar to other studies with fingolimod.

Discussion
A four-way crossover study is necessary to determine the absolute bioavailability of a compound that undergoes reversible metabolism. In such a study the parent and metabolite are administered separately by oral and intravenous routes.

Intravenous administration of fingolimod-phosphate to humans is not feasible based on a single-dose toxicity study of intravenous fingolimod-phosphate in rats.

For a given individual, route-related difference in the interconversion could yield a higher fingolimod AUC after oral than after intravenous administration. Without corresponding oral and intravenous pharmacokinetic data from fingolimod-phosphate, it is not possible to determine further how this interconversion contributes to the oral/intravenous AUC ratios. The values derived for fingolimod clearance and distribution volume in this study are not definitive values given the restrictions on study design and interpretation mentioned above. The pharmacokinetic and response data collected in this study suggest that presystemic phosphorylation of fingolimod may be an important contributor to the formation of fingolimod-phosphate.

Without corresponding oral and intravenous pharmacokinetic data from fingolimod-phosphate, it is not possible to determine further how this interconversion contributes to the oral/intravenous AUC ratios. The values derived for fingolimod clearance and distribution volume in this study are not definitive values given the restrictions on study design and interpretation mentioned.

Evidence from the lymphocyte and heart rate responses suggests that systemic exposure to fingolimod-phosphate was lower after intravenous administration of fingolimod. The lymphocyte nadir response was 35 percent weaker and the heart rate nadir response was 11 percent weaker after intravenous compared with oral administration (these exposure-response relationships are sigmoidal in shape, not linear/proportional). Since these two effects are driven by fingolimod-phosphate, they suggest that fingolimod-phosphate blood levels after intravenous administration of fingolimod-even when dose adjusted-were likely lower than after oral administration.

Therefore this study does not provide absolute value for bioavailability after oral administration. However, the PK parameters indicate that fingolimod is well absorbed from the hard gelatin capsule formulation.

CONCLUSIONS:
- Fingolimod has a high bioavailability from the capsule formulation; however, due to the reversible interconversion between fingolimod and fingolimod-phosphate in vivo, a definitive value for fingolimod absolute bioavailability could not be derived.
- A conventional evaluation of the pharmacokinetic data yielded an overall average oral/intravenous AUC-ratio of 94 percent. Of the eleven subjects, three had a higher AUC after oral versus intravenous administration yielding AUC-ratios greater than 100 percent.

FTY720A0107: An open label, randomized, single dose, crossover study to evaluate the effect of food on the pharmacokinetics of FTY720 and FTY720-P from 1.25 mg Final Market Image (FMI) capsules in healthy subjects

Introduction

The effect of food on FTY720 bioavailability has been assessed in a previous study (Study FTY720A0106). The results showed that a high-fat meal had no effect on FTY720 peak or total exposure. However, in view of a new drug application (NDA), the effect of food should be investigated on the to-be-marketed formulation, at the highest dose strength, with measurements of the active moiety. As none of these conditions were fulfilled in the above mentioned study (clinical service form, 1 mg dose and measurements of FTY720 only), this investigation was conducted again.

Objectives:

Primary objective
To examine the effect of food on the pharmacokinetics of FTY720 and FTY720-P from 1.25 mg FMI capsules in healthy subjects.

Secondary objective
To assess the safety and tolerability of FTY720 in healthy subjects.

<table>
<tr><td>Study Design</td><td>This study was an open-label, randomized, single dose, two period, two sequence, crossover design. The study was planned to compare FTY720 and FTY720-P pharmacokinetics in healthy subjects under fasting and fed conditions.</td></tr>
<tr><td>Study Population</td><td>Healthy male and female
Age: 18-45 years
BMI: 18-30 kg/m^2
34 subjects were planned, and 29 completed the study</td></tr>
<tr><td>Treatment Groups</td><td>Test= The investigational drug, FTY720 1.25mg FMI capsules with batch no. H378BD was used in the trial.
Reference = The investigational drug, FTY720 1.25mg FMI capsules with batch no. H378BD was used in the trial.
The two treatments were separated by 36 days (t1/2=146 hrs).

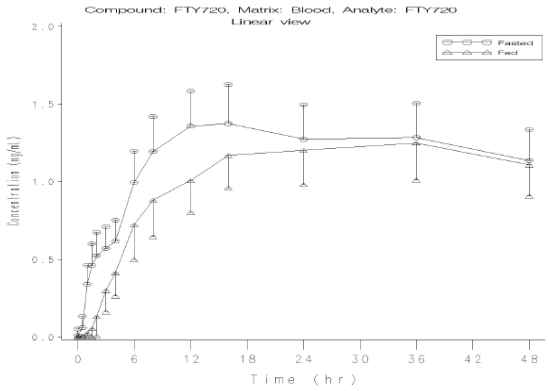
Treatment Sequences
<table>
<tr><th></th><th>Sequence Description</th><th>Period 1</th><th>Period 2</th></tr>
<tr><td>Sequence A</td><td>FTY720 1.25 mg Fasted / FTY720 1.25 mg Fed</td><td>Treatment 1</td><td>Treatment 2</td></tr>
<tr><td>Sequence B</td><td>FTY720 1.25 mg Fed / FTY720 1.25 mg Fasted</td><td>Treatment 2</td><td>Treatment 1</td></tr>
<tr><td colspan="4">Treatment 1: Single 1.25 mg oral dose of FTY720 FMI capsules (Under Fasted conditions)
Treatment 2: Single 1.25 mg oral dose of FTY720 FMI capsules (Under Fed conditions)</td></tr>
</table></td></tr>
<tr><td>Sampling: Blood</td><td>Blood samples were collected at pre-dose on Day 1 and Day 37, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96h post-dose. Subjects are discharged after collection of the 96-h sample, then return for sampling in period 1 on Day 8 (168h), Day 11 (240h), Day 15 (336h), Day 22 (504h), Day 29 (672h) and Day 37 (~864h) post-dose. In period 2, these same return visits occurred on Days 44, 47, 51, 58, 65, and 73.</td></tr>
</table>

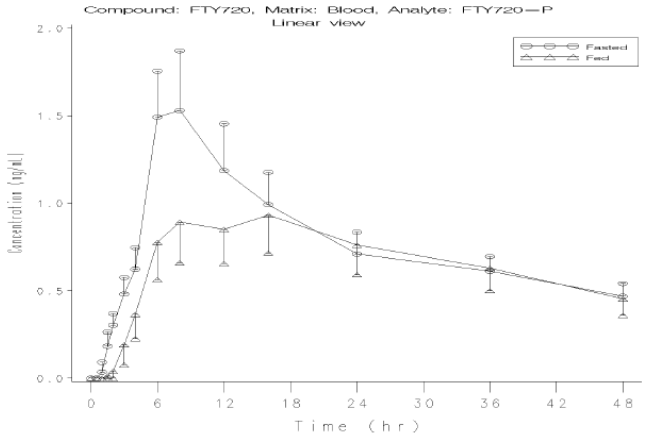
<table>
<tr><td>Analysis</td><td>Fingolimod concentration was determined in blood samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 0.08 ng/mL.

Fingolimod
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (µg/mL)</td><td>0.24, 5 and 25 ng/mL</td><td>0.08, 0.2, 1, 3, 10, 20, and 30 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>4.2 to 6.5</td><td>2.3 to 6.1</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.996</td></tr>
<tr><td>Linear Range (µg/mL)</td><td colspan="2">0.08 to 30 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, µg/mL)</td><td colspan="2">0.08 ng/mL</td></tr>
</table></td></tr>
<tr><td>Feces</td><td>None</td></tr>
<tr><td>PK Evaluations</td><td>FTY720 and FTY720-P PK parameters AUClast, AUC0-∞, and Cmax are compared between the fed and fasted periods. For each of those PK parameters, the log-transformed data are analyzed using a linear mixed effect model including treatment (fed or fasted), period, and sequence as fixed factors and subject as a random factor. A point estimate and a 2-sided 90% confidence interval for the geometric mean food effect ratio (fed/fasted) on the original scale are provided. The molar ratios of FTY720/FTY720-P is calculated, listed, and summarized by treatment. PK parameters determined: tlag, tmax, Cmax, AUC0-∞, AUC0-24h, AUClast, t1/2, CL/F, t1/2, Vz/F using non-compartmental analysis in WinNonLin.</td></tr>
<tr><td>Safety Evaluations</td><td>All subjects who received at least one treatment are included in the safety and tolerability evaluation based on vital signs, ECG, Clinical laboratory evaluations and adverse events.</td></tr>
<tr><td>Statistical Methods</td><td>Pharmacokinetic and response parameters were compared in ANOVA. Lack of a food effect was based on conventional bioequivalence criteria.</td></tr>
</table>

RESULTS:

PK profile of fingolimod and fingolimod phosphate in healthy subjects under fasting and fed conditions.





Geometric mean and 90% confidence intervals for the fingolimod (FTY720) parameter ratios (fed/fasted) - Pharmacokinetic Analysis Population

	Adjusted geo-mean		Geo-mean ratio		
Parameter (Unit)	**Fed**	**Fasted**	**Estimate**	**Lower 90% CL**	**Upper 90% CL**
$C_{max,b}$ (ng/mL)	1.264	1.378	0.92	0.88	0.96
$AUC_{last,b}$ (ng/mL.h)	225.887	230.104	0.98	0.91	1.06
$AUC_{0-\infty,b}$ (ng/mL.h)	252.171	255.459	0.99	0.92	1.06

Geometric mean and 90% confidence intervals for the FTY720-P PK parameter ratios (fed/fasted) - Pharmacokinetic Analysis Population

	Adjusted geo-mean		Geo-mean ratio		
Parameter (Unit)	**Fed**	**Fasted**	**Estimate**	**Lower 90% CL**	**Upper 90% CL**
$C_{max,b}$ (ng/mL)	1.019	1.537	0.66	0.62	0.71
$AUC_{last,b}$ (ng/mL.h)	87.724	93.295	0.94	0.87	1.01
$AUC_{0-\infty,b}$ (ng/mL.h)	119.461	121.793	0.98	0.92	1.04

FTY720 pharmacokinetics in fasted and fed state

In fasted state, after a median lag-time of 0.5 hour (range: 0 to 0.5 hour), the median tmax ranged from 12 to 36 hours post-dose with a median of 16 hours. In fed state, FTY720 median lag time and median tmax, were greater than in fasted state.

FTY720 Cmax exhibited inter-individual variability with coefficients of variation of geometric mean of 16% and 19%, in fasted and fed state, respectively. On average, Both AUC exhibited inter-individual variability with coefficients of variation of geometric mean ranging from 32% to 39%. The geometric mean t1/2 was similar in fasted and fed state (about 132 hours) with an inter-individual variability (about 45%).

FTY720-P pharmacokinetics in fasted and fed state
In fed state, FTY720-P lag time and median tmax were greater than in fasted state. Cmax exhibited small inter-individual variability in both states, with coefficients of variation of geometric mean of 20-21%.

Both AUC exhibited moderate to high inter-individual variability with coefficients of variation of geometric mean ranging from 33% to 40%. The geometric mean t1/2 was similar in fasted and fed state (about 130 hours) with the same high interindividual variability (about 45%).

Comparison of FTY720 and FTY720-P pharmacokinetics
In both fasted and fed state, FTY720-P lag time was slightly greater than that of FTY720. In both states, FTY720-P peaked earlier than FTY720, with a greater peak concentration. Independently from the state, the geometric mean FTY720-P AUC0-∞ and AUClast, were about 47% and 40%, respectively, that of FTY720 on a ng-per-mL basis. The corresponding geometric mean molar ratios of FTY720-P/FTY720 AUC0-∞ and AUClast in both states, were 0.37 and 0.31-0.32, respectively. The associated CV% geometric means were low for both AUC0-∞ and AUClast in both states: about 17%. FTY720 and FTY720-P blood concentrations declined in parallel resulting in a similar t1/2.

Reviewer's Comment:
Median Tmax and the lag time for fingolimod-phosphate were twice that of observed time under fasted conditions indicating slower absorption and interconversion. Peak plasma concentration of fingolimod-phosphate was approximately 34% less under fed conditions though total exposure was similar under fasted and fed conditions.

CONCLUSIONS:

- A high-fat meal had no effect on FTY720 peak or total exposure.
- A high-fat meal had no effect on FTY720-P total exposure. However, peak plasma concentration of FTY720-P was higher in fasted state when compared to fed state.
- FTY720 capsules can be taken without regard to timing of meals

4.1-1. BIOPHARMACEUTICS STUDIES
4.1-1.2 Comparative BA/BE

FTY720A0116: An open label, single dose, crossover study to evaluate the relative bioavailability of 1.25 mg and 2.5 mg FTY720 Final Market Image (FMI) formulations in comparison to Clinical Service Formulations (CSF) in healthy volunteers

Objectives: The primary objective was to determine the relative bioavailability of FTY720 from the FMI and CSF formulations at the 1.25 mg and 2.5 mg dose strengths.

<table>
<tr><td>Study Design</td><td>This was a randomized, open-label, two-way crossover study in two parallel groups of 14 healthy subjects each (total, 28 subjects). Group 1 subjects received single 1.25-mg doses and Group 2 received single 2.5-mg doses of FMI and CSF. The study consisted of a screening period (days -21 to -2), two baseline periods (day -1), two 7-day treatment periods, a 28-day interdose interval, and an end-of-study evaluation. The end-of-study evaluations were performed on day 7 of period 2.</td></tr>
<tr><td>Study Population</td><td>Healthy male and female
Age: 18-45 years
BMI: 18-30 kg/m^2
28 subjects were enrolled, and 25 completed the study</td></tr>
<tr><td>Treatment Groups</td><td>Treatment A: FTY720 1.25 mg hard gelatin oral capsules, Final Market Image, Lot US-02029
Treatment B: FTY720 1.25 mg hard gelatin oral capsules, clinical service formulation, Lot H-05673
Treatment C: FTY720 2.5 mg hard gelatin oral capsules, Final Market Image, Lot US-02030
Treatment D: FTY720 2.5 mg hard gelatin oral capsules, clinical service formulation, Lot H-05929
The two treatments were separated by 28 days (t1/2=146 hrs).</td></tr>
<tr><td>Sampling: Blood</td><td>A venous blood sample was obtained before FTY720 administration and then at 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, and 144 hours postdose. Each blood sample was 2.7 ml in volume collected in an EDTA-containing vacuum tube.</td></tr>
<tr><td>Analysis</td><td>Fingolimod and Fingolimod phosphate concentration was determined in blood samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 0.08 ng/mL.
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (µg/mL)</td><td>0.24, 1.5, 40 ng/mL</td><td>0.08, 0.2, 1, 5, 10, 25 and 50 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>3.8 to 6.6</td><td>2.8 to 5.8</td></tr>
</table></td></tr>
</table>

	Linearity	Weighted linear equation ($1/X^2$), mean r= 0.9997
	Linear Range (µg/mL)	0.08 to 50 ng/mL
	Sensitivity (LLOQ, µg/mL)	0.08 ng/mL
Feces	None	
PK Evaluations	Standard noncompartmental pharmacokinetic parameters were derived and FMI/CSF parameter ratios calculated. PK parameters determined: tlag, tmax, Cmax,b, AUC0-∞,b, AUC0-24h,b, AUClast,b, t1/2, CLb/F, t1/2, Vz,b/F using non-compartmental analysis in WinNonLin.	
Safety Evaluations	Safety and tolerability were assessed via vital signs, ECG, safety laboratory evaluations (biochemistry, hematology, urinalysis), and adverse event recording.	
Statistical Methods	Log-transformed Cmax,b, AUC(0-tz)b, and AUCb were compared in an ANOVA. Test (FMI) and reference (CSF) treatments were contrasted to obtain the geometric mean ratio and 90% confidence interval. Bioequivalence was concluded if the ratio and confidence interval were contained in the range 0.80 to 1.25.	

RESULTS

Drug carryover from period 1 to period 2 occurred in eight subjects: three at 1.25 mg and five at 2.5 mg. The carryover blood concentrations ranged from 0.09 to 0.26 ng/ml corresponding to 5.0% to 15.4% of the Cmax. Concentration-time profiles from these subjects in period 2 were corrected for carryover before bioequivalence testing as foreseen in the protocol.

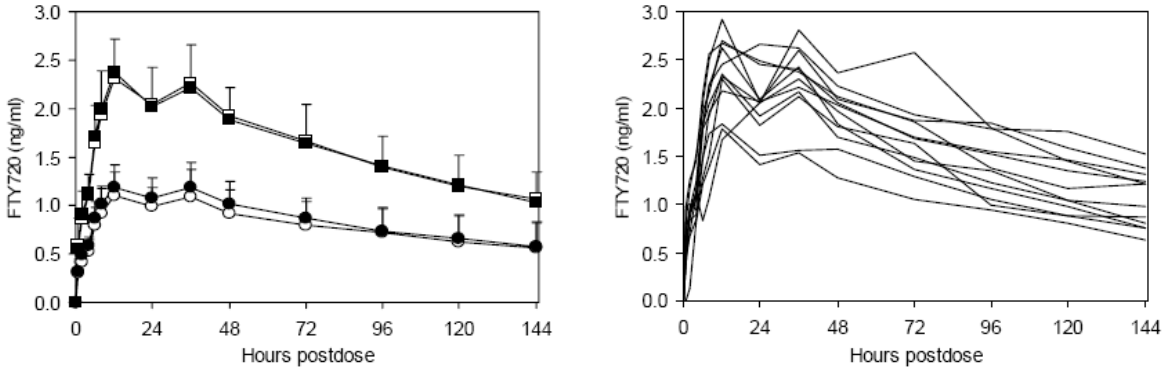
Following table represents PK parameters for different treatments.
Summary of Pharmacokinetic Parameters

Dose and parameter	FMI	CSF	Ratio (90%CI)
1.25 mg			
t_{max} (h)	12 (8 – 36)	12 (12 – 36)	--
$C_{max,b}$ (ng/ml)	1.2 ± 0.3	1.1 ± 0.2	1.09 (1.03 – 1.17)
AUC(0-t_z)$_b$ (ng.h/ml)	123 ± 32	115 ± 32	1.08 (0.97 – 1.20)
AUC_b (ng.h/ml)	256 ± 168	229 ± 121	1.08 (0.90 – 1.29)
2.5 mg			
t_{max} (h)	12 (12 – 36)	12 (12 – 36)	--
$C_{max,b}$ (ng/ml)	2.4 ± 0.4	2.4 ± 0.3	1.02 (0.97 – 1.08)
AUC(0-t_z)$_b$ (ng.h/ml)	233 ± 42	232 ± 43	1.00 (0.94 – 1.07)
AUC_b (ng.h/ml)	416 ± 137	413 ± 144	1.02 (0.93 – 1.11)

Parameters are arithmetic mean ± sd except tmax which is median (range)
Ratio is ratio of FMI/CSF geometric means.

The primary analysis based on data from all 25 completing subjects corrected for carryover demonstrated bioequivalence for all three pharmacokinetic parameters at both dose levels with the exception of AUC at 1.25 mg (due to a single outlier).

A secondary analysis that included only the subjects without carryover (n = 17) yielded similar results.



Left Panel: Mean FTY720 concentration profiles from the 1.25 mg CSF and FMI (open and filled circles) and the 2.5 mg CSF and FMI (opened and filled squares) based on all completing subjects. Bars represent standard deviations.

Right Panel: Individual FTY720 plasma concentration time profile

Following table provides primary pharmacokinetic parameters, ratios of point estimates and 90% confidence intervals.

Bioequivalence anlaysis: Fingolimod 1.25 mg and 2.5 mg capsules CSF (A) vs Fingolimod 1.25 mg and 2.5 mg capsules FMI (B)

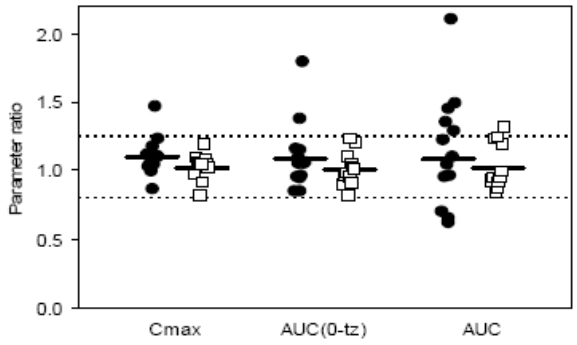
Table 7-4 Bioequivalence results

Parameter	1.25 mg capsules	2.5 mg capsules
All subjects:	13	12
$C_{max,b}$ (ng/ml)	1.09 (1.03 – 1.17)	1.02 (0.97 – 1.08)
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	1.08 (0.97 – 1.20)	1.00 (0.94 – 1.07)
AUC_b (ng.h/ml)	1.08 (0.90 – 1.29)	1.02 (0.93 – 1.11)
Subjects without carryover:	10	7
$C_{max,b}$ (ng/ml)	1.09 (0.99 – 1.19)	0.96 (0.88 – 1.04)
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	1.11 (0.97 – 1.27)	0.95 (0.84 – 1.08)
AUC_b (ng.h/ml)	1.12 (0.91 – 1.38)	0.95 (0.83 – 1.08)

Data are geometric mean ratio (90% confidence interval).

Following figure represents distribution of the individual PK parameter ratios

Fingolimod PK parameter ratios



Individual FMI/CSF parameter-ratios from the 1.25 mg dose (*filled circles*) and the 2.5 mg dose (*open squares*). *Solid bars* are the geometric means. *Dashed lines* demarcate the equivalence interval. Additional

Discussion

Drug carryover and populations for pharmacokinetic analysis

In period 1, all predose blood samples had no interfering substances or FTY720 present. In period 2, eight subjects had FTY720 blood concentrations in the predose blood sample above the assay quantification limit of 0.08 ng/ml. Generally, these low concentrations were between 5 and 10 percent of the subsequent Cmax in period 2. Specifically, for the 1.25 mg dose, there were three subjects with drug carryover of 0.09, 0.14, and 0.26 ng/ml representing 5.0%, 8.3%, and 15.4% of Cmax. For the 2.5 mg dose, there were five subjects with carryover of 0.12, 0.14, 0.15, 0.19, 0.21 ng/ml representing 5.3%, to 9.5% of Cmax. The primary pharmacokinetic evaluation was performed on all subjects who completed both periods after correcting for the drug carryover in period 2 as foreseen in the protocol. This resulted in 13 subjects at 1.25 mg and 12 subjects at 2.5 mg. A secondary pharmacokinetic evaluation was performed by removing the 8 subjects with carryover, resulting in 10 subjects at 1.25 mg and 7 subjects at 2.5 mg.

Reviewer's Comment

Fingolimod-phosphate concentrations were not determined in this study.

CONCLUSIONS

Both peak and total exposure to fingolimod were bioequivalent between the Final Market Image and the Clinical Service Form capsule at 2.5 mg. However, total exposure of FMI was not bioequivalent to CSF at 1.25 mg dose, though 90% CI of Cmax were within 80-125%

FTY720A2309: An open-label, single dose, crossover study to evaluate the bioequivalence of FTY720 2 x 1.25 mg and 2.5 mg Final Market Image (FMI) capsules in healthy volunteers.

Objective:

The objective of this study was to assess whether two 1.25 mg versus one 2.5 mg FTY720 final market image capsules are bioequivalent.

Study Design	This was an open-label, two-period, two-sequence, crossover, single-dose bioequivalence study intended for 32 healthy subjects. The reference treatment A was two 1.25 mg FTY720 capsules and the test treatment B was one 2.5 mg FTY720 capsule. In period 1 (days 1-36) and period 2 (days 37-72), subjects remained in the clinical center from the day before each period began until 5 days postdose for pharmacokinetic and clinical assessments. They returned each week over the next 5 weeks for assessments to characterize fully the pharmacokinetics of fingolimod due to its prolonged half-life. The end-of-study evaluations occurred on day 72.
Study Population	Healthy male and female Age: 18-45 years BMI: 18-30 kg/m^2 35 subjects were enrolled, and 34 completed the study
Investigational Drug	FTY720 final market image hard gelatin capsules: 1.25 mg (lot US03079; KN3761319.005) and 2.5 mg (lot US03082; KN3752938.006)
Treatment Groups	Treatment A: FTY720 1.25 mg (x2) hard gelatin oral capsules, Final Market Image Treatment B: FTY720 2.5 mg hard gelatin oral capsules, Final Market Image The two treatments were separated by 36 days (t1/2=146 hrs).
Sampling: Blood	For fingolimod 3 ml venous blood samples were collected into EDTA vacuum tubes and for fingolimod-phosphate 3 ml venous blood samples were collected in sodium citrate vacuum tubes predose and then 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 168, 240, 336, 504, 672, and 840 hours postdose.
Analysis	Fingolimod and Fingolimod phosphate concentration was determined in blood samples using a validated method for high performance liquid chromatography- with tandem mass spectrometric detection with a lower limit of quantification of 0.08 ng/mL. Fingolimod

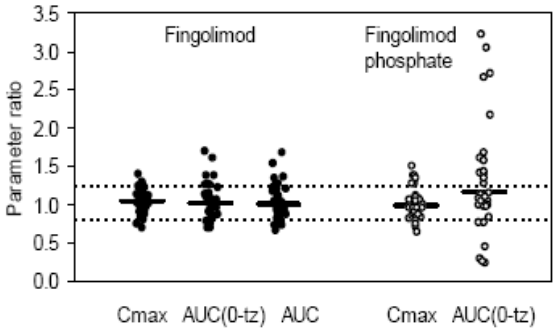
Parameter	**Quality Control Samples**	**Standard Curve Samples**
Quality Control or Standard Curve Concentration (µg/mL)	0.24, 2, 5 and 25 ng/mL	0.08, 0.2, 1, 3, 10, 20 and 30 ng/mL

<table>
<tr><td></td><td>
<table>
<tr><td>Between Batch Precision (%CV)</td><td>4.3 to 6.8</td><td>2.8 to 6.8</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.996</td></tr>
<tr><td>Linear Range (µg/mL)</td><td colspan="2">0.08 to 30 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, µg/mL)</td><td colspan="2">0.08 ng/mL</td></tr>
</table>

Fingolimod-Phosphate
<table>
<tr><th>Parameter</th><th>Quality Control Samples</th><th>Standard Curve Samples</th></tr>
<tr><td>Quality Control or Standard Curve Concentration (µg/mL)</td><td>2, 4, 7.5 and 40 ng/mL</td><td>1, 2.5, 5, 10, 20, 30 and 50 ng/mL</td></tr>
<tr><td>Between Batch Precision (%CV)</td><td>4.3 to 6.8</td><td>2.9 to 5.0</td></tr>
<tr><td>Linearity</td><td colspan="2">Weighted linear equation ($1/X^2$), mean r= 0.995</td></tr>
<tr><td>Linear Range (µg/mL)</td><td colspan="2">1 to 50 ng/mL</td></tr>
<tr><td>Sensitivity (LLOQ, µg/mL)</td><td colspan="2">1 ng/mL</td></tr>
</table>
</td></tr>
<tr><td>Feces</td><td>None</td></tr>
<tr><td>PK Evaluations</td><td>Standard noncompartmental pharmacokinetic parameters were derived and FMI/CSF parameter ratios calculated.
PK parameters determined: tmax, Cmax,, AUC0-∞, AUC0-24h, AUClast, t1/2, CL/F, t1/2, Vz,/F using non-compartmental analysis in WinNonLin.</td></tr>
<tr><td>Safety Evaluations</td><td>Monitoring of adverse events and concomitant medications, laboratory safety parameters, vital signs, standard 12-lead ECGs, and physical examinations.</td></tr>
<tr><td>Statistical Methods</td><td>Fingolimod Cmax, AUC(0-tz), AUC and fingolimod-phosphate Cmax, AUC(0-tz) were log-transformed and compared between treatments using a linear mixed-effects model including treatment, period and sequence as fixed factors and subject-within-sequence as a random factor. The point estimates and 90% confidence intervals for the ratios of treatment geometric means on the original scale were provided for each comparison. Average bioequivalence was declared if the test/reference parameter ratio and 90% confidence intervals were contained in the range 0.80 to 1.25.</td></tr>
</table>

RESULTS:

Parameter Ratios



Individual parameter ratios (test/reference). Not shown but included in the evaluation is a fingolimod-phosphate AUC(0-tz) ratio of 4.95. *Solid bars* are the point estimates. *Dashed lines* demarcate the bioequivalence interval.

Fingolimod pharmacokinetics: The mean concentration-time curves of fingolimod were similar from the two capsule dose strengths. Both peak and total drug exposure satisfied average bioequivalence criteria as tabulated below.

PK parameters, geometric mean and 90% confidence intervals for the fingolimod (FTY720) and fingolimod-phosphate parameter ratios

Parameter	Fingolimod			Fingolimod-phosphate		
	1.25 mg	2.5 mg	Ratio (90%CI)	1.25 mg	2.5 mg	Ratio (90%CI)
t_{max} (h)	12	12	--	8	8	--
$C_{max,b}$ (ng/ml)	1.9	2.0	1.05 (1.00, 1.10)	2.5	2.5	1.00 (0.95, 1.06)
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	269	276	1.02 (0.96, 1.09)	27.8	32.4	1.17 (0.96, 1.41)
AUC_b (ng.h/ml)	294	297	1.01 (0.95, 1.08)	--	--	--

Values are geometric means except for tmax which is median.
Subscript *b* denotes parameter based on blood concentrations.

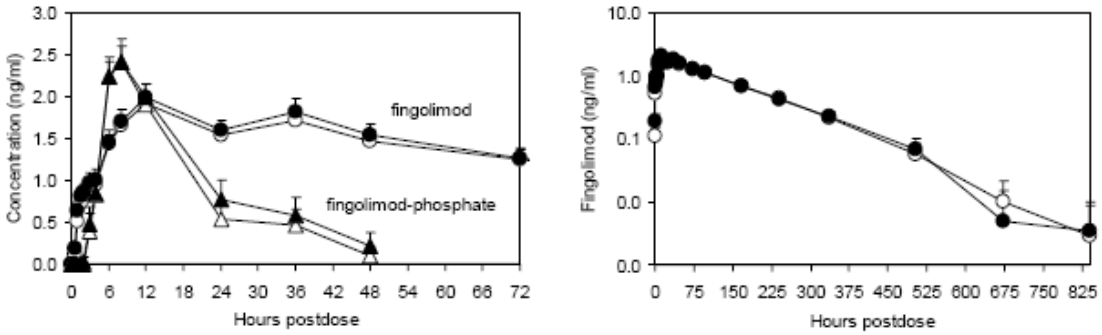
Fingolimod-phosphate pharmacokinetics: The pharmacologically-active moiety fingolimod-phosphate reached higher peak concentrations at an earlier time postdose compared to the parent prodrug fingolimod. Peak exposure to fingolimod-phosphate satisfied average bioequivalence criteria.

Fingolimod-phosphate was quantifiable generally to 48 hours postdose precluding the full characterization of total exposure to this analyte. Quantifiable AUC(0-tz) was 17% higher after administration of a 2.5 mg capsule compared to two 1.25 mg capsules.

Reviewer's Comment:
Bioequivalence was not established for AUC(0-tz) between administration of a 2.5 mg capsule compared to two 1.25 mg capsules as sampling frequency to a accurately characterize total exposure of fingolimod-phosphate were inadequate and intersubject variability. Furthermore, the assay was not sensitive enough to characterize PK beyond 48 hrs.

Figure below shows mean concentration-time profiles for fingolimod and fingolimod-phosphate over the first 4 days postdose; the right panel shows the mean fingolimod concentration-time curves over the full study course.

Concentration Profiles



Left: Mean concentration profiles of fingolimod and fingolimod-phosphate over 72 hours after administration of the 1.25 mg capsule (open symbols) and 2.5 mg capsule (filled symbols).

Right: As in left panel for fingolimod profiles over the full time course on a logarithmic concentration scale.

CONCLUSIONS:

- The 1.25 mg and 2.5 mg final market image capsules were bioequivalent with respect to fingolimod peak and total exposure.
- As supportive information, peak exposure to fingolimod-phosphate was bioequivalent between capsule strengths. The quantifiable total exposure AUC(0-tz) was 17% higher from the 2.5 mg capsule.

4.1-2. IN VITRO STUDIES
4.1-2.1 In vitro metabolism

Study Title	Evaluation of FTY720 as an inhibitor of human P450 enzymes
Study number	DMPK-r99201
Objective	To evaluate the ability of FTY720 to inhibit the major P450 enzymes (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A9/11) in human liver microsomes.
Test System	Human liver microsomes

METHODS

Following probe substrates were used for each CYP enzyme.

Evaluation of FTY20 as a metabolism-Independent Inhibitor of human P450 enzymes

CYP1A2	7 -Ethoxyesorufin O-dealkylation
CYP2A6	Coumarin 7-hydroxylation
CYP2B6	7 -ethoxy-4-trifluoromethylcoumarin
CYP2C9	Diclofenac 4' -hydroxylation
CYP2C19	S-Mephenytoin 4'-hydroxylation
CYP2D6	Dextromethorphan O-demethylation
CYP2E1	Chlorzoxazone 6-hydroxylation
CYP3A415	Testosterone 6ß-hydroxylation
CYP4A9/11	Lauric acid 12-hydroxylation

Conditions used for in vitro studies below follow the Agency's guidance. These methods are acceptable.

- Evaluation of FTY20 as a metabolism-independent inhibitor of human P450 enzymes: Determination of IC50 values
- Evaluation of FTY20 as a metabolism-Independent Inhibitor of human P450 enzymes: Determination of Ki values
- Evaluation of FTY20 as an irreversible metabolism-dependent inhibitor of human P450 enzymes

RESULTS
Following table indicates validation of the test system.

Appendix 1: Characterization of the test system

Enzyme	P450 Activity	Individual Samples (pmol/mg protein/min) †							Pool of 16, 17, 21, 23, 28, 29 and 30 ¥		
									(pmol/mg protein/min)		(µmol/L)
		#16	#17	#21	#23	#28	#29	#30	Theoretical rates ‡	Observed Vmax ¥	Observed Km ¥
CYP1A2	7-Ethoxyresorufin *O*-dealkylase	50.7	53.7	139	48.3	80.4	139	206	102	110 ± 3	0.33 ± 0.02
CYP2A6	Coumarin 7-hydroxylase	1930	2410	1050	1680	966	1760	1220	1580	1700 ± 3	0.54 ± 0.00
CYP2B6	7-EFC £ *O*-dealkylase	730 ●	271 ●	227 ●	115 ●	199 ●	529 ●	275 ●	335 ●	620 ± 10	2.9 ± 0.1
CYP2C9	Diclofenac 4´-hydroxylase	8070	11000	2230	7980	6880	4110	8120	6920	5400 ± 50	4.7 ± 0.1
CYP2C19	*S*-Mephenytoin 4´-hydroxylase	261	205	225	129	156	140	160	182	180 ± 2	24 ± 1
CYP2D6	Dextromethorphan *O*-demethylase	355	277	122	237	268	460	929	378	450 ± 20	4.1 ± 0.3
CYP2E1	Chlorzoxazone 6-hydroxylase	1660	2320	1740	1840	1220	2590	2060	1920	1300 ± 0	27 ± 2
CYP3A4/5	Testosterone 6β-hydroxylase	7810	8460	3860	5670	2310	17900	3060	7010	5700 ± 200	52 ± 5
CYP4A9/11	Lauric acid 12-hydroxylase	2060	1960	2340	3130	2830	2160	2330	2400	2700 ± 200	7.6 ± 1

Summary: Evaluation of FTY20 as an inhibitor of P450 enzymes in human liver microsomes

Concentrations of FTY720 studied: 0, 1.0, 5.0, 10, 25, 50 and 100 µmol/L

Enzyme	P450 Activity	Metabolism-independent		Irreversible Metabolism-dependent
		IC50 (µmol/L)	Ki (µmol/L)	
CYP1A2	7-Ethoxyresorufin *O*-dealkylase	80	89 ± 8 NCI	Little or no inhibition observed
CYP2A6	Coumarin 7-hydroxylase	53	62 ± 7 NCI	Little or no inhibition observed
CYP2B6	7-EFC § *O*-dealkylase	36	37 ± 3 UCI	Little or no inhibition observed
CYP2C9	Diclofenac 4´-hydroxylase	110	55 †	Little or no inhibition observed
CYP2C19	*S*-Mephenytoin 4´-hydroxylase	> 900 *	> 450 *	Possible mechanism-based inhibitor
CYP2D6	Dextromethorphan *O*-demethylase	51	53 ± 6 NCI	Little or no inhibition observed
CYP2E1	Chlorzoxazone 6-hydroxylase	180	90 †	Little or no inhibition observed
CYP3A4/5	Testosterone 6β-hydroxylase	35	9.0 ± 1.8 CI, 33 ± 4 NCI	Little or no inhibition observed
CYP4A9/11	Lauric acid 12-hydroxylase	80	170 ± 30 NCI	Little or no inhibition observed

Constants shown were calculated by GraFit using the matrix inversion method, which utilized rates of product formation.

* Under the conditions examined, FTY720 did not inhibit these P450 enzymes. Therefore, the IC50 and Ki values for inhibition of these P450 enzymes by FTY720 are greater than 100 µmol/L, which is the highest concentration of FTY720 examined. Based on the conservative assumption that 10% inhibition of P450 activity by 100 µmol/L FTY720 could have been masked by experimental error, the IC50 and Ki values for FTY720 could be as low as 900 µmol/L and 450 µmol/L, respectively.

† Predicted Ki value if FTY720 were a competitive inhibitor of these enzymes (IC50 values were determined at substrate concentration equal to Km).

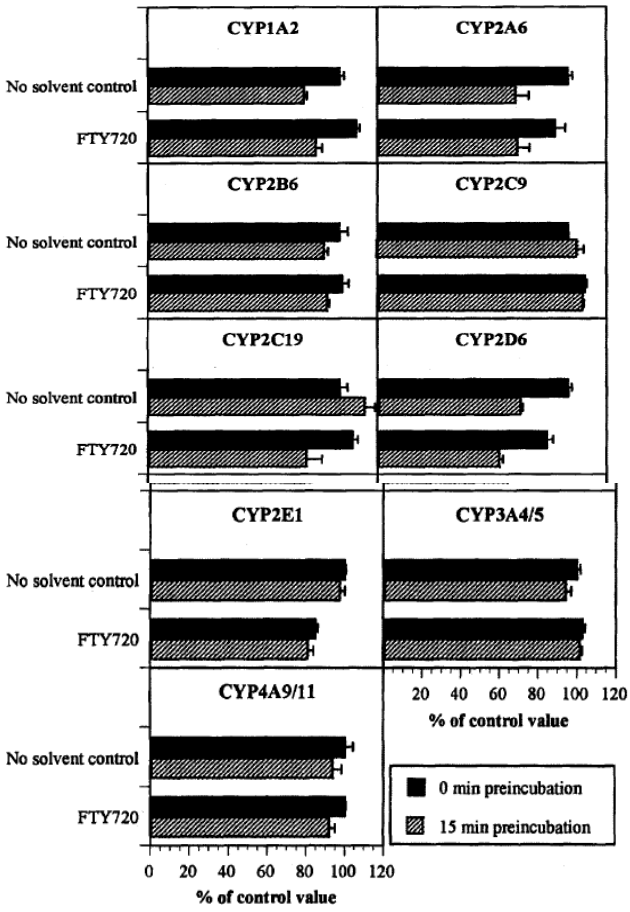
CI Competitive inhibition

NCI Non-competitive inhibition

UC Uncompetitive inhibition

The results of this study showed that as a direct acting (metabolism-independent) reversible inhibitor,
(1) FTY720 does not inhibit CYP2C19,
(2) FTY720 appears to be an inhibitor of CYP2C9 and CYP2E1 with Ki values (estimated from IC50) of approximately 55 and 90 µmol/L, respectively.
(3) FTY720 appears to be a non-competitive inhibitor of CYP1A2, CYP2A6, CYP2D6 and CYP4A9/11 with Ki values of 89, 62, 53 and 170 µmol/L, respectively.
(4) FTY720 appears to be an uncompetitive inhibitor of CYP2B6 with a Ki value of 37 µmol/L.
(5) FTY720 appears to inhibit CYP3A4/5 either competitively or noncompetitively with a Ki value of 9.0 or 33 µmol/L, respectively.

Metabolism-dependent inhibition of human P450 enzymes by FTY20



Values are mean ± standard deviation of three determinations. Individual data are shown in **Appendix 5**.

The concentration of FTY720 present in the preincubations was as follows: CYP1A2, 25 µmol/L; CYP2A6, 25 µmol/L; CYP2B6, 25 µmol/L; CYP2C9, 50 µmol/L; CYP2C19, 100 µmol/L; CYP2D6, 25 µmol/L; CYP2E1, 100 µmol/L; CYP3A4/5, 12.5 µmol/L; CYP4A9/11, 50 µmol/L.

Discussion

An estimated [I]/Ki ratio of greater than 0.1 is considered positive and a follow-up in vivo evaluation is recommended. Maximum plasma concentration at 1 mg fingolimod dose which is twice the dose sought for labeling was approximately 10 ng/ml which corresponds to 0.0325 µmol/L. A drug interaction is remote as [I]/Ki ratio is less than 0.1.

Reviewer's Comment:

Fingolimod's potential to inhibit metabolism of CYP2C8 was not evaluated in this study or other studies submitted in this NDA package. However, fingolimod-phosphate's ability to inhibit CYP2C8 was evaluated in study dmpk 0201484.

CONCLUSIONS

- FTY720 is unlikely to reduce the in vivo clearance of drugs mainly cleared though metabolism by CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2El, CYP4A9/11, CYP2B6 and/or CYP3A4/5 mediated metabolic clearance of co-administered drugs.
- FTY720 did not function as an irreversible metabolism-dependent inhibitor of any of the P450 enzymes except weak inhibition of CYP2C19.

Study Title	Identification of the human cytochrome P450 isozyme(s) involved in the biotransformation of FT720 in vitro: role of CYP4F
Study number	DMPK-r99202
Objective	The purpose of this study was to determine the human cytochrome P450 enzymes responsible for metabolizing the test substance FTY720 at therapeutically relevant concentrations.
Test System	Human and rat liver microsomes

METHODS

Several methods were used to determine which cytochrome P450 enzymes are principally responsible for the metabolism of drugs. These include:

1. Correlation of the rates of metabolism for marker, enzyme-selective activity in a panel of characterized human liver microsomes.
2. Inhibition of specific cytochrome P450 enzymes with selective chemical and immunochemical (antibody) inhibitors.
3. Analysis of enzyme kinetic parameters using individual, cDNA-expressed human cytochrome P450 enzymes and human liver microsomes.

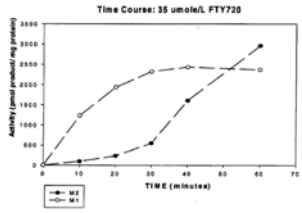
Enzyme kinetic parameters, apparent Km and apparent Vmax, are measured under initial rate conditions using enzyme concentrations and incubation times which are in the linear portions of the respective concentration and time-dependence determinations. The in vitro intrinsic clearance, calculated as Vmax/Km, is a parameter for determining which enzyme is the principal enzyme for the metabolism of the drug at low (<< Km) therapeutic concentrations. When multiple enzymes are capable of metabolizing a drug, the enzyme with the highest in vitro intrinsic clearance is the most active at low drug concentrations and hence, the most likely to be the principal enzyme for metabolism in vivo.

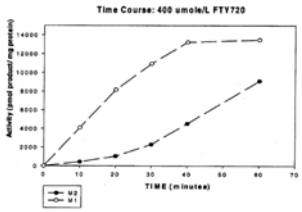
RESULTS

Time Course and Protein-Dependence of FT720 Metabolism Catalyzed by Human Liver Microsomes

Experiments were conducted to determine conditions of linearity for the metabolism of FTY720 with respect to time and protein concentration. The formation of the major metabolite was linear for up to about 20 min at both a low and high FTY720 concentration. Latency was observed for the minor metabolite, suggesting that it may be a secondary product of the major metabolite. The metabolism of FTY720 was linear with respect to increasing protein concentrations up to 0.8 mg/mL for the major metabolite, and 1 mg/mL for the minor metabolite.

Time course of fingolimod metabolism in HLM





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P450 Phenotyping via Recombinant Human P450 Enzymes

None of the cDNA expressed P450s (Supersomes™) had significant activity towards FTY720 metabolism. P450s 2D6, 2C9 and 2El produced a small peak with the same retention time as the minor metabolite in HLM. The positive control activities were validated. Additional recombinant enzymes tested included CYP1A1, 2C9*3, 3A5, and FMO3; none of these enzymes were able to metabolize FTY720.

The preliminary metabolite structures of FTY720 from human incubations further suggests the involvement of CYP4A (fatty acid terminal hydroxylation). However, expressed 4A11 was shown here not to metabolize FTY720.

Expressed
CYP4F2 showed slight activity for the formation of the major peak, but only one of the three CYP4F2 preparations were able to form the product. Like 4A9/l1, CYP4F metabolizes fatty acids, however, CYP4F enzymes have been found to be more specific for longer chain fatty acids; both arachidonic acid (C20) and prostaglandins are substrates for CYP4F2 and 4F3 (L3). Leukotriene B4 omega-hydroxylation is also cleared out by both 4F enzymes. CYP4F3 is a low Km enzyme for this reaction (about 1 µmol/L), while 4F2 shows a much higher Km value of about 75 µmol/L. CYP4F3 has been isolated in human neutrophils and detected in human liver. CYP4F2 has been isolated from human liver tissue.

Reaction Phenotyping using cDNA Expressed P450 Enzymes

		FTY720 Metabolism (pmol/min/pmol P450)		CYP Enzyme Positive Control
CYP Enzyme	pmol P450/ml	M2	M1	pmol/min/pmol P450
CYP1A2	75	0	0	24
CYP2A6	50	0	0	14.9
CYP2B6	35	0	0	3.4
CYP2C8	60	0	0	4.9
CYP2C9(*1)	140	0	0	40.5
CYP2C19	35	0.04	0	9.6
CYP2D6	15	0.72	0	36.5
CYP2E1	50	0.16	0	11.9
CYP3A4	200	0	0	65
Control Cells	Not Applicable	0	0	Not Applicable
HLM (pooled)	Not Applicable	39.7	155.2	Not Applicable

Inhibition of FTY720 by Chemical and Antibody Inhibitors Specific for CYP1A2, 2A6, 286, 2C9, 2D6, 2E1 and 3A4

Ketoconazole, a specific inhibitor of CYP3A4 at low ketoconazole concentrations, was found to inhibit FTY720 metabolism about 70% using 1 µmol/L ketoconazole. Troleandomycin inhibited FTY720 metabolism by about 20%.

However, the anti-3A4 antibody was shown not to inhibit FTY720 metabolism, a result consistent with the cDNA expressed P450 data and with the HLM correlation data showing that CYP3A4 is not involved in FTY720 metabolism. Thus, the FTY720 hydroxylase in HLM is sensitive to low ketoconazole concentrations where this compound was thought to be specifically inhibiting CYP3A4. Antibodies specific for CYP1A2, 2A6, 2B6 and 2El were found not to inhibit FTY720 metabolism in HLM.

Chemical Inhibition of FTY720 Metabolism in HLM by Lauric Acid, LTB4 and 17-Octadecynoic Acid

Lauric acid is shown here to be a weak inhibitor of FTY720 metabolism with IC50 values of about 200 µmole/L for both metabolites. This result, coupled with the lack of turnover by expressed 4A11, strongly suggests that CYP4A9/11 is not involved in FTY720 metabolism in HLM.

LTB4 is a specific substrate for CYP4F2 and CYP4F3. The IC50 value for FTY720 inhibition by LTB4 was about 61.5 µmole/L for combined Ml and M2 formation. This result suggests that the CYP enzyme involved in FTY720 metabolism is more specific for longer chain fatty acids. 17-Octadecynoic acid (17-ODYA) is an 18-carbon terminal acetylenic fatty acid mechanism based inhibitor of specific P450 reactions. It is able to inhibit both CYP4A and 4F reactions. 17-ODYA is a potent inhibitor of LTB4 omega-hydroxylase in human polymorphonuclear leukocytes (complete inactivation at < 5 µmole/L), suggesting it is highly specific for CYP4F3. Shorter chain acetylenic fatty acids (C-10) have no inhibitory effect on LTB4 hydroxylation. The results shown here demonstrate that 17-ODYA is a potent inhibitor of FTY720 metabolism. The IC50 values decreased significantly when the microsomes were pre-incubated with 17-ODYA in the presence of NADPH, suggesting mechanism-based inhibition.

Inhibition of FTY720 Metabolism in HLM and RLM by Anti-Human 4F and 4A Antibody.

Anti-4F2 antibody (IgG) was a very potent inhibitor of FTY720 metabolism. Whereas, anti-1A2 antibody did not inhibit metabolism of FTY720 approximately more than 50%.

Inhibition of FTY720 Metabolism in HLM by Cyclosporin A and RAD001: Potential Co-Medications

Cyclosporin could not produce any inhibition of FTY720 metabolism in HLM while 100 µmole/L RAD001produced about 30% inhibition when FTY720 was 5 µmole/L. The lack of inhibition by cyclosporin confirms that CYP3A4 is not involved in FTY720 metabolism.

Reviewer's Comment:

This is an exploratory study additional investigations on identification of human CYPs involved in the oxidative metabolism of fingolimod was carried out in two studies including dmpk 0301153 and dmpk 0400708.

CONCLUSIONS

- FTY720 is metabolized by human liver microsomes to two metabolites, Ml and M2. Ml is about 5-fold more abundant than M2 under the experimental conditions used. The formation of the two metabolites is completely dependent on the presence of NADPH. Carbon monoxide inhibited the formation of both peaks. Preliminary Mass Spectral data indicated that the major metabolite is a terminal (omega) alcohol, while the minor peak is the carboxylic acid.
- Time course experiments demonstrate linear formation of the major peak up to about 20 min. The minor peak showed latency in its formation, suggesting it is a secondary metabolite.
- None of the expressed enzymes tested showed significant metabolism of FTY720, including recombinant CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYPC9, CYPC19, CYP2D6, CYP2El, CYP3A4, CYP4A11 or CYP4F2. The latter two P450s are known to metabolize fatty acid and various eicosanoid substrates.
- Chemical and antibody inhibitors specific for P450s lA2, 2A6, 2B6, 2C9, 2D6, 2E1, and 3A4 did not inhibit FTY720 metabolism in HLM, confirming the lack of involvement of the major hepatic CYP enzymes in FTY720 metabolism.
- Lauric acid, a known specific substrate of CYP4A11, was found to be a weak inhibitor of FTY720 metabolism. 17-octadecynoic acid, a mechanism-based inhibitor of P450s of the 4A and 4F families, was a very potent inhibitor of FTY720 metabolism. LTB4, a known substrate of the 4F family was found to be an intermediate inhibitor of FTY720 metabolism in HLM.
- Anti-4F2 antibody (IgG) was a very potent inhibitor of FTY720 metabolism.

- Cyclosporin A and RAD001, drugs which could be co-administered with FTY720, were both unable to produce significant inhibition of FTY720 metabolism in HLM.
- 4-Methylpyrazole, a potent inhibitor of alcohol dehydrogenase was able to inhibit secondary metabolism of FTY720 (using Ml as substrate). Ethanol was also shown to inhibit secondary metabolite formation.
- Precise identification of the individual enzymes responsible for FTY720 metabolism were not established in this study, however, several pieces of evidence are presented which implicate CYP4F2/3 and alcohol dehydrogenase.

Study Title	In vitro assessment of cytochrome P450 enzyme inhibition by the FTY720 phosphates AML627 and AML629
Study number	DMPK-r0201483
Objective	The objective of this study was to determine the potential of AML627 and AML629 to function as an in vitro inhibitor of cytochrome P450 (CYP)-mediated reactions.
Test System	Human liver microsomes

Introduction

The enantiomers AML627 and AML629 are phosphorylated forms of the prochiral parent compound FTY720. In humans FTY720 is phosphorylated almost exclusively to AML629 *in vivo*. AML629 is the pharmacologically active isomer, whereas AML627 and the parent compound FTY720 are essentially biologically inactive. FTY720 in its phosphorylated form is a potent agonist of a subgroup of sphingosine 1-phosphate receptors (S1P) and is intended for therapeutic prophylaxis of allograft rejection after transplantation.

METHODS

A pool of human liver microsomes prepared from 48 individual donors was obtained from commercial source. The total P450 content was 270 pmol/mg protein and the cytochrome b_5 content was 550 pmol/mg protein. The incubations were conducted in a 96-well plate format at 37 °C.

Following table represents microsomal incubation composition for IC50 determination

Microsomal incubation composition for IC50 determination

Chemical	Final concentration
0.5 M potassium phosphate buffer, pH 7.4	50 mM
glucose-6-phosphate	130 µM
NADP	50 µM
$MgCl_2$	130 µM
glucose-6-phosphate dehydrogenase	0.4 U / mL
CHAPS	0.1% (w/v)
AML627 or AML629	0, 0.019, 0.039, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10 µM
probe substrates	2.5, 5, 10 or 40 µM (see Table 4-2)
pooled human liver microsomes in 50 mM potassium phosphate buffer, pH 7.4	0.1or 0.5 mg protein / mL (see Table 4-2)

Eight CYP-isoenzyme selective substrate probes were incubated in the absence and presence of increasing amounts of AML627 or AML629 up to a concentration of 10 µM. Probe substrate concentrations were per guidance.

RESULTS

Inhibitory effect of AML627 and AML629 on CYP isoenzyme-selective metabolic reactions			
CYP enzyme	**Probe reaction**	**IC_{50} value for AML627 [µM]**	**IC_{50} value for AML629 [µM]**
CYP1A2	phenacetin O-deethylation	> 10	> 10
CYP2A6	coumarin 7-hydroxylation	> 10	> 10
CYP2C8	paclitaxel 6α-hydroxylation	> 10	> 10
CYP2C9	diclofenac 4'-hydroxylation	> 10	> 10
CYP2C19	S-mephenytoin 4'-hydroxylation	> 10	> 10
CYP2D6	bufuralol 1'-hydroxylation	> 10	> 10
CYP2E1	chlorzoxazone 6-hydroxylation	12.5 ± 1.1	9.9 ± 0.4
CYP3A4	midazolam 1'-hydroxylation	> 10	> 10
CYP3A4	testosterone 6β-hydroxylation	17.0 ± 3.0	> 10

Both compounds displayed comparable moderate inhibition of CYP2E1 related activity (IC_{50} of 12.5 µM for AML627 and 9.9 µM for AML629). AML627 additionally inhibited CYP3A4-mediated testosterone hydroxylation with a moderate IC_{50} of 17.0 µM, but did not inhibit CYP3A4-mediated midazolam biotransformation. CYP3A4 inhibition was not seen with AML629. No inhibition was observed for CYP enzymes 1A2, 2A6, 2C8, 2C9, 2C19 and 2D6 at concentrations of AML627 and AML629 up to 10 µM.

Discussion

Based on the in vitro inhibition results of this study, the therapeutic whole blood concentrations of FTY720 phosphate below 0.1 µM and the absence of AML627 in human metabolism, inhibition of cytochrome P450-mediated metabolic clearance by AML627 and AML629 is not expected in man. Although inhibition of CYP2E1 and CYP3A4 was observed in vitro at concentrations of 10 µM or greater, FTY720 phosphate concentrations of this magnitude are unlikely to occur in man based on the exposure reached at therapeutic doses.

CONCLUSIONS

AML629 are unlikey to inhibit the metabolic clearance of comedications metabolized by CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19 and CYP2D6.

Study Title	In vitro biotransformation of AML629 in human liver microsomes, S9 fraction and cytosol
Study number	DMPK-r0201484
Objective	The objective of this study was to investigate the biotransformation of AML629 in human liver microsomes and to identify the human cytochrome P450 isoenzymes involved in the oxidative metabolism of AML629.
Test System	Human liver microsomes

AML629 (S-enantiomer of phospho-FTY720) is the pharmacologically active metabolite of FTY720.

METHODS

Human liver microsomes, S9 fraction and cytosol

A pool of liver microsomes prepared from 22 individual donors was obtained from a commercial source. Catalytic activities (pmol/(mg x min)) for the following assays were provided: phenacetin O-deethylase, coumarin 7-hydroxylase, (S)-mephenytoin N-demethylase, paclitaxel 6α-hydroxylase, diclofenac 4'-hydroxylase, (S)- mephenytoin 4'-hydroxylase, bufuralol 1'-hydroxylase, chlorozoxazone 6-hydroxylase, testosterone 6β-hydroxylase, lauric acid 12-hydroxylase, methyl p-Tolyl sulfide oxidase and cytochrome c reductase. A pool of human liver S9 fraction was obtained from (b) (4). The human liver cytosol (pooled fraction) was from (b) (4)

Incubation of [^{14}C]AML629 with human liver microsomes

Conditions and procedures used for incubation with HLM, time dependence, protein dependence, and to determine enzyme kinetics were as per Agency's guidance.

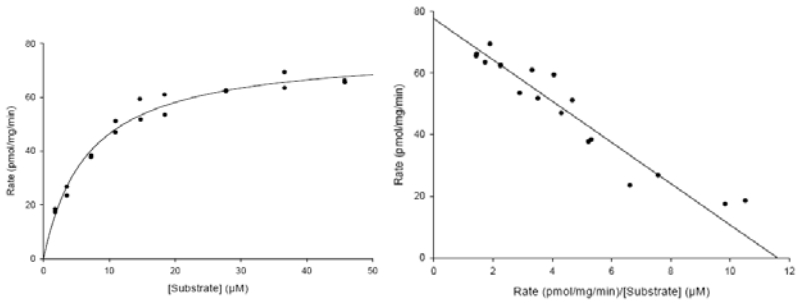
Concentration-dependent biotransformation of [^{14}C]AML629 in human liver microsomes

After establishing linear reaction conditions, AML629 enzyme kinetic parameters K_m and V_{max} were determined by incubating pooled human liver microsomes (0.2 mg/ml) with various substrate concentrations ranging from 1 to 50 μM for 40 min. The kinetics of the formation of [$_{14}$C]FTY720 were analyzed by nonlinear regression analysis considering different kinetic models (Michaelis-Menten, Hill, isoenzyme, random substrate activation, substrate inhibition) as provided by the Enzyme Kinetics module, SigmaPlot.

RESULTS

The concentration-dependent kinetics of [14C]AML629 metabolism in HLM (0.2 mg/ml) after 40 min incubation. The rate of FTY720 formation was plotted as Michaelis-Menten plot (upper) and as Eadie-Hofstee plot (bottom) using substrate concentration 1 to 50 μM.

Enzyme kinetics of [14C]AML629 biotransformation in human liver microsomes



V_{max} [pmol/min/mg]	K_m^{app} [µM]	V_{max}/K_m µl/mg/min
77.7 ± 2.2	6.7 ± 0.6	11.6

The apparent Michaelis-Menten constant K_m^{app} of 6.7± 0.6 µM and Vmax of 77.7 ± 2.2 pmol/min/mg were calculated with the Michaelis-Menten kinetic model. The derived intrinsic clearance (V_{max}/K_m) as calculated for the formation of FTY720 was 11.6 µl/min/mg. This indicates the low biotransformation rate by the metabolite patterns.

The only biotransformation of AML692 observed in the incubates with human liver microsomes (and human liver S9 and cytosol fractions) was its dephosphorylation to FTY720, probably catalyzed by a phosphatase.

No evidence of any direct oxidative metabolism of AML629 was obtained in these *in vitro* systems. A previous study in hepatocytes also reported FTY720 as the major primary metabolite of AML629.

CONCLUSIONS

- In human liver microsomes AML629 was metabolized to FTY720 by dephosphorylation. The biotransformation was slow and follows Michaelis-Menten kinetics with apparent kinetic constants Km of 6.7±0.6 µM and Vmax of 77.7±2.2 pmol/min/mg.

- A low in vitro intrinsic clearance (CLint) of 11.6 µL/min/mg protein was determined for this pathway. No evidence of any direct oxidative metabolism of AML629 was obtained in these in vitro systems.

Study Title	Additional investigations on the identification of human cytochrome P450 enzymes involved in the oxidative metabolism of FTY720
Study number	DMPK-r0301153
Objective	The objective of this study was to investigate the human cytochrome P450 isoenzymes involved in the oxidative metabolism of FTY720.
Test System	Human liver microsomes

INTRODUCTION

The present study was conducted to investigate the human cytochrome P450 isoenzymes involved in the oxidative metabolism of FTY720. Although a P450 reaction phenotyping study was carried previously (DMPK(CH) R99-202) showing evidence of CYP4F2/3 involvement, the exact identity of the major enzyme isoforms was not determined in that investigation.

METHODS

Human liver microsomes

A pool of liver microsomes prepared from 22 individual donors was obtained from (b) (4) a commercial source. Catalytic activities (pmol/(mg x min)) for the following assays were provided: phenacetin O-deethylase, coumarin 7-hydroxylase, (S)-mephenytoin N-demethylase, paclitaxel 6α-hydroxylase, diclofenac 4'-hydroxylase, (S)- mephenytoin 4'-hydroxylase, bufuralol 1'-hydroxylase, chlorozoxazone 6-hydroxylase, testosterone 6β-hydroxylase, lauric acid 12-hydroxylase, methyl p-Tolyl sulfide oxidase and cytochrome c reductase.

The incubations of HLM recombinant human CYPs, determination of time dependence, protein dependence, enzyme kinetics and inhibition by specific inhibitors were performed as per Agency's guidance

RESULTS

In human liver microsomes FTY720 was metabolized to the two mono-hydroxylated metabolites M12 and M15 with M12 being the major metabolite. The oxidative metabolism of FTY720 in HLM was slow and showed very low intrinsic clearance.

Inhibition of [^{14}C]FTY720 biotransformation by chemical inhibitors

The biotransformation of FTY720 was tested at 1 and 50 μM substrate concentration in the presence of 8 individual chemical inhibitors. The metabolism of FTY720 was significantly inhibited by 35-49% with 1μM ketoconazole and by 63-77% with 10 μM ketoconazole. 10 μM quinidine was also inhibiting the metabolism of 1 μM FTY720 by 41%. Slight inhibitions were observed at 1 μM FTY720 with TAO and DETC (mechanism-based inhibitors for 3A4 and 2E1) respectively. No significant inhibition was detected with furafylline, taxol, sulphaphenazole and tranylcypromine.

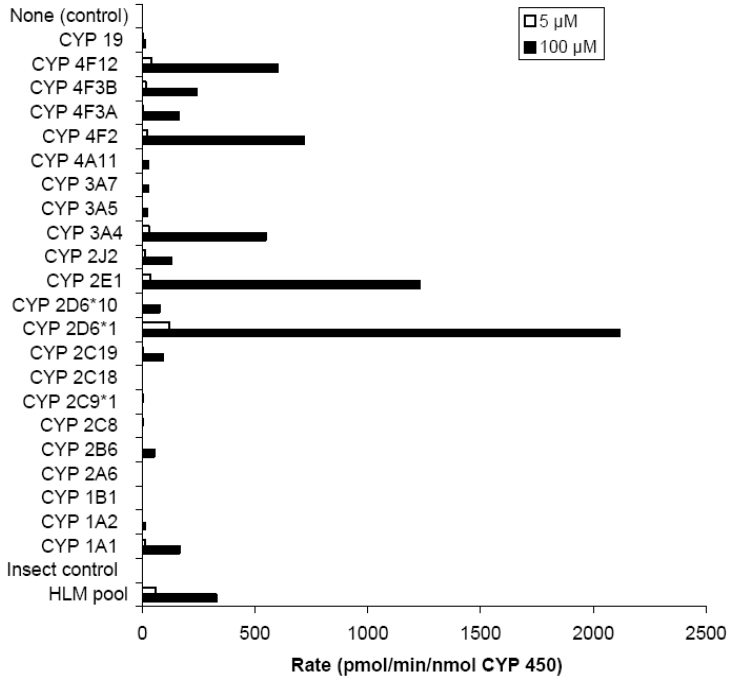
The involvement of multiple cytochrome isoforms in the oxidative pathways suggest that the biotransformation of FTY720 is not readily inhibited completely by a single specific inhibitor.

Inhibition of metabolism by specific inhibitors in human liver microsomes

	1 µM FTY720		50 µM FTY720	
Inhibitor	Rate	Relative activity	Rate	Relative activity
	(pmol/min/mg)	(%)	(pmol/min/mg)	(%)
No Inhibitor (A)	1.58	100	121.3	100
No Inhibitor (B)	2.16	100	104.7	100
2 µM Furafylline	2.51	116	130.0	124
10 µM Furafylline	2.12	98	106.3	101
2 µM Taxol	1.38	88	125.0	103
10 µM Taxol	1.63	103	86.6	71
2 µM Sulfaphenazole	1.95	124	85.0	70
10 µM Sulfaphenazole	1.68	106	115.6	95
2 µM Tranylcypromine	1.65	105	128.1	106
10 µM Tranylcypromine	1.90	121	126.9	105
1 µM Quinidine	1.95	124	140.6	116
10 µM Quinidine	0.93	59	128.8	106
1 µM Ketoconazole	1.03	65	61.3	51
10 µM Ketoconazole	0.36	23	44.7	37
2 µM TAO	2.14	99	145.9	139
20 µM TAO	1.51	70	142.8	136
5 µM DETC	1.28	59	123.4	118
30 µM DETC	2.03	94	159.7	153

A. normal blank; B: Blank with 15 min pre-incubation

Biotransformation of [^{14}C]FTY720 by recombinant human CYP P450s



Enzyme kinetic constants and estimated contribution of various CYPs to the metabolic clearance of FTY720 in human liver microsomes

Enzyme	Km	Vmax	CL(Vm/Km)	rhCYP[a]	abundance[b]	Specific Conc.	Rel. CL	Contribution[c]
	(μM)	(pmol/(min.nmol CYP)	μl/(min. nmol CYP)	factor	(pmol P450/mg)	(% total CYP)	μl/(min.nmol total CYP)	(% total Rel. CL)
CYP 1A1	117	359	3.1					
CYP 1A2	nd	nd			52	9.0%		
CYP 1B1	nd	nd						
CYP 2A6	nd	nd			36	6.3%		
CYP 2B6	nd	nd			11	1.9%		
CYP 2C8	nd	nd			24	4.2%		
CYP 2C9*1	nd	nd			73	12.7%		
CYP 2C18	nd	nd						
CYP 2C19	nd	nd			14	2.4%		
CYP 2D6*1	87	9442	108.4	36.7%	8	1.4%	0.55	7.8
CYP 2E1	73	442	6.1	100.0%	61	10.6%	0.64	9.1
CYP 2J2	76	231	3.0					
CYP 3A4	107	1423	13.3	3.1%	155	26.9%	0.11	1.6
CYP 3A5	nd	nd						
CYP 3A7	nd	nd						
CYP 4A11	nd	nd						
CYP 4F2	45	1049	23.4	100.0%	141.8	24.6%	5.76	81.5
CYP 4F3A	nd	nd						
CYP 4F3B	288	932	3.2	100.0%				
CYP 4F12	19	481	25.7	40.0%				
Total					575.8	100%	7.07	100

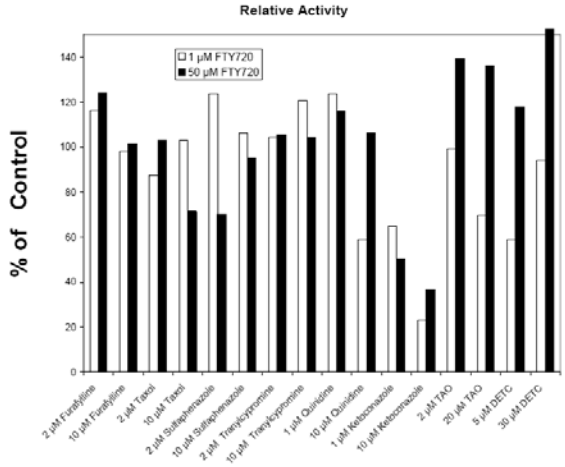
Enzyme kinetic parameters V_{max} and K_m of CYP2D6*1, 3A4, 2E2, 4F2 and 4F12 were obtained from kinetic experiments (rates of parent drug disappearance). The values of other isoenzymes were estimated by solving the Michaelis-Menten equation V=Vmax*S/(Km+S) for the 2 concentrations of substrate and their rate numbers.

a: rhCYP factor: ratio of HLM metabolites (M12+M15) to the total metabolites catalyzed by the rhCYP

b: The abundance of 4F2 was from (Jin,et al 1998); others were from (Rowland, et al 2004). For 3A4, the abundance of CYP3A was used.

c: The contribution of other CYPs (4F12, 2J2, 4F3B) were not taken into consideration due to the unknown abundance in HLM.

Inhibition of FTY720 metabolism in HLM by specific inhibitors



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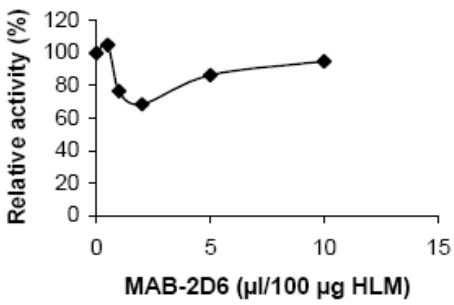
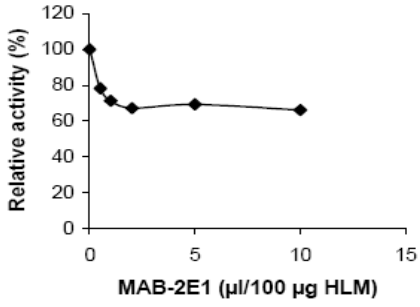
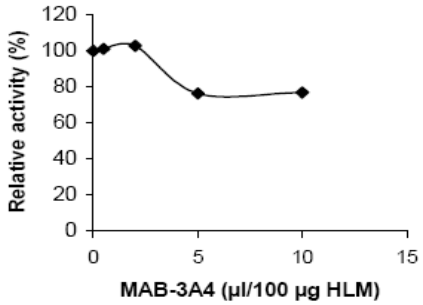
Inhibition of FTY720 metabolism in HLM by inhibitory antibodies







CONCLUSIONS

- FTY720 is metabolized at 5 and 100 µM substrate concentration by recombinant human CYP2D6*1, 2E1, 3A4, 4F2, 4F3B and 4F12 with measurable turnover. Slight metabolic activity was also observed in incubations with CYP1A1 and 2J2.
- The metabolism of FTY720 in human liver microsomes was inhibited by chemical inhibitors such as ketoconazole and quinidine. Combination of several inhibitors resulted in more inhibition than a single inhibitor. Ketoconazole inhibited the biotransformation of FTY720 by recombinant CYP4F2 and 4F12 with IC50 of 1.6 and 0.6 µM, respectively.
- Monoclonal antibodies inhibitory to CYP2D6, 3A4 and 2E1 also reduced partially the biotransformation rate of FTY720.

FTY720 was metabolized via multiple cytochrome P450 enzymes (CYP4F2, 4F3B, 4F12, 2E1, 2D6, 3A4, etc.). Abundance of CYP4F2 content in human liver microsomes is high; therefore the contribution of CYP4F2 to the total intrinsic clearance is estimated to be predominant, although other CYP enzymes may also contribute to metabolism of FTY720. The involvement of multiple cytochrome isoforms in the oxidative pathways suggest that the metabolism of FTY720 is not readily inhibited by a single specific CYP inhibitor.

Study Title	In vitro inhibition of FTY720 metabolism by ketoconazole in human liver microsomes
Study number	DMPK-r040708
Objective	The present study was conducted to determine the Ki of FTY720 metabolism by ketoconazole in human liver microsomes and to estimation of the risk of potential drug-drug interactions by co-medications.
Test System	Human liver microsomes

INTRODUCTION

In previous investigations, CYP4F2 was found as predominant isoenzyme with major contribution to the biotransformation of FTY720 in HLM. It was also reported in the same study (PCS(EU) R0301153) that ketoconazole, a well known CYP3A strong inhibitor, also had a significant inhibitory effect on oxidative metabolism of FTY720 by HLM and by recombinant CYP4F2.

In order to distinguish the inhibition effect of ketoconazole to both CYP3A and 4F2, sponsor has selected to test the more selective 3A inhibitor azamulin, which was recently shown to be a highly selective, potent and irreversible CYP3A inhibitor (Stresser, et al 2004). In vitro, Azamulin was reported to show strong inhibition to CYP3A (IC_{50} < 1 μM) but only a moderate inhibition to CYP4F2 (IC_{50} = 46 μM).

METHODS

In vitro incubation of [14C] FTY720 with human liver microsomes, Ki determination and determination of inhibition by azamullin was conducted per Agency's guidance. The conditions used in these studies are acceptable.

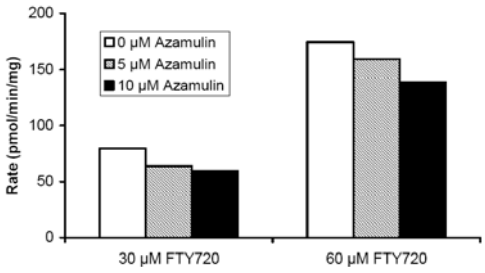
RESULTS

Effects of ketoconazole and azamulin

^{14}C-FTY720 was metabolized in human liver microsomes to several metabolite peaks. The major metabolite M12 and another peak M15 were previously reported to be monohydroxylated metabolites (PCS(EU) R0301153).

Figure below shows the effect of azamulin on FTY720 metabolism in HLM. In the presence of 5 and 10 μM of this compound, the biotransformation rate of FTY720 was reduced by 9 to 25 %.

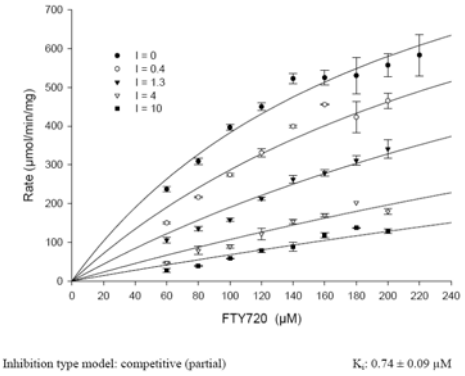
Effect of azamulin on FTY720 metabolism in HLM



Inhibition of [^{14}C] FTY720 metabolism in human liver microsomes by ketoconazole - Ki determination

The concentration-dependent kinetics of [^{14}C]FTY720 metabolism in HLM (0.6 mg/ml) in the presence of four concentrations of ketoconazole (I = 0.4, 1.3, 4, 10 µM) after 20 min incubation was plotted as Michaelis-Menten plot (mean value from 2 incubations) using substrate concentration 60-200 µM. The kinetic profile in the absence of inhibitor was obtained from means of 2-6 incubations.

Kinetic profiles of FTY720 metabolism in the presence of ketoconazole



Inhibition type model: competitive (partial) K_i: 0.74 ± 0.09 µM

Discussion

Metabolism of FTY720 by CYP4F2 is consistent with its selective inhibition in human liver microsomes by ketoconazole but much less by the more specific CYP3A inhibitor azamulin (Stresser, et al 2004). K_i and IC_{50} values were consistent with reported effects on CYP4F2. The data thus indicate that CYP4F2 inhibitors such as ketoconazole would have the potential to inhibit competitively the FTY720 metabolic clearance with a K_i-value of 0.74 µM. The plasma concentrations of ketoconazole found *in vivo* were 1-6 µg/ml or 2-12 µM. These values are 3 to 16-fold above the K_i-value and therefore competitive P450 inhibition of ketoconazole on the oxidative metabolism of FTY720 may occur in man.

Reviewer's Comment
Sponsor's inference related to metabolism of the fingolimod appears to be reasonable. Furthermore, effect of ketoconazole was evaluated in a drug interaction study conducted in healthy volunteers.

CONCLUSIONS

- In vitro the oxidative metabolism of ^{14}C-FTY720 in pooled human liver microsomes was inhibited by ketoconazole with an apparent K_i value of 0.74 µM (partial competitive inhibition model).
- Azamulin, which was recently shown to be a more selective CYP3A inhibitor than ketoconazole, showed only slight inhibitory effect on FTY720 metabolism in HLM.
- CYP4F2 has a major role in the metabolism of FTY720 and CYP3A has comparatively less involvement in FTY720 metabolism.

Study Title	Evaluation of FTY720 as an inducer of cytochrome P450s and ABCB1 (Pgp) in human hepatocytes
Study number	DMPK-r05000157
Objective	The objective of this study was to evaluate induction potential of FTY720 treatment on CYP3A4, CYP1A2, CYP4F2, and ABCB1 (Pgp) mRNA and CYP3A, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP4F activity in primary human hepatocytes.
Test System	Human hepatocytes

METHODS
Rifampicin (RIF), evaluated at the same concentrations as FTY720 (including a 10 and/or 25 μM treatment), was used as a positive control for CYP3A/2B/2C and ABCB1 induction. Phenobarbital (PB) at a concentration of 1000 μM, was also included as well-known positive control for CYP3A/CYP2B/2C induction and phenytoin (PHY) was used as a positive control for CYP3A/2B induction. β-napthoflavone (BNF) was included at a concentration of 10 μM as positive control for CYP1A induction and 9-cis retinoic acid (9-cisRA) was included as a potential positive control for CYP4F induction in human hepatocytes.

Conditions used for primary human hepatocyte incubations and measurement of P450 activity in primary human hepatocytes were per Agency's guidance.

MTT cell viability assay
The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay was used to spectrophotometrically, as a function of mitochondrial activity in living cells, assess the viability of the hepatocytes at the end of the induction period.

Relative quantitation of CYP3A4, CYP1A2, CYP4F2, and ABCB1 (Pgp) mRNA by RT-PCR
Relative quantification of human CYP3A4, CYP1A2, CYP4F2, and ABCB1 (Pgp) mRNA were determined by RT-PCR using the Comparative CT Method, based upon determined similar amplification efficiencies of the target and endogenous primers/probes. In the Comparative CT Method, changes in gene expression, relative to a calibrator sample (vehicle control), are calculated from the equation: $2^{-\Delta\Delta CT}$. The derivation of this equation is presented in the Appendix, Section 8. The ΔΔCT value is derived by subtraction of the ΔCT value of the calibrator sample (vehicle control) from the ΔCT value of the treatment group.

Protein concentration determination
The amount of cellular protein in the wells were determined by the Bradford protein assay method.

RESULTS
RT-PCR analysis of CYP3A, CYP1A2, CYP4F2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or ABCB1 (Pgp) mRNA expression in treated human hepatocytes relative to the vehicle control and increase in CYP3A, CYP1A2, CYP4F2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or ABCB1 (Pgp) activity showed that treatment of primary human hepatocytes with FTY720 (0.01, 0.1, or 1 μM) for 72 h did not induce mRNA (< 2-fold) in all the three human livers examined or increase in the enzyme activity or ABCB1 activity compared to positive control.

CONCLUSIONS
FTY720 is not an inducer of CYP3A, CYP1A2, CYP4F2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or ABCB1 (Pgp) at therapeutic concentrations.

Study Title	Evaluation of [^{14}C]FTY720 metabolism after induction of cytochrome P450s in human hepatocytes
Study number	DMPK-r0500655
Objective	The present study was conducted to determine if changes in FTY720 metabolism would be observed in primary human hepatocyte incubations after a treatment period with known P450 inducers
Test System	Human hepatocytes

METHODS

Conditions used for primary human hepatocyte incubations, measurement of P450 activity in primary human hepatocytes, MTT cell viability assay and [^{14}C]FTY720 metabolism in human hepatocytes after 72h induction followed the Agency's guidance. These methods are acceptable.

Relative quantification of human P450 enzymes mRNA expression by RT-PCR of cDNA from treated and control human hepatocytes

Relative quantification of human CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4, CYP3A5, CYP4F2, CYP4F3B, and CYP4F12 mRNA was determined by RT-PCR using the Comparative C_T Method, based upon the similar amplification efficiencies of the target and endogenous primers/probes.

RESULTS

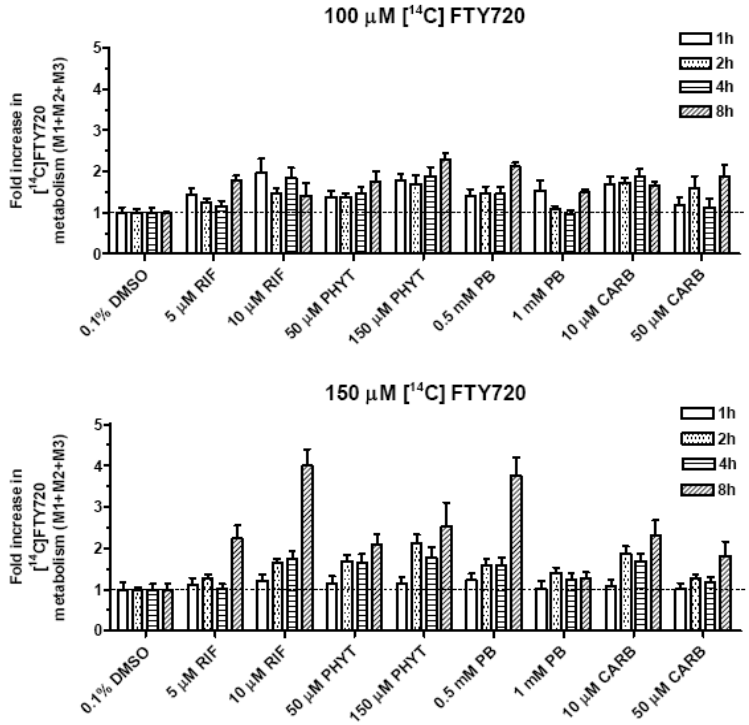
Changes in [^{14}C]FTY720 metabolism in primary human hepatocytes after treatment with P450 inducers

RIF (10 or 25 μM) treatment increased [^{14}C]FTY720 metabolism by 4.12- and 3.31-fold, respectively at 8h. PHYT (50 or 100 μM) increased [^{14}C]FTY720 metabolism by 2.46- and 2.60-fold, respectively, and PB (1000 μM) increased the metabolism by 3.28-fold, with respect to the vehicle control treated cells.

Increases in metabolism of [14C]FTY720 were more apparent using 100 or 150 μM [14C]FTY720 to assess induction, particularly after incubation for 8h with 150 μM FTY720. RIF (5 or 10 μM) treatment increased [14C]FTY720 (150 μM) metabolism by 2.23- and 3.99-fold, respectively at 8h. PHYT (50 or 150 μM) increased [14C]FTY720 metabolism by 2.09- and 2.52-fold, respectively, and PB (500 or 1000 μM) increased the metabolism by 3.75- and 1.28-fold, respectively. CARB (10 or 50 μM) increased [14C]FTY720 metabolism by 2.30- and 1.80- fold, respectively, with respect to the vehicle control treated cells.

Fold change of [^{14}C]FTY720 metabolism in primary human hepatocytes (Exp 2)

[^{14}C]FTY720 (50, 100, or 150 μM) was incubated for 1, 2, 4, or 8h with primary human hepatocytes after 72h treatment with inducers. Data is expressed as the mean [^{14}C]FTY720 metabolism activity (formation of M1, M2, and M3) of the samples related to the vehicle control and standard deviation (error bars) of triplicate wells.

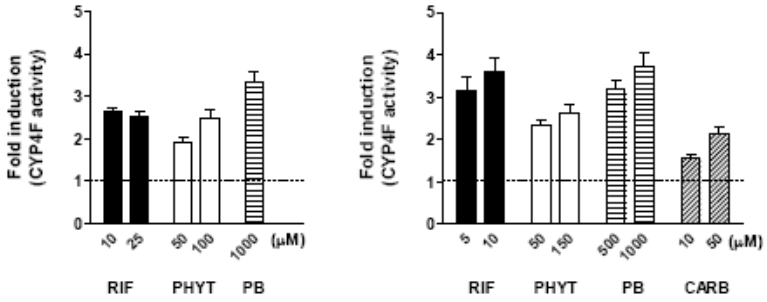


Induction of specific P450 mRNA and activity

The P450s CYP2B6, CYP2C8/9/19, and CYP3A mRNA and activities were increased by all these inducers, as expected. CYP4F activity was also increased in human hepatocytes after all inducer treatments (~2- to 4-fold). CYP4F2 and CYP4F12 mRNAs were not induced by PHYT, PB, or CARB and RIF caused a minimal increase in these mRNAs (<1.4-fold).

Fold increase in CYP4F activity

Data is expressed as the mean CYP4F activity (20-OH-LTB4 formation) of the treatment groups relative to the vehicle control and standard deviation (error bars) of quadruplicate wells. (A) Exp 1 (B) Exp 2.



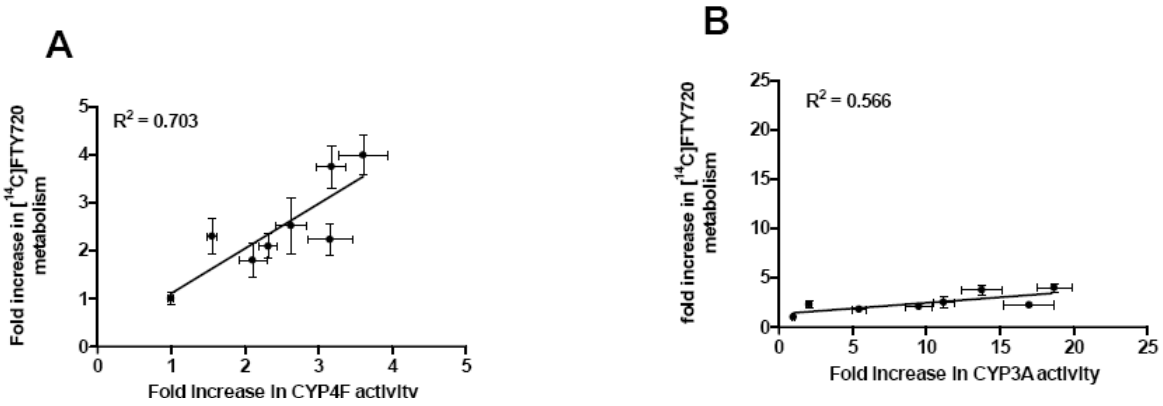
Fold changes in mRNA levels (mean ± SD) from treated and control human hepatocytes (Exp 2)

Treatment	CYP3A4 mRNA	CYP3A5 mRNA	CYP2B6 Mrna	CYP2C8 mRNA	CYP2C9 mRNA	CYP2C19 mRNA	CYP4F2 mRNA	CYP4F3B mRNA	CYP4F12 mRNA
DMSO (0.1%)	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
RIF, 5 µM	170 ± 9.2	3.89 ± 0.13	8.54 ± 0.49	4.07 ± 0.31	1.83 ± 0.36	5.90 ± 0.53	1.24 ± 0.069	1.39 ± 0.067	1.09 ± 0.055
RIF, 10 µM	180 ± 6.1	4.16 ± 0.40	9.05 ± 0.78	4.26 ± 0.43	1.90 ± 0.35	5.94 ± 0.56	1.36 ± 0.18	1.28 ± 0.030	1.29 ± 0.20
PHYT, 50 µM	47.4 ± 1.2	4.62 ± 0.26	14.0 ± 0.69	2.84 ± 0.27	1.29 ± 0.20	1.61 ± 0.15	0.900 ± 0.088	1.12 ± 0.045	1.02 ± 0.10
PHYT, 150 µM	93.3 ± 3.9	5.67 ± 0.36	16.7 ± 1.3	2.57 ± 0.063	1.36 ± 0.16	2.16 ± 0.14	0.827 ± 0.091	1.58 ± 0.053	1.05 ± 0.092
PB, 500 µM	126 ± 4.3	5.79 ± 0.39	13.7 ± 1.1	3.62 ± 0.38	1.63 ± 0.25	3.12 ± 0.18	0.938 ± 0.079	1.23 ± 0.031	0.929 ± 0.092
PB, 1000 µM	129 ± 6.7	6.28 ± 0.52	17.2 ± 0.67	3.87 ± 0.24	1.69 ± 0.14	4.43 ± 0.25	0.957 ± 0.059	1.51 ± 0.10	1.02 ± 0.090
CARB, 10 µM	9.51 ± 0.18	2.10 ± 0.13	5.80 ± 0.44	2.14 ± 0.19	1.15 ± 0.13	1.23 ± 0.083	0.857 ± 0.11	0.903 ± 0.047	0.872 ± 0.10
CARB, 50 µM	65.6 ± 4.6	3.87 ± 0.25	11.9 ± 1.2	3.23 ± 0.19	1.68 ± 0.18	1.99 ± 0.044	0.947 ± 0.14	1.29 ± 0.11	0.904 ± 0.061

The increase in FTY720 metabolism is due to increases in CYP4F activity as the magnitude of increases of FTY720 and LTB4 metabolism were more similar (~2- to 4- fold) than, for instance, increases in CYP3A activity (~2- to 19-fold).

Correlation of induction of P450 activity and [14C]FTY720 metabolism

Fold increases in [^{14}C]FTY720 metabolism (8h time point) by the various inducers was plotted against the fold increases in CYP4F (A) or CYP3A (B) activities from Exp 2. There was a better correlation between increases in FTY720 metabolism with induction of CYP4F activity compared to CYP3A activity. The PB (1000 µM) data point was not included in the comparison as it appeared to be an outlier. Inclusion of this point decreased R2 values to 0.257 and 0.258, for CYP3A and CYP4F activity comparisons, respectively.



Conclusions

- These results indicated that the inducers, RIF, PHYT, PB, and CARB have the potential to increase the hepatic oxidative metabolic clearance of FTY720 in vivo.
- RIF, PHYT, PB, and CARB treatment of human hepatocytes for 72h increased [^{14}C]FTY720 metabolism up to 2.5- to 4-fold and it is likely this increase was due to induction of CYP4F activity.

Study Title	Assessment of efflux transporter (MDR1, MXR, MRP2, BSEP) inhibition by FTY720 and its active main metabolite AML629
Study number	DMPK-r0700867
Objective	The present study was conducted to determine the potential of FTY720 and its active main metabolite FTY720-phosphate (AML629) to inhibit human ATP-binding cassette (ABC) transporter-mediated efflux via the multidrug-resistant protein 1 (MDR1), the breast cancer resistant protein (MXR), the multidrug resistance-associated protein 2 (MRP2) and the bile salt export pump (BSEP), respectively.
Test System	MDCKII cells and Sf9 derived vesicles

METHODS

The potential of FTY720 and AML629 to inhibit human ATP-binding cassette transporter activity was assessed using recombinant MDCKII cells and Sf9 derived vesicles by determining the compound's ability to inhibit (sub)cellular uptake of the probe substrates cyclosporine A (CsA for MDR1), mitoxantrone (MTX for MXR), taurochlate (TCH for BSEP) and estradiol 17β-D-glucuronide (E17G for MRP2). Probe substrate concentrations used for these determinations were less than or equal to their reported Km values as shown in the table below.

Probe Substrate Concentrations

Probe substrate	ABC transporter	Conc. (µM)	Literature K_m value (µM)	Incubation time (min)
Cyclosporin A	MDR1	0.1	0.2 [a)]	40
Mitoxantrone	MXR, BCRP	0.1	7 [b)]	40
Estradiol 17β-D-glucuronide	MRP2	0.1	7.2 [c)], 9.4 [d)]	5
Taurocholate	BSEP	5	4.3 [e)]	2

[a)] (Adachi, et al 2001) [b)] (Ozvegy, et al 2001) [c)] (Cui, et al 1999) [d)] (Hirohashi, et al 2000) [e)] (Byrne, et al 2002)

Transpoter Inhibitors

Inhibitor	Conc. (µM)	ABC transporter(s)	Literature K_i value (µM)	Substrate
Cyclosporin A	10	MDR1	1.3 [a)], 2.2 [b)]	yes
STI571	100	MXR	0.5 [c, d)]	yes
Estradiol 17β-D-glucuronide	100	MRP2	not assessed	yes
Taurocholate	50	BSEP	not assessed	yes

[a)] (Ekins, et al 2002) [b)] (Tang, et al 2002) [c)] (PCS(EU) R0400181) [d)] (Ozvegy-Laczka, et al 2004)

Inhibition of ABC-mediated probe uptake by FTY720 as well as AML629 in vitro is summarized in the table below. AML629 displayed inhibition of neither MDR1, MXR, BSEP nor MRP2 in the (sub)cellular uptake assay.

FTY720 is not an inhibitor of MXR, BSEP and MRP2. FTY720 showed strong inhibition of MDR1 (about 2-fold more potent with respect to positive control CsA) with an IC50 values of about 84 µM.

OATP transporter	Probe substrate	IC_{50} value (µM) [a]	
		FTY720	AML629
MDR1	Cyclosporin A	84±22 [b]	not observed [c]
MXR, BCRP	Mitoxantrone	not observed [d]	not observed [c]
MRP2	Estradiol 17β-D-glucuronide	not observed [c]	not observed [c]
BSEP	Taurocholate	not observed [d]	not observed [c]

[a] FTY720 and AML629 concentration estimated to inhibit probe substrate uptake by 50%

[b] Maximal observed inhibition with respect to positive control (CsA) was about 200% at the highest concentration tested (100 µM)

[c] No inhibition observed up to the highest concentration tested (100 µM)

[d] Maximal observed inhibition with respect to positive control was ≤ 50% at the highest concentration tested (100 µM)

Sample data analysis

Analysis of activation kinetics

Kinetic uptake parameters can be estimated from the Michaelis-Menten equation including the nonsaturable (passive) transport clearance (Sasaki, et al 2004):

$$V_0 = CL_{app} \cdot S = CL_m \cdot S \pm CL_c \cdot S = CL_m \cdot S \pm \frac{V_{max} \cdot S}{K_m + S} \quad \text{Eq. 1}$$

where V_0 represent the initial uptake velocity/rate (pmol/min) of a test compound at 37°C. S is the concentration (µM) of the test compound in the medium, V_{max} is the maximum uptake velocity/rate (pmol/min), K_m is the Michaelis-Menten constant (µM), CL_m is the nonspecific (passive) uptake clearance (µL/min), CL_c is the transporter-mediated (saturable) uptake clearance (µL/min), and CL_{app} is the whole (apparent) uptake clearance (µL/min).

Analysis of inhibition kinetics

The transport in the presence of a competitive and non-competitive inhibitor can be described as follows (Gao, et al 2000):

$$V_{0(+inhibitor)} = CL_m \cdot S \pm \frac{V_{max} \cdot S}{S + K_m \cdot (1 + I / K_i)} \quad \text{Eq. 2}$$

where K_i is the inhibitor constant or inhibition constant (µM) and I is the concentration of the inhibitor in the medium (µM).

IC_{50} calculations

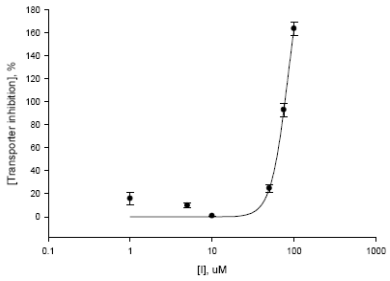
All absolute transporter uptake data were converted into relative values (y) by defining transporter activities without addition of test compounds as 100%. The IC_{50} values (inhibitor concentration that causes 50% inhibition of the maximal drug effect) were calculated using the following equation (Rautio, et al 2006):

$$y = \frac{a \cdot I^n}{IC_{50}^n + I^n} \quad \text{Eq. 3}$$

RESULTS

FTY720 was shown to be a potent inhibitor of MDR1 as shown in figure below. The calculated IC50 value for MDR1 inhibition was 84 µM. Inhibition at the highest investigated concentration was about 200% compared to background uptake activity.

Effect of FTY720 on probe substrate uptake by MDR1-transporter expressing MDCKII cells



No or only partial (< 50%) inhibition of MXR, BSEP and MRP2 could be obtained up to 100 µM FTY720.

Inhibitory effect of FTY720 on the probe substrate uptake in recombinant (inside-out) membrane vesicles overexpressing MRP2 or BSEP

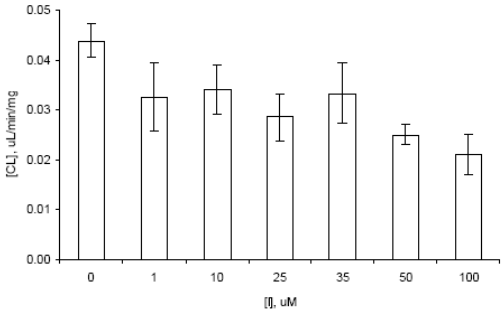
FTY720 [µM]	Uptake Clearance [µL/min/mg protein]					
	MRP2 [a]			BSEP [b]		
0 (= baseline)	2.07	±	0.44	4.35	±	0.57
1	2.96	±	0.24	4.02	±	0.39
10	2.21	±	0.24	3.38	±	0.82
25	1.97	±	0.66	3.52	±	0.33
35	1.92	±	0.19	3.88	±	0.54
50	2.04	±	0.53	3.99	±	0.31
100	2.31	±	0.54	3.16	±	0.16
Background [c]	4.68	±	0.52	1.26	±	0.13

[a] Probe substrate: Estradiol 17β-D-glucuronide [0.1 µM]
[b] Probe substrate: Taurocholate [5 µM]
[c] Estradiol 17β-D-glucuronide [100 µM] for MRP2 and Taurocholate [50 µM] for BSEP

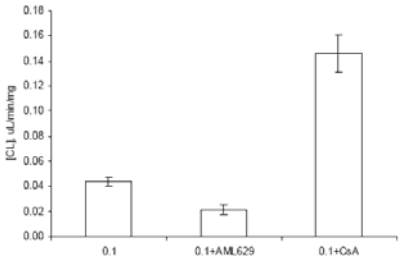
Using (sub)cellular assay systems no inhibition of MDR1, MXR, BSEP or MRP2 dependent transport was found at AML629 concentrations up to 100 µM.

Effect of AML629 on probe substrate uptake by MDR1-transporter expressing MDCKII cells

Concentration Dependency



Uptake in the presence of positive control



CONCLUSIONS

- FTY720 shows inhibition on MDR1-mediated transport at concentrations above 10 µM. But, AML629 did not inhibit MDR1 mediated transport
- AML629 and FTY720 did not inhibit the efflux transport by MXR, BSEP or MRP2.

Study Title	Assessment of uptake transporter (OATP1B1, OATP1B3, OATP2B1, NTCP) inhibition by FTY720 and its active main metabolite AML629
Study number	DMPK-r0700868
Objective	The objective of this in vitro study was to determine the potential of FTY720 and its active main metabolite FTY720-phosphate (AML629) to inhibit human solute carrier (SLC) transporter-mediated uptake via the human anion transporting polypeptide family (OATPs) and the sodium taurocholate cotransporting polypeptide (NTCP).
Test System	MDCKII cells and Sf9 derived vesicles

METHODS

The potential of FTY720 and AML629 to inhibit human SLC transporter activity was assessed using recombinant HEK293 cells and xenopus laevis transportocytes by determining the compound's ability to inhibit (sub)cellular uptake of the probe substrates estradiol 17β-D-glucuronide (E17G for OATP1B1 and OATP1B3) and taurocholate (TCH for NTCP). Probe substrate concentrations used for these determinations were less than or equal to their reported Km values

Probe Substrate Concentrations

Probe substrate	OATP transporter	Conc. (µM)	Literature K_m value (µM)	Incubation time (min)
Estradiol 17β-D-glucuronide	OATP1B1	0.1	8.2 [a], 3.7 [b]	2
Estradiol 17β-D-glucuronide	OATP1B3	0.1	5.4 [c]	2
Taurocholate	NTCP	1	6.2 [d]	60

[a] (Koenig, et al 2000a) [b] (Tamai, et al 2001) [c] (Koenig, et al 2000b) [d] (Hagenbuch and Meier 1994)

Uptake studies into HEK293 cells

Probe substrates

Probe substrate	OATP transporter	Conc. (µM)	Literature K_m value (µM)	Incubation time (min)
Estradiol 17β-D-glucuronide	OATP1B1	0.1	8.2 [a], 3.7 [b]	2
Estradiol 17β-D-glucuronide	OATP1B3	0.1	5.4 [c]	2
Taurocholate	NTCP	1	6.2 [d]	60

[a] (Koenig, et al 2000a) [b] (Tamai, et al 2001) [c] (Koenig, et al 2000b) [d] (Hagenbuch and Meier 1994)

Transporter Inhibitors

Inhibitor	Conc. (µM)	SLC transporter(s)	Literature K_i value (µM)	Substrate
Rifamycin SV	10	OATP1B1	2 [a]	no
Rifamycin SV	10	OATP1B3	3 [a]	no
Taurocholate	50 [b]	NTCP	not investigated	yes

[a] (Vavricka, et al 2002) [b] (Hagenbuch and Meier 1994)

Xenopus laevis oocyte uptake

Transport studies

Conditions used in these studies are acceptable.

Analysis of inhibition kinetics

The transport in the presence of a competitive and non-competitive inhibitor can be described as follows (Gao, et al 2000):

$$V_{0(+inhibitor)} = CL_m \cdot S + \frac{V_{max} \cdot S}{S + K_m \cdot (1 + I / K_i)}$$ Eq. 2

where K_i is the inhibitor constant or inhibition constant (µM) and I is the concentration of the inhibitor in the medium (µM).

IC_{50} calculations

All absolute transporter uptake data were converted into relative values (y) by defining uptake without addition of inhibitor (baseline) minus nonspecific (passive) uptake (background) as 100% and recalculating the other carrier-mediated uptake data relative to this number. The IC_{50} values (inhibitor concentration that causes 50% inhibition of the maximal drug effect) were calculated using the following equation (Rautio, et al 2006):

$$y = \frac{a \cdot I^n}{IC_{50}^n + I^n}$$ Eq. 3

where n is the slope factor (Hill coefficient) and a is the maximal transporter inhibition (%).

RESULTS

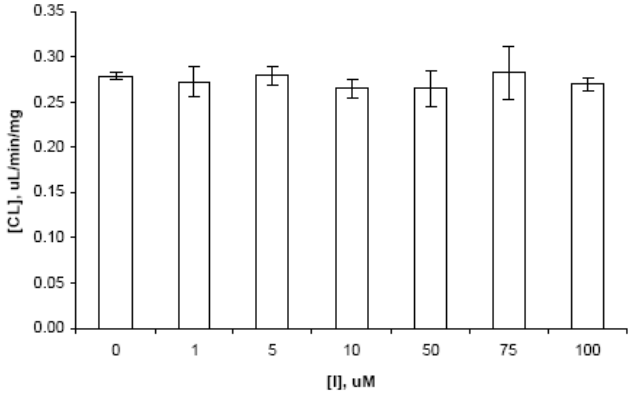
Based on the *in vitro* inhibition results of this study, FTY720 as well as AML629 are not expected to inhibit the uptake of co-medications and/or biologics transported by OATP1B1, OATP1B3 or NTCP.

OATP as well as NTCP probe substrates were incubated in the absence and presence of increasing amounts of FTY720 or AML629. The transporter activities without addition of test substances represent the control values (100% activity).

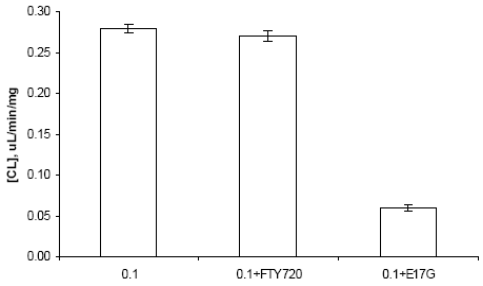
FTY720 as well as AML629 displayed no significant inhibition of OATP1B1, OATP1B3 and NTCP in the cellular assay up to concentrations of at least 100 µM.

Effect of FTY720 on probe substrate uptake by OATP1B1-transporter expressing HEK293 cells

Concentration Dependency



Uptake in the presence of positive control



Inhibition of OATP- as well as NTCP-mediated probe uptake by FTY720 or AML629 in vitro is summarized in the table below. FTY720 as well as AML629 displayed no significant inhibition of OATP1B1, OATP1B3 and NTCP in the cellular assay up to concentrations of at least 100 µM.

Summary of Results

OATP transporter	Probe substrate	IC_{50} value (µM) [a]	
		FTY720	AML629
OATP1B1	Estradiol 17β-D-glucuronide	not observed [b]	not observed [b]
OATP1B3	Estradiol 17β-D-glucuronide	not observed [b]	not observed [b]
NTCP	Taurocholate	not observed [c]	not observed [b]

[a] FTY720 or AML629 concentration estimated to inhibit probe substrate uptake by 50%
[b] Up to the highest concentration tested (100 µM)
[c] Up to the highest concentration tested (200 µM)

Conclusions

FTY720 and AML629 do not inhibit the uptake of OATP1B1, OATP1B3 or NTCP.

Study Title	Mechanistic transport studies across Caco-2 cell monolayers
Study number	DMPK-r982366
Objective	Determination of the predictive absorption and particularly the involvement of a non-passive diffusion system in the transport of FTY720 across the intestinal barrier. In addition, transporter identification and estimation of transporter affinity if FTY720 would be identified as a substrate for an active transporter system.
Test System	Human intestinal cell line Caco-2 grown on permeable filter support

Result summary and conclusions

Due to strong binding effects to the supporting filter device, no reliable conclusions can be drawn from this Caco-2 experiment.

4.1-2. IN VITRO STUDIES
4.1-2.2 Protein binding

Study Title	Ex vivo protein binding of [^{3}H]FTY720 in plasma from healthy volunteers and from patients with moderate hepatic insufficiency, before and after administration of 1 mg of FTY720.
Study number	DMPK-r002017
Objective	Investigate the ex vivo protein binding of [^{3}H]FTY720 in plasma from healthy volunteers and from patients with moderate hepatic insufficiency, before and after administration of 1 mg non-labeled FTY720.

METHODS
Plasma was collected from 32 volunteers enrolled in an open-label, single-dose, case-control study (study FTY720 0112). The subjects represented two groups: 16 subjects (1-16) with moderate hepatic impairment were matched according to gender, age, height and weight with volunteers with normal liver function (19-34). The subjects received a single oral 1 mg dose of non-labeled FTY720. Before and 24 h and 36 h after administration, 5 mL whole blood was collected into regular vacutainer tubes containing EDTA as anticoagulant. Plasma was prepared by centrifugation. The plasma samples were stored frozen at -20°C until analysis.

Plasma Protein Binding Method:

Plasma protein binding of figolimod was determined by ultracentrifugation method.

RESULTS

Bound fraction in plasma
• 99.83% (CV 15%) in hepatically impaired subjects (group 1, n=16), irrespective of the time point of the blood sampling (pre-dose or 24/36 h after peroral administration of 1 mg FTY720).
• 99.85% (CV 17%) in healthy subjects (group 2, n=16), irrespective of the time point of the blood sampling (pre-dose or 24/36 h after peroral administration of 1 mg FTY720).

CONCLUSIONS

The *ex vivo* binding of fingolimod to plasma proteins in healthy volunteers was 99.85% (CV 17%) and in patients with hepatic insufficiency 99.83% (CV 15%).

Study Title	In vitro plasma protein binding of [14C]FTY720 and its active metabolite [14C]AML629 to isolated human plasma proteins
Study number	DMPK-r0301139
Objective	The objectives of this study were to identify the plasma proteins which bind FTY720 and its pharmacologically active metabolite AML629.
Test System	Lipoprotein deficient human serum and solutions of isolated human plasma proteins (human serum albumin, α1-acid glycoprotein, γ-globulins, high density lipoprotein, low density lipoprotein and very low density lipoprotein) in PBS.

METHODS

Ultrafiltration ([^{14}C]AML629), Ultracentrifugation ([^{14}C]FTY720) and Equilibrium dialysis ([^{14}C]FTY720) were used to determine plasma protein binding.

RESULTS

Following table represents in vitro plasma protein binding of fingolimod and its active metabolite in lipoprotein deficient human serum.

In vitro plasma protein binding of [^{14}C]FTY720 and [^{14}C]AML629 in lipoprotein deficient human serum

Nominal concentration [ng/mL]	Actual concentration		Bound fraction [%]	Unbound fraction ± SD [%]
	Plasma [ng/mL]	Plasma water [ng/mL]		
		[^{14}C]FTY720		
5000	4677	16.8	99.6	0.4 ± 0.02
50	51	0.22	99.6	0.4 ± 0.1
		[^{14}C]AML629		
5000	4806	60.9	98.7	1.3 ± 0.06
500	492	5.9	98.8	1.2 ± 0.01
50	49	0.63	98.7	1.3 ± 0.07

Following tables represents in vitro binding of fingolimod and it active metabolite to isolated plasma proteins.

Binding of [^{14}C]FTY720 to isolated human plasma proteins

Plasma Protein/ concentration [mg/mL]	Nominal concentration [ng/mL]	Actual concentration		Unbound fraction ± SD [%]
		Plasma [ng/mL]	Plasma water [ng/mL]	
Serum albumin/ 40	5000	4696	19.8	0.4 ± 0.01
	50	51	0.26	0.5 ± 0.1
α_1-acid glycoprotein/ 1	5000	3402	341	10 ± 0.3
	50	24	4.0	17 ± 2
γ-globulins/ 12	5000	1813	457	25 ± 4
	50	22	8.5	39 ± 3
High density lipoprotein/ 3.6	5000	4378	27	0.6 ± 0.1
	50	44	0.20	0.5 ± 0.2
Low density lipoprotein/ 3.9	5000	4628	43	0.9 ± 0.1
	50	47	0.56	1.2 ± 0.1
Very low density lipoprotein*/ 1.3	5000	4414	257*	-
	50	47	6.6*	-

* no separation possible for this protein with the employed method

Binding of [^{14}C]AML629 to isolated human plasma proteins

Plasma Protein	Nominal concentration [ng/mL]	Actual concentration		Unbound fraction ± SD [%]
		Plasma [ng/mL]	Plasma water [ng/mL]	
Serum albumin	5000	4473	51	1.1 ± 0.1
	500	485	5.3	1.1 ± 0.04
	50	47	0.54	1.2 ± 0.1
α_1-acid glycoprotein	5000	3202	355	11 ± 1
	50	45	3.3	7 ± 1
γ-globulins	5000	4281	283	7 ± 2
	50	45	5.1	11 ± 1
High density lipoprotein	5000	4315	170	3.9 ± 0.9
	50	38	17	44 ± 4
	5000	4314	170	3.9 ± 0.2
	500	445	23	5.1 ± 0.2
	50	46	11	25 ± 7
Low density lipoprotein	5000	4109	218	5.3 ± 0.2
	500	435	32	7 ± 1
	50	44	17	39 ± 4
Very low density lipoprotein	5000	4375	281	6.4 ± 0.3
	500	460	33	7 ± 0.2
	50	46	23	49 ± 9

CONCLUSIONS

Plasma protein binding of [^{14}C]FTY720

- [^{14}C]FTY720 was highly bound to serum albumin as well as the three tested lipoproteins (high density lipoprotein, low density lipoprotein and very low density lipoprotein).
- High protein binding of [^{14}C]FTY720 in human plasma is mainly to its binding to serum albumin as well as to the different lipoproteins.

Plasma protein binding of [^{14}C]AML629

- High binding of [^{14}C]AML629 was seen in lipoprotein deficient human plasma and in a solution of human serum albumin; a high to moderate binding was seen for all other plasma proteins employed in this study (α1-acid glycoprotein, γ-globulins, high density lipoprotein, low density lipoprotein and very low density lipoprotein).
- High protein binding of [^{14}C]AML629 in human plasma is mainly to its binding to serum albumin, but a fraction is also bound to other plasma proteins mainly to lipoproteins.

Study Title	Protein binding of [^{3}H]FTY720 and its active metabolite [^{14}C]AML629 in plasma samples from subjects with severe hepatic impairment and in healthy control subjects
Study number	DMPK-r00400235
Objective	To investigate the protein binding of [3H]FTY720 and its active metabolite [14C]AML629 in plasma from healthy volunteers and patients with severe hepatic impairment, before and after administration of 5 mg non-labeled FTY720.
Test System	Plasma samples from clinical study FTY720A2204

METHODS

Test system

Plasma was collected from 12 volunteers enrolled in an open-label, single-dose, parallel-group study (study FTY720A2204). The subjects represented two groups: 6 subjects with severe hepatic impairment (5101-5106) were matched by gender, age, body height and lean body mass (height and frame size) with volunteers with normal liver function (5107-5112). The subjects received a single oral 5 mg dose of non-labeled FTY720. Before and 24 and 36 h after administration, 2.7 mL whole blood were collected into heparin tubes. Plasma was prepared by centrifugation. The plasma samples were stored frozen at 20°C until analysis.

Ultracentrifugation and ultracentrifugation were used to determine plasma protein binding.

The radioactivity in the biological samples was measured by liquid scintillation counting (LSC).

RESULTS

Plasma protein binding of [^{3}H]FTY720

Results on the plasma protein binding of [^{3}H]FTY720 in samples from subjects with severe hepatic impairment and healthy control subjects are summarized in the table below.

Subject No.	Pre-dose		24 h post-dose		36 h post-dose		Mean Unbound fraction
	Total concentration [ng/mL]	Unbound fraction ± SD [%]	Total concentration [ng/mL]	Unbound fraction ± SD [%]	Total concentration [ng/mL]	Unbound fraction ± SD [%]	
5101	1.54	0.18 ± 0.04	1.38	0.36 ± 0.19	1.57	0.61 ± 0.26	0.38 ± 0.22
5102	1.45	0.19 ± 0.05	1.36	0.21 ± 0.08	1.71	0.25 ± 0.05	0.22 ± 0.03
5103	1.61	0.52 ± 0.16	1.52	0.81 ± 0.20	1.91	0.35 ± 0.05	0.56 ± 0.23
5104	1.29	0.38 ± 0.13	1.45	0.39 ± 0.10	1.64	0.15 ± 0.05	0.31 ± 0.13
5105	1.58	1.38 ± 0.36	1.83	0.28 ± 0.10	1.77	0.58 ± 0.25	0.75 ± 0.57
5106	1.58	0.12 ± 0.01	1.61	0.18 ± 0.03	1.66	0.13 ± 0.02	0.14 ± 0.03
5107	1.45	0.14 ± 0.03	1.74	0.46 ± 0.30	1.89	0.46 ± 0.17	0.35 ± 0.18
5108	1.67	0.24 ± 0.09	1.77	0.29 ± 0.04	1.79	0.21 ± 0.04	0.25 ± 0.04
5109	ND	ND	1.51	0.42 ± 0.16	1.72	0.20 ± 0.05	0.31 ± 0.16
5110	1.73	0.18 ± 0.04	1.56	0.44 ± 0.16	1.82	0.15 ± 0.04	0.28 ± 0.14
5111	1.97	0.24 ± 0.03	1.75	0.41 ± 0.07	ND	ND	0.32 ± 0.12
5112	1.71	0.40 ± 0.08	ND	ND	ND	ND	0.40
Overall Mean ± SD of subject with severe hepatic impairment (5101 – 5106)							0.39 ± 0.31
Overall Mean ± SD of subject with severe hepatic impairment (5101 – 5106) pre-dose							0.46 ± 0.47
Overall Mean ± SD of subject with severe hepatic impairment (5101 – 5106) post-dose							0.36 ± 0.21
Overall Mean ± SD of volunteers with normal liver function (5107-5112)							0.30 ± 0.12
Overall Mean ± SD of volunteers with normal liver function (5107-5112) pre-dose							0.24 ± 0.10
Overall Mean ± SD of volunteers with normal liver function (5107-5112) post-dose							0.34 ± 0.13

Plasma protein binding of [^{14}C]AML629

Subject No.	24 h post-dose		36 h post-dose		Mean Unbound fraction ± SD [%]
	Total concentration [ng/mL]	Unbound fraction ± SD [%]	Total concentration [ng/mL]	Unbound fraction ± SD [%]	
5101	521	1.4 ± 0.1	519	0.9 ± 0.2	1.2
5102	506	1.4 ± 0.3	533	0.9 ± 0.1	1.1
5103	504	1.3 ± 0.2	558	0.9 ± 0.2	1.1
5104	528	1.4 ± 0.2	512	0.8 ± 0.1	1.1
5105	517	1.4 ± 0.1	517	1.0 ± 0.05	1.2
5107	526	1.0 ± 0.3	531	0.6 ± 0.005	0.8
Overall Mean ± SD of subjects with severe hepatic impairment (5101 – 5105)					1.14 ± 0.25

CONCLUSIONS

FTY720

The unbound fractions of [^{3}H]FTY720 in plasma of subjects with severe hepatic impairment were similar to healthy subjects. Based on total radioactivity measurements the unbound fraction at 2 ng/mL [^{3}H]FTY720 was 0.4 ± 0.3% for the six severely hepatic impaired patients and 0.3 ± 0.1% in the six control subjects. For both groups no significant difference was seen between pre-dose and post-dose means of the unbound fractions.

AML629

The unbound fractions in plasma of subjects with severe hepatic impairment were similar to healthy subjects. Based on total radioactivity measurements the unbound fraction at 500 ng/mL [^{14}C]AML629 was in the range of 0.8- 1.4 % in five severely hepatic impaired patients.

Study Title	Protein binding of [^{14}C]FTY720 and its active metabolite [$^{3}H_4$]AML629 in plasma samples from subjects with renal impairment and in healthy control subjects
Study number	DMPK-r0800403
Objective	To investigate the protein binding of [14C]FTY720 and its active metabolite [$^{3}H_4$]AML629 in plasma from subjects with renal impairment and from healthy control subjects after single oral administration of 1.25 mg non-labeled FTY720.
Test System	Plasma samples from clinical study CFTY720D2108

METHODS

Plasma protein binding was determined by equilibrium gel filtration

RESULTS

Fraction unbound (%, mean±SD) of [^{14}C]FTY720 and [$^{3}H_4$]AML629 in plasma of subjects with renal impairment and in healthy control subjects.

Test compound	Subjects with renal impairment (n=9)	Healthy control subjects (n=9)	P-value[a)]
[^{14}C]FTY720	0.098 ± 0.015	0.106 ± 0.014	0.25
[$^{3}H_4$]AML629	0.532 ± 0.059	0.494 ± 0.046	0.15

a: determined by the two-tailed t-test for equal variances

Protein binding of [^{14}C]FTY720 and [$^{3}H_4$]AML629 in plasma of subjects with renal impairment

Subject	[^{14}C]FTY720		[$^{3}H_4$]AML629	
	Mean	SD	Mean	SD
5101	0.105	0.010	0.607	0.010
5102	0.072	0.005	0.463	0.022
5103	0.095	0.003	0.477	0.113
5104	0.106	0.004	0.526	0.108
5105	0.117	0.003	0.570	0.116
5106	0.080	0.001	0.471	0.109
5107	0.105	0.001	0.614	0.005
5108	0.092	0.001	0.567	0.024
5109	0.111	0.003	0.497	0.111
Mean	0.098		0.532	
SD	0.015		0.059	

Protein binding of [^{14}C]FTY720 and [$^{3}H_4$]AML629 in plasma of healthy

Subjects

Subject	[^{14}C]FTY720		[$^{3}H_4$]AML629	
	Mean	SD	Mean	SD
7101	0.123	0.003	0.568	0.011
7102	0.088	0.004	0.472	0.076
7103	0.102	0.008	0.489	0.096
7104	0.121	0.008	0.483	0.092
7105	0.107	0.001	0.563	0.008
7106	0.084	0.003	0.425	0.097
7107	0.120	0.006	0.474	0.091
7108	0.113	0.009	0.511	0.094
7109	0.100	0.005	0.465	0.046
Mean	0.106		0.494	
SD	0.014		0.046	

CONCLUSIONS

- Both [^{14}C]FTY720 and [$^{3}H_4$]AML629 were highly bound to plasma proteins.
- No significant difference in the fraction unbound was observed between subjects with renal impairment and in healthy control subjects.
- The fractions unbound in plasma for both test compounds were in line with values determined earlier by ultracentrifugation or by ultrafiltration.

4.1-3. HUMAN PK STUDIES
4.1-3.1 Healthy subject PK

Study FTY720A 2215*:* **A randomized, single-blind, placebo-controlled, timelagged, ascending, single oral dose, pharmacokinetic, safety and tolerability study of FTY720 in healthy volunteers**

Dose proportionality SD 5-40 mg (PK and PD: lymphocyte, heart rate and pulmonary)

A brief overview of some essential components of the study design is given below:

Study Design	Single-dose, ascending, randomized, single-blind, placebo-controlled
Study Population	N=56 enrolled, 56 completed (42 active, 14 placebo) Age: 18-45 years (mean 28.5 years) Gender: 35 males (62.5%), 21 females (37.5%) Weight: 73.2-101.6 kg pounds (mean 73.9 kg) Race: 37 White(66%),1 Black(1.8%),1 Asian(1.8 %) and 17 Other(30%)
Dosage and Administration	7 cohorts (n=8/cohort), the maximum proposed dose changed from 80 mg to 40 mg due to the decreases in pulmonary function tests and reports of chest tightness and discomfort. Cohort 1: FTY720 5 mg capsule (n=6) or placebo (n=2) Cohort 2-3: FTY720 7.5 mg capsule (n=6) or placebo (n=2) Cohort 4: FTY720 10 mg capsule (n=6) or placebo (n=2) Cohort 5: FTY720 15 mg capsule (n=6) or placebo (n=2) Cohort 6: FTY720 25 mg capsule (n=6) or placebo (n=2) Cohort 7: FTY720 40 mg capsule (n=6) or placebo (n=2) Study drugs were given under fasted state. Batch No. FYT720 2.5 mg capsules (FMI): 1-137US, KN3752938.00.002 Placebo capsules: H-05666, KN3755030.00.016 Diet: Subjects fasted for approximately 10 hours before dosing and 4 hours after dose. All subjects drank at least 240 mL of water for dosing. Alcohol was prohibited for 72 hours prior to dosing until study completion. Intake of xanthine-containing foods or beverages was permitted and had to be recorded.
PK Sampling: Blood	At predose (0 hour), and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 240, 576 and 912 hours postdose. The samples were analyzed for blood concentrations of FTY720 and FTY720-P.
PD Sampling: Blood	**Lymphocyte counts:** at screening, day -2, predose (0 hour), and 1, 2, 3, 4, 5, 6, 12, 24, 48, 72, 96 hours and in the morning of days 7, 11, 25 and 39. **Heart rate:** heart rate data was collected continuously over each 24-hour period. Mean hourly heart rate was derived. **Pulmonary function:** pulmonary function testing was performed on days -1, 1, 2 and 4.

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 15.4% Inter-day accuracy: < 7.9 % FTY720-P: Linear range : 0.987-329 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 15.1% Inter-day accuracy: < 3.6 %
PK Assessment	FTY720 and FTY720-P in blood: Tlag, Cmax,b, Tmax, Cap,b, Tap, Tdur, T ½, AUC, AUCinf, CL,b/F and Vz,b
PD Assessment	ECG recordings, 12-lead digital Holter monitoring, continuous telemetry monitoring, exercise oximetry testing, pulmonary function testing, and absolute lymphocyte counts. Descriptive response parameters were derived including predose values, nadir values, and area under the effect vs time curve. Predose, Nadir, Tnadir and AUE
Safety Assessment	Physical examinations, vital signs, ECGs, clinical laboratory parameters (hematology, biochemistry, urinalysis), and adverse events monitoring.

Pharmacokinetic Results:

FTY720 pharmacokinetics in blood:

Mean FTY720 PK parameters after 5 mg to 40 mg FTY720 single dose administration are shown in the following table:

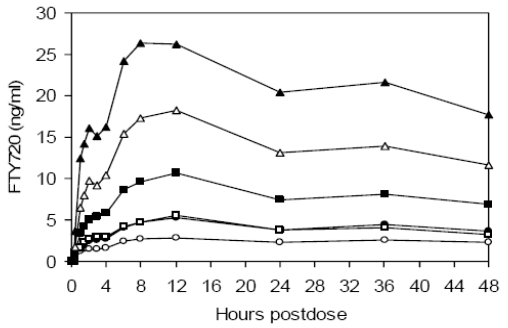
Table 7-6 FTY720 pharmacokinetic parameters

Parameter	5mg	7.5mg	10mg	15mg	25mg	40mg
N	6	12	5	6	6	6
Peak exposure:						
t_{max} (h)	12 (8-36)	12 (8-36)	12 (12-12)	12 (8-12)	12 (8-12)	12 (8-12)
$C_{max,b}$ (ng/ml)	3.0 ± 0.3	5.3 ± 0.9	5.6 ± 1.2	11.0 ± 2.3	18.4 ± 3.5	26.9 ± 6.5
$C_{max,b}$/Dose (ng/ml)	0.59 ± 0.06	0.71 ± 0.12	0.56 ± 0.12	0.73 ± 0.15	0.74 ± 0.14	0.67 ± 0.16
t_{ap} (h)	18 (8-30)	12 (8-36)	10 (8-12)	10 (8-24)	9 (8-30)	10 (8-10)
$C_{ap,b}$ (ng/ml)	2.7 ± 0.3	4.9 ± 0.9	5.2 ± 1.4	10.2 ± 2.0	17.1 ± 3.4	25.1 ± 6.2
t_{dur} (h)	33 (6-66)	28 (4-42)	4 (0-28)	5 (4-24)	6 (4-64)	24 (6-30)
Total exposure:						
AUC(0-t_z)$_b$ (ng.h/ml)	624 ± 235	1088 ± 390	928 ± 90	2193 ± 721	3899 ± 468	5445 ± 2553
AUC_b (ng.h/ml)	662 ± 240	1136 ± 429	972 ± 107	2319 ± 801	4084 ± 497	5753 ± 2874
AUC_b/Dose (ng.h/ml)	132 ± 48	152 ± 57	97 ± 11	155 ± 53	163 ± 20	144 ± 72
CL_b/F (L/h)	8.5 ± 3.4	7.4 ± 2.4	10.4 ± 1.2	7.6 ± 4.1	6.2 ± 0.7	8.6 ± 4.1
$V_{z,b}$/F (L)	1797 ± 376	1609 ± 309	2944 ± 563	1943 ± 233	1863 ± 314	2206 ± 594
$t_{1/2}$ (days)	6.7 ± 2.5	6.8 ± 2.1	8.4 ± 2.3	8.4 ± 2.4	8.7 ± 1.0	8.3 ± 2.2

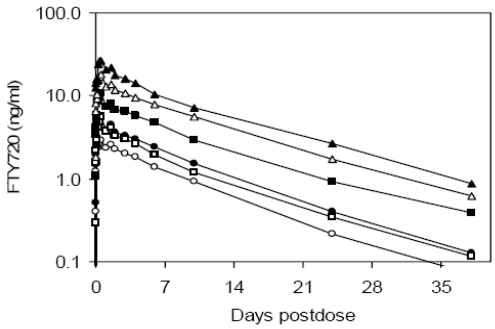
Values are mean ± sd except for times which are median (range).
Data source: Appendix 4, Table 1 and Table 2.

Mean FTY720 blood concentration-time plots after 5 mg to 40 mg FTY720 single dose administration are shown in the following figure:

Figure 7-1 FTY720 concentration profiles



Mean FTY720 profiles on a linear concentration scale out to 48 hours postdose: 5mg (*open circles*), 7.5mg (*filled circles*), 10mg (*open squares*), 15mg (*filled squares*), 25mg (*open triangles*), 40mg (*filled triangles*).



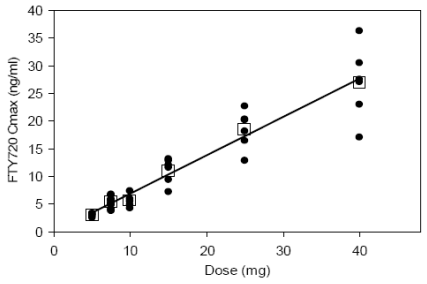
As in left panel on a log concentration scale over the full blood sampling period of 39 days postdose. Individual and group plots are in Appendix 4, Figure 1 and Figure 2.

- An absorption shoulder was observed at ~ 2 hours postdose as a first peak.
- Major Tmax reached generally at 12 hours postdose by a broad absorption phase.
- The Cmax,b was embedded in a broad plateau region between 8 to 36 hours postdose. The concept of the plateau region was to quantify the apical concentration (Cap,b), that is, the mean of all concentrations within 20% of Cmax,b.
- Cmax,b/dose exhibited moderate intersubject variability with a coefficient of 20%.
- The apical duration (Tdur) averaged around 30 hours at the lower doses while at higher doses, the peak region tended to be sharper as manifested by shorter apical durations of around 4 hours.
- Mean blood concentrations declined in parallel at all dose levels.
- CLb/F was similar across dose levels (averaged 8.0 ± 3.0 L/h).
- Vz,b/F was similar among dose levels except at the 10 mg for which the volume was significantly larger than at doses above and below. Since there was no consistent indication for dose-dependency, Vz,b/F was pooled across all subjects yielding an average value of 1973 ± 558 L.
- T1/2 was similar across all dose levels and averaged 7.7 ± 2.2 days.

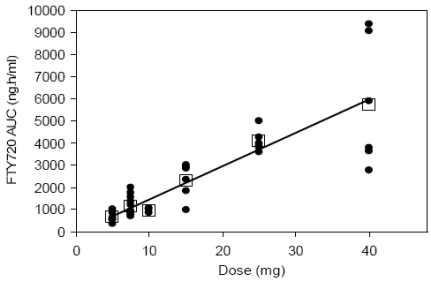
<u>Dose Proportionality of FTY720</u>

The relationship of doses of FTY720 versus FTY720 Cmax and AUC are shown in the figures below:

Figure 7-2 FTY720 dose-proportionality: 5 – 40 mg



FTY720 Dose-Cmax relationship. Shown are the individual values (*filled circles*), group means (*open squares*), and the linear regression line: Cmax = 0.69 x Dose – 0.05 (r^2 = 0.889).



As in left panel for FTY720 Dose-AUC relationship. Regression line: AUC = 150 x Dose – 57 (r^2 = 0.719).

- Cmax,b increased in a dose-proportional manner over the dose range of 5 mg to 40 mg and this is confirmed by the power model dose-Cmax slope of 1.04 (90% CI, 0.96-1.12).
- Similarly to AUCb with dose-AUC slope of 1.05 (90% CI, 0.91-1.19).

FTY720-P exposure in blood:

Mean FTY720-P PK parameters after 5 mg to 40 mg FTY720 single dose administration are shown in the following table:

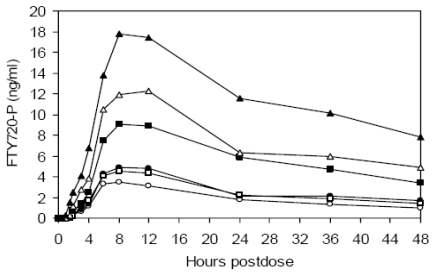
Table 7-7 FTY720-phosphate pharmacokinetic parameters

Parameter	5mg	7.5mg	10mg	15mg	25mg	40mg
N	6	12	5	6	6	6
Peak exposure:						
t_{lag} (h)	2 (1.5-4)	2 (1.5-4)	2 (1.5-2)	1.8 (1.5-3)	1.5 (1-2)	1 (0.5-2)
t_{max} (h)	8 (6-8)	10 (6-12)	8 (6-12)	8 (8-24)	10 (6-12)	10 (8-12)
$C_{max,b}$ (ng/ml)	3.9 ± 0.9	5.3 ± 1.2	5.7 ± 1.8	9.8 ± 1.5	12.8 ± 3.9	18.3 ± 4.8
$C_{max,b}$/Dose (ng/ml)	0.77 ± 0.18	0.71 ± 0.16	0.57 ± 0.18	0.65 ± 0.10	0.51 ± 0.16	0.46 ± 0.12
Total exposure:						
AUC(0-t_z)$_b$ (ng.h/ml)	115 ± 59	231 ± 105	226 ± 51	666 ± 192	1101 ± 539	2311 ± 1367
AUC(0-t_z)$_b$/Dose (ng.h/ml)	23 ± 12	31 ± 14	23 ± 5	44 ± 13	44 ± 22	58 ± 34
AUC_b (ng.h/ml)	--	--	--	1012 ± 387	1719 ± 617	2871 ± 1573
AUC_b/Dose (ng.h/ml)	--	--	--	67 ± 26	69 ± 25	72 ± 39
$t_{1/2}$ (days)	--	--	--	6.3 ± 2.0	8.7 ± 3.3	8.6 ± 3.8

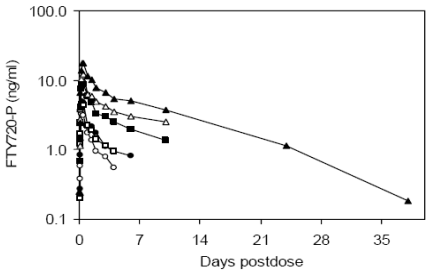
Values are mean ± sd except for times which are median (range).
Data source: Appendix 4 Table 1 and Table 3.

Mean FTY720-P blood concentration-time plot after the 5 mg to 40 mg FTY720 single dose administration is shown in the following figure:

Figure 7-3 FTY720-phosphate concentration profiles



Mean FTY720-phosphate profiles on a linear concentration scale to 48 hours postdose: 5mg (*open circles*), 7.5mg (*filled circles*), 10mg (*open squares*), 15mg (*filled squares*), 25mg (*open triangles*), 40mg (*filled triangles*).



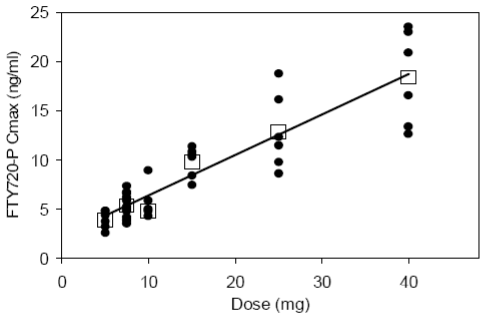
As in left panel on a log concentration scale over the full blood sampling period of 39 days postdose. Individual and group plots are in Appendix 4 Figure 1 and Figure 2.

- FTY720-P became quantifiable in blood by 2 to 3 hours postdose which lead to a lag time for absorption.
- Tmax occurred at 8 hours postdose (6-24 h).
- AUC of FTY720-P can not be properly characterized at low doses of 5 mg to 10 mg due to the low blood concentrations and the assay quantification limit.
- At the higher doses of 15 mg to 40 mg, blood concentrations were quantifiable between 11 to 39 days postdose thereby allowing an adequate half-life estimate and AUC extrapolation.
- T1/2 across dose levels of 15 mg to 40 mg averaged 7.9 ± 3.1 days.

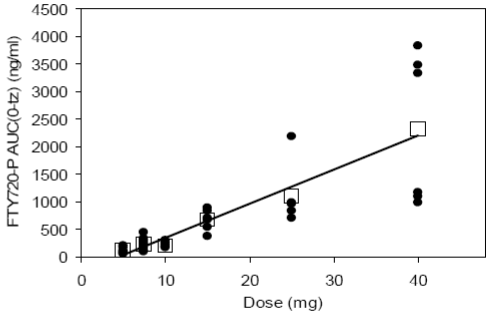
Dose Proportionality of FTY720-P

The relationship of doses of FTY720 versus FTY720-P Cmax and AUC are shown in the figures below:

Figure 7-4 FTY720-phosphate dose-proportionality: 5 – 40 mg



FTY720-phosphate Dose-Cmax relationship. Shown are the individual values (*filled circles*), group means (*open squares*), and the linear regression line: Cmax = 0.41 x Dose + 2.29 (r^2 = 0.794).



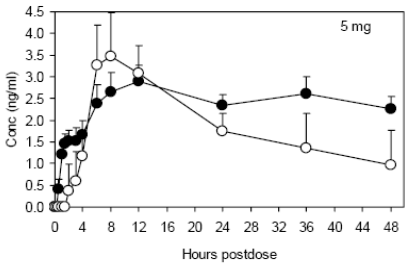
As in left panel for FTY720-phosphate Dose-AUC(0-tz) relationship. Regression line: AUC = 62 x Dose – 274 (r^2 = 0.657).

- The increase in Cmax,b with dose was underproportional over the full range of 5 mg to 40 mg and this is confirmed by the power model dose-Cmax slope which was significantly less than unity: 0.75 (90% CI, 0.64-0.86).
- Since AUC(0-tz)b was truncated at 2-6 days at the lower doses (5 mg to 10 mg) but adequately characterized to 25-39 days at the higher doses (15 mg to 40 mg), the dose-AUC(0-tz)b relationship was overproportional with a slope of 1.38 (90%CI, 1.17 – 1.58).
- When restricting the evaluation to 15 mg to 40 mg, AUC(0-tz)b remained slightly overproportional [1.13 (0.65-1.61)] but upon extrapolation, AUCb was consistent with dose-proportionality [0.98 (0.52 – 1.45)].

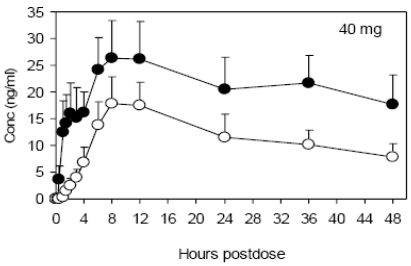
FTY720 and FTY720-P relationship

The early portion of the FTY720 and FTY720-P mean concentration profiles are shown below at the lowest (5 mg) and highest (40 mg) doses.

Figure 7-5 Comparative plots of FTY720 and FTY720-phosphate peaks



Mean concentration profiles of FTY720 (*filled circles*) and FTY720-phosphate (*open circles*) to 48 hours postdose at 5mg.



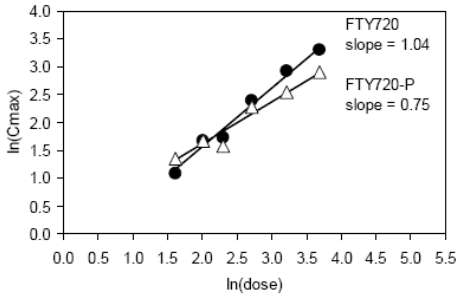
As in the left panel for 40mg.

- Tmax of FTY720-P generally occurred earlier (8 hours) than that of FTY720 (12 hours) at all dose levels.

- FTY720-P Cmax,b exceeded that of FTY720 at 5 mg; similar to that of FTY720 at 7.5 mg and 10 mg; and became progressively less than FTY720 with rising doses from 15 mg to 40 mg.
- The mean concentration profiles of these two analytes declined in parallel after 24 hours postdose, with FTY720-P always at lower concentrations compared with FTY720.

The dose-dependency of the relationship between FTY720 and FTY720-P in the peak region over this dose range is captured in the dose-Cmax regressions shown below:

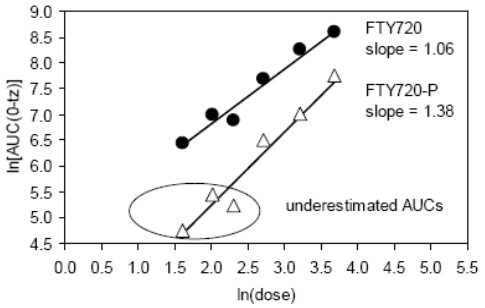
Figure 7-6 Dose-Cmax regressions for FTY720 and FTY720-phosphate



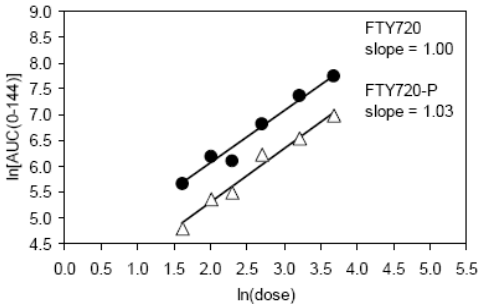
Dose-Cmax regression for FTY720 (*filled circles*) and FTY720-phosphate (*open triangles*).

- FTY720-P Cmax,b is greater than that of FTY720 at doses <7.5 mg; whereas, the FTY720 Cmax,b is greater than that of FTY720-phosphate at doses >10 mg.

Figure 7-7 Dose-AUC regressions for FTY720 and FTY720-phosphate



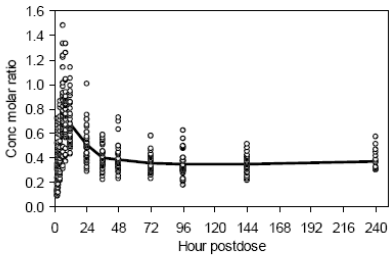
Dose-AUC(0-tz) regression for FTY720 (*filled circles*) and FTY720-phosphate (*open triangles*).



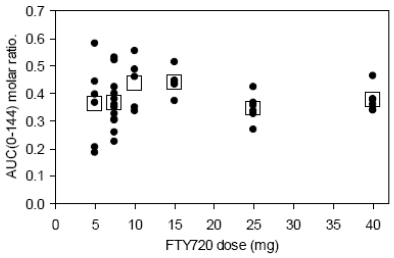
As in left panel for dose-AUC(0-144) regressions.

- The FTY720-P dose-AUC(0-tz)b regression with a slope of 1.38 (1.17 – 1.58) did not run parallel with the regression of FTY720. Based on the sponsor, this was likely due to underestimation of the area at the lower doses given the higher assay quantification limit for FTY720-P.
- When the AUC was truncated at a common end timepoint (144 hours) up to which most concentration-time profiles were measurable at all dose levels, the regression slopes for FTY720 and FTY720-P ran in parallel and both were consistent with dose proportionality: FTY720 slope 1.00 (0.92 – 1.09), FTY720-phosphate slope 1.03 (0.90 – 1.16).

Figure 7-8 FTY720-phosphate/FTY720 molar ratios with respect to time and dose



FTY720-phosphate/FTY720 concentration molar ratios with respect to time postdose pooled across all dose levels.



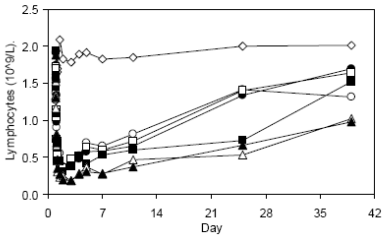
FTY720-phosphate/FTY720 AUC(0-144) molar ratios with respect to FTY720 dose. Shown are the individual ratios (*filled circles*) and group means (*open squares*).

- FTY720-P is generally not quantifiable before 3 hours postdose.
- Between 3 and 4 hours FTY720-P becomes quantifiable and is lower than FTY720 yielding a mean molar ratio of 0.39.
- Between 6 and 12 hours peak concentrations of both analytes are reached and the highest molar ratios are attained averaging 0.74.
- By 36 hours postdose and thereafter, the ratio stabilizes at 0.38.
- The FTY720-P/FTY720 AUC(0-144)b molar ratios were relatively dose independent over the range 5 mg to 40 mg and averaged 0.38 (n = 41 subjects).

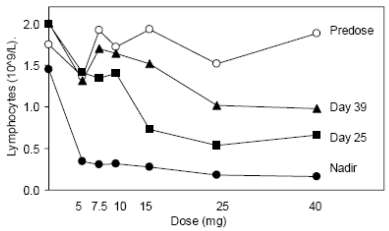
Pharmacodynamics results:

Lymphocyte response: Dose-response relationships

Figure 7-9 Lymphocyte dose-response plots



Lymphocyte trajectories after placebo (*open diamonds*) and FTY720 doses of 5mg (*open circles*), 7.5mg (*filled circles*), 10mg (*open squares*), 15mg (*filled squares*), 25mg (*open triangles*), 40mg (*filled triangles*).



Mean predose, nadir, day 11, and day 39 lymphocyte counts with respect to FTY720 dose. Individual trajectory plots are in Appendix 4 Figure 3.

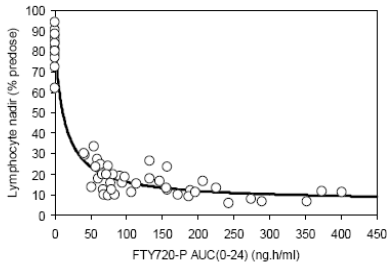
- Lymphocytes counts exhibited slight fluctuations averaging ±16% over the 39-day period in the placebo group based on the nadir.
- All FTY720 dose cohorts had a notable decrease in lymphocyte count and reaching the absolute nadir by 24 hours postdose.
- A dose-dependency was evident ranging from 26% at 5 mg to 9% at 40 mg when the nadir was expressed as a percent of the predose count. (decrease from predose ranging from 74% to 91%).
- On day 11, lymphocyte counts were recovered to 61% of predose at 5 mg, around 35% of predose at doses of 7.5 mg to 25 mg, and 20% of predose at 40 mg.
- At the end-of-study visit on day 39, lymphocyte counts had fully recovered for doses of 5 mg to 10 mg and were returning to predose counts for doses of 15 mg (79%), 25 mg (68%), and 40 mg (58%) showing the dose-response relationships.
- The lymphocyte area-under-the-effect curve, AUE(1-39) showed that at the lower end of the dose range (5 mg to 10 mg) AUE(1-39) were similar while at the upper end of

the dose range (15 mg to 40 mg), a clearer dose-response relationship in the mean values was evident.

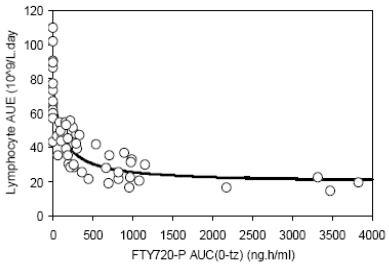
Descriptive exposure-response relationships

An inhibitory effect Emax model was utilized to relate FTY720-P AUC as exposure versus the lymphocyte nadir and AUE as responses.

Figure 7-10 Lymphocyte exposure-response plots



Inhibitory effect Emax model of the relationship between FTY720-phosphate AUC(0-24) and the lymphocyte nadir expressed as percent of predose count. Details on the model fit are in Appendix 4 Figure 4.



As in left panel for the relationship between overall exposure vs overall response parameterized as FTY720-phosphate AUC(0-tz) vs the lymphocyte AUE(1-39).

	Estimate	%CV
A		
E0 (10^9/L)	1.5	3.8
EC50 (ng.h/ml)	9.8	62.8
Emax (10^9/L)	0.16	50.1
B		
E0 (% predose)	84.1	2.3
EC50 (ng.h/ml)	13.9	29.5
Emax (% predose)	6.8	42.9
C		
E0 (10^9/L.day)	74	4.3
EC50 (ng.h/ml)	125	34.7
Emax (10^9/L.day)	19	22.9

- E0 was estimated to be 84% of the predose value.
- EC50 was estimated to be 14 ng.h/ml.
- Emax was estimated to be 7% of the predose count (93% reduction from predose). These exposure-response parameters are in agreement with the dose-response data shown above.
- Similarly, the overall effect on lymphocyte AUE(1-39) was related to the overall FTY720- P exposure AUC(0-tz)b as shown in the right panel of the figure above.

An indirect-response model was used to describe a mechanistic relationship between total lymphocyte counts in blood and FTY720-phosphate blood concentrations.

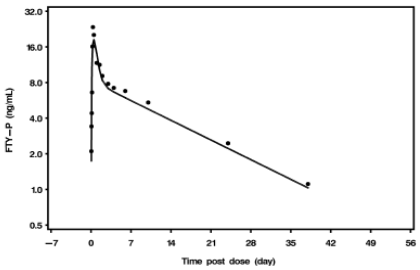
Table 7-9 Indirect-response model parameters

Parameter	Estimate	Standard error (%CV)	Intersubject (%CV)
Pharmacokinetics:			
t_{lag} (h)	1.590	7.3	30.8
k_a (h^{-1})	0.121	5.3	5.6
α	0.208	20.0	-
$V_{c,b}$ (L)	640.0	8.3	15.6
k_{12} (h^{-1})	0.088	6.3	23.7
k_{21} (h^{-1})	0.023	6.8	-
k_e (h^{-1})	0.016	8.4	27.7
Lymphocyte responses:			
k_{out} (h^{-1})	0.587	7.3	-
k_{in} (10^9/L/h)	1.020	8.1	19.7
IC_{50} (ng/ml)	0.455	11.9	33.5
I_{max}	0.903	1.9	-

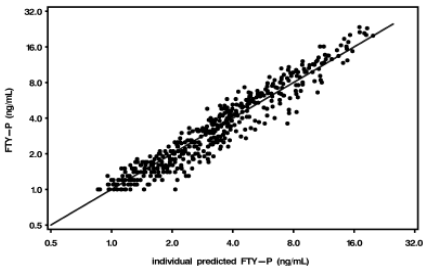
- The population pharmacokinetics of FTY720-P was adequately described by a two-compartment model.
- The population means of the parameters were estimated with standard error CVs <20%.
- The intersubject variation was moderate with CVs ≤30%.
- The parameter α, related to the relative bioavailability by F = 1 – [α × log(Dose/5 mg)], indicated a 20% underproportionality in FTY720-P blood concentrations over the range achieved in this study. This is in agreement with the 25% under-proportionality observed in FTY720-P Cmax,b from the noncompartmental pharmacokinetic evaluation described previously.
- CL/F of FTY720-P calculated as Vc,b × ke was 10.4 L/h. The CV of the residual error was 23%.
- For lymphocyte response, the population means of the parameters were estimated with standard error CVs of 2% to 12%.
- The steady-state baseline value of lymphocyte counts in the absence of FTY720-P exposure (*R0*) was 1.74 × 10^9/L as calculated from the quotient *kin*/*kout*.
- The FTY720-P blood concentration elicited a *IC50* of 0.46 ng/ml (1.2 nM).
- The maximum inhibitory effect was 90.3% which is in agreement with the mean 91% reduction from predose in lymphocyte counts observed in the 40 mg dose group described previously.

The fitting for a representative subject and for all observed versus predicted FTY720-P concentrations were shown below:

Figure 7-11 Indirect-response diagnostic plots: pharmacokinetics



FTY720-phosphate concentration-time profile for subject 5153 receiving 40mg FTY720. Shown are the measured concentrations (*points*) and model-fitted curve (*line*). Individual plots for all subjects are in Appendix 4 Figure 5.



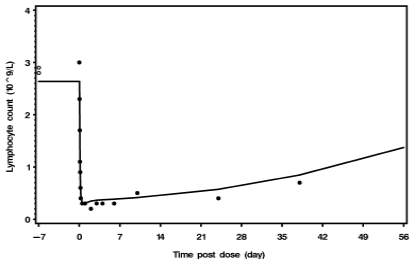
Individual model-predicted versus measured FTY720-phosphate blood concentrations across all subjects. Shown is the identity line.

Reviewer's note:

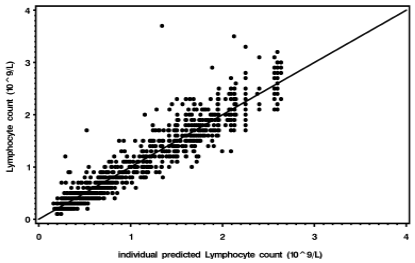
Although the fitting (left) tend to miss the peak concentrations in quite a few subjects which is also evident from the predicted vs observed plot at the very high concentrations (right), the overall observed and predicted concentrations show good correlation.

A representative lymphocyte trajectory and the measured versus model-predicted lymphocyte counts across all subjects are shown below:

Figure 7-12 Indirect-response diagnostic plots: lymphocytes



Blood lymphocyte trajectory for subject 5153 receiving 40mg FTY720. Shown are the measured lymphocyte counts (*points*) and model-fitted trajectory (*line*). Individual plots for all subjects are in Appendix 4 Figure 5.



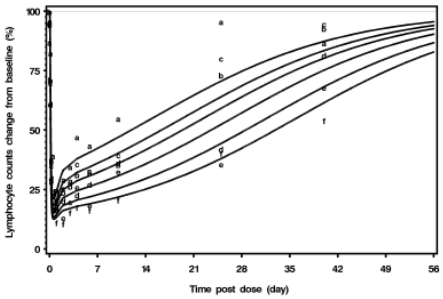
Individual model-predicted versus measured blood lymphocyte counts across all subjects. Shown is the identity line.

- The model seems to describe the blood lymphocyte count trajectory as a function of FTY720-P blood concentrations.

Reviewer's note:
Although the diagnostic plot looks OK, the individual fittings tend to miss the baseline and the recovery curve. While the observed data were well described, the model is suggested to use only for the supportive purpose.

For a typical subject with pharmacokinetic and response parameters equal to the population estimates, the expected time course of lymphocyte counts relative to baseline is shown in the figure below for single FTY720 doses of 5, 7.5, 10, 15, 25, and 40 mg.

Figure 7-13 Model-predicted lymphocyte trajectories



Model-predicted lymphocyte trajectories for escalating single FTY720 doses. Overlaid are average counts relative to baseline for 5 mg (*a*), 7.5 mg (*b*), 10 mg (*c*), 15 mg (*d*), 25 mg (*e*), and 40 mg (*f*).

Conclusions for lymphocyte counts:

- This study confirmed previous finding that the maximum effect of FTY720 on blood lymphocyte counts is 80 to 90 percent reduction from predose using doses up to 5 mg, by direct measurements with a dose escalation up to 40 mg at which the lymphocyte reduction from baseline averaged 91 percent.
- The physiologic exposure-response model indicated that low FTY720-P blood levels of 0.46 ng/ml are associated with a half-maximal lymphocyte response. This IC50 concentration is

below the assay quantification limit (1 ng/ml), but based on the observed FTY720-P concentrations, it is generally exceeded within the first 2 hours after FTY720 single doses of 5 mg to 40 mg. This explains the rapid drop in lymphocyte counts in blood which is evident within hours after these doses.

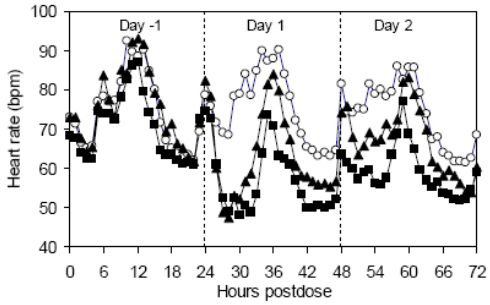
- T1/2 of lymphocyte disappearance from blood (kout = 0.587, half-life = 1.2 h) is consistent with the near-nadir lymphocyte counts occurring around 6 hours postdose.
- The model indicated that the recovery of lymphocyte counts is concentration dependent consistent with the dose-dependency in recovery observed during the follow-up visits.

Heart rate response

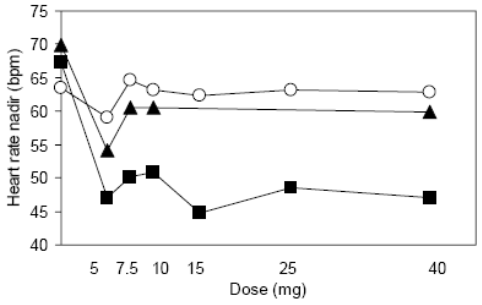
Acute heart rate response

Change of the teart rate response from baseline day -1 and treatment day 1 is shown below:

Figure 7-14 Heart rate response plots



Mean heart rate trajectories from telemetry. All subjects received placebo on day -1 and FTY720/placebo on day 1. Shown are data from the placebo group (*open circles*), FTY720 5mg (*filled squares*), and FTY720 40mg (*filled triangles*). Mean trajectories from all dose levels are in Appendix 4 Figure 6.



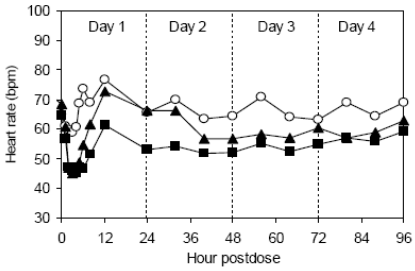
Dose-response plots for nadir heart rate on day -1 (*open circles*), day 1 (*filled squares*), and day 2 (*filled triangles*). Day 2 telemetry was not performed at 15mg and 25mg dose levels.

- The typical circadian rhythm in heart rate was shown on day -1 whereby heart rate declines to a morning nadir of about 63 bpm 2 to 3 hours after the placebo dose. Thereafter, the heart rate rises to a maximum around 12 hours postdose and undergoes a similar decline and rise in the evening and night.
- On day 1 the heart rate trajectory in the placebo cohort reached a similar nadir (67 ± 9 bpm) as on day -1; whereas, FTY720-treated cohorts had a lower and slightly delayed morning nadir of 48 ± 6 bpm at 4 hours postdose.
- Mean nighttime nadirs were similar to the corresponding daytime nadirs on day 1 and exhibited no dose-dependency across FTY720 dose levels.
- The right panel of figure above demonstrates that between 5 mg and 40 mg, the dose-response relationship for nadir heart rate was flat as was also the case for AUE(0-4) and AUE(0-12) as summarized in the table above. A maximum effect on heart rate appears to occur at the 5 mg dose with no further increase in effect at higher doses.

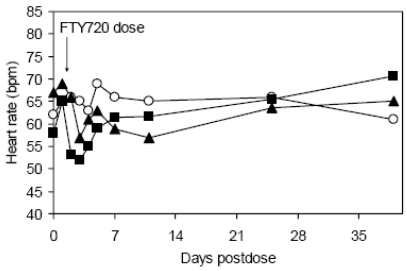
Heart rate recovery

The left panel of the figure below shows the heart rate measured frequently on day 1 and every 8 hours on days 2 to 4 based on pulse rates from vital signs. Only the two FTY720 dose extremes were included.

Figure 7-15 Heart rate recovery plots



Mean heart rate trajectories from vital signs pulse recording in subjects receiving placebo (*open circles*), 5mg FTY720 (*filled squares*), and 40mg FTY720 (*filled triangles*).



As in left panel for morning heart rate trajectories measured at about 07:00-08:00.

- In the placebo cohort, a normal and consistent circadian rhythm is apparent over the 4-day observation period.
- A similar circadian pattern is apparent for the FTY720 cohorts but the trajectories are lower at all time points with no particular dose-dependency.
- Longer term observations at follow-up and end-of-study visits were made for the morning heart rate. Mean morning heart rates remained stable over time in the placebo group. Mean heart rates remained numerically depressed in FTY720-treated subjects until day 4 after which visit they were similar to those in the placebo group.

The observation from previous figure was quantified further as the area under the heart rate-time effect curve for each day.

Table 7-11 Heart rate recovery parameters

Day	AUE(0-24) (bpm.h)		Morning heart rate (bpm)	
	Placebo (n = 14)	All FTY720 (n = 41)	Placebo (n = 14)	All FTY720 (n = 41)
-1	1632 ± 241	1629 ± 180	62 ± 8	63 ± 9
1	1682 ± 266	1490 ± 184	65 ± 11	66 ± 8
2	1586 ± 223	1388 ± 159	66 ± 11	59 ± 8
3	1591 ± 261	1393 ± 148	65 ± 9	57 ± 7
4	1597 ± 236	1435 ± 141	63 ± 11	58 ± 7
5	-	-	69 ± 10	63 ± 9
7	-	-	66 ± 10	62 ± 7
11	-	-	65 ± 9	61 ± 5
25	-	-	66 ± 11	65 ± 10
39	-	-	61 ± 6	67 ± 8

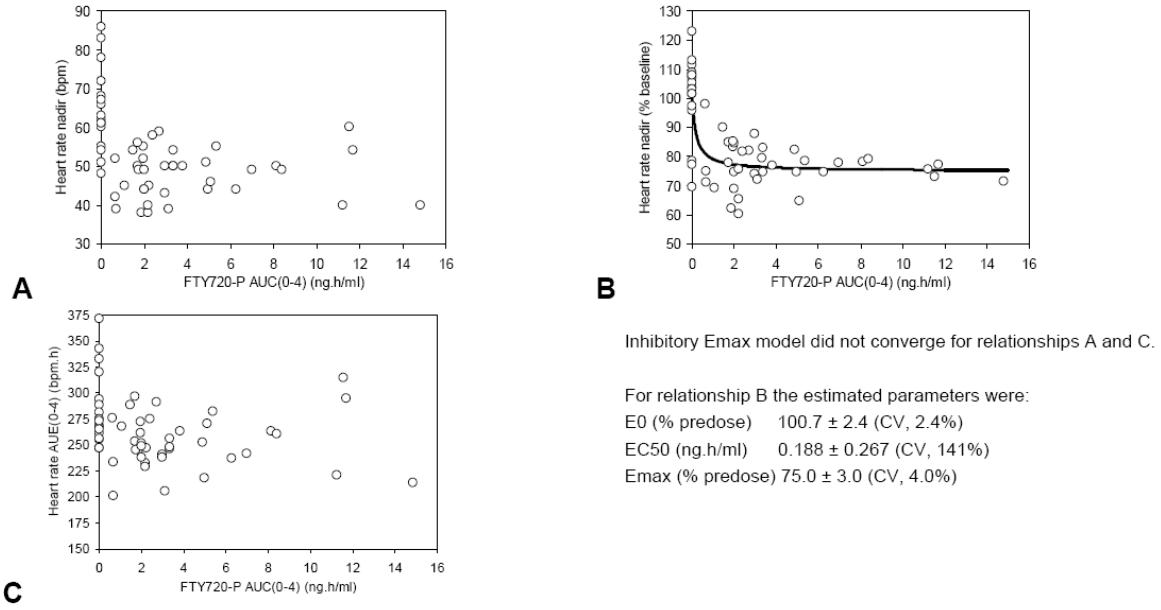
Values are mean ± sd except for time of nadir which is median (range).
Data source: Appendix 4, Table 5 from vital signs measurements in Appendix 3 Table 3.4.2.

- The AUE(0- 24) was lower in all FTY720 cohorts compared to placebo on days 1 through 4.
- There was an increase in AUE(0-24) from days 2 to 4 but full recovery of this parameter did not occur by day 4, the last day of AUE(0-24) measurement.

The inhibitory effect Emax model was applied to the dose versus nadir heart rate across single doses of 0.25 mg to 40 mg and shown below:

Heart rate exposure-response plots

Figure 7
Descriptive exposure-response relationships for heart rate from telemetry data



Inhibitory Emax model did not converge for relationships A and C.

For relationship B the estimated parameters were:
E0 (% predose) 100.7 ± 2.4 (CV, 2.4%)
EC50 (ng.h/ml) 0.188 ± 0.267 (CV, 141%)
Emax (% predose) 75.0 ± 3.0 (CV, 4.0%)

- The inhibitory effect Emax model indicated that the maximal negative chronotropic effect of FTY720 occurs around 5 mg with no further augmentation in response when escalating to doses up to 40 mg.
- This first-dose negative chronotropy is generally modest reaching a mean morning nadir of 44 bpm compared with 57 bpm in the absence of FTY720.

Pulmonary function response

Table 7-12 Pulmonary function response parameters

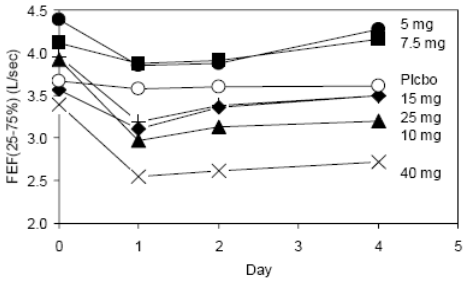
Parameter	Placebo	5mg	7.5mg	10mg	15mg	25mg	40mg
N	14	6	12	5	6	6	6
FEV_1							
Day -1 (L)	3.82 ± 0.96	4.26 ± 0.70	4.28 ± 0.75	3.55 ± 0.63	3.78 ± 0.39	3.56 ± 0.66	3.73 ± 1.00
Day 1 (L)	3.79 ± 0.94	4.04 ± 0.74	4.10 ± 0.77	3.29 ± 0.62	3.54 ± 0.33	3.35 ± 0.53	3.21 ± 1.20
Ratio day 1 / -1	1.00 ± 0.05	0.95 ± 0.04	0.96 ± 0.04	0.92 ± 0.02	0.94 ± 0.03	0.95 ± 0.04	0.83 ± 0.17
$FEF_{25-75\%}$							
Day -1 (L/sec)	3.67 ± 1.20	4.39 ± 1.17	4.11 ± 1.13	3.92 ± 0.88	3.56 ± 0.70	3.95 ± 1.07	3.39 ± 1.04
Day 1 (L/sec)	3.58 ± 1.19	3.85 ± 1.15	3.88 ± 1.36	2.97 ± 0.67	3.11 ± 0.69	3.18 ± 0.64	2.55 ± 1.13
Ratio day 1 / -1	0.98 ± 0.07	0.87 ± 0.08	0.93 ± 0.10	0.76 ± 0.07	0.87 ± 0.07	0.82 ± 0.12	0.71 ± 0.19
AUE(0-78) (L/sec.h)	77 ± 5	71 ± 6	75 ± 8	63 ± 3	74 ± 8	68 ± 10	58 ± 14

Values are mean ± sd. Data for 10mg is for the 5 subjects included in the pharmacokinetic evaluation.
Data source: Appendix 3 Table 2.5.6 and Appendix 4 Table 6.

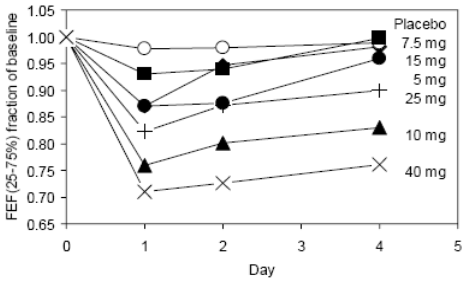
- Forced expiratory flow in 1 second (FEV1) was consistent from baseline day -1 to day 1 in placebo-treated subjects based on the ratio of 1.00.
- Small reductions or inter-day fluctuations were evident in the FTY720 5 mg to 25 mg cohorts (ratios, 0.92 to 0.96); whereas, a more prominent signal was apparent at 40mg with an average 17% reduction in FEV1 postdose (ratio, 0.83).
- Mid-expiratory flow (FEF25-75%) was also consistent between days in the placebo group; whereas, mean 7% to 24% reductions occurred after doses of 5 mg to 25 mg; and a mean 29% reduction after 40 mg.

The left panel of figure below shows the temporal course for mean midexpiratory flow in each dose cohort and the right panel shows the values as fraction of baseline.

Figure 7-16 Mid-expiratory flow trajectories



Mean FEF(25-75%) over 4 days postdose in subjects receiving placebo (*open circles*) or FTY720 5mg (*circles*), 7.5mg (*squares*), 10mg (*triangles*), 15mg (*diamonds*), 25mg (+), or 40mg (*X*).

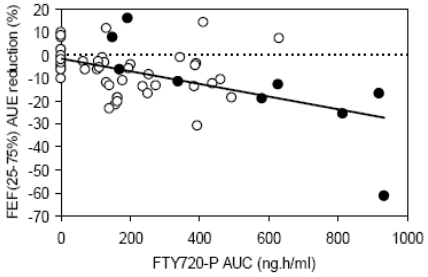


As in left panel for FEF(25-75%) expressed as the fraction of the baseline value. Group synoptic plots are in Appendix 4 Figure 8.

- The mean value on day -1 exhibited notable intergroup differences. No change was noted over 4 days in the placebo group.
- Distinct decreases from baseline were noted in all FTY720 groups with a rough dose-dependency and a nadir reached on day 1 (6 hours postdose). By day 4, mean values had recovered back to baseline except in the 10 mg, 25 mg, and 40 mg groups.
- The area under the effect curve AUE over the 4- day observation period was calculated based on the FEF(25-75%) values expressed as fraction of baseline for each subject (see right panel of the figure above for the mean trajectories).

The mean AUE(0-78) for placebo-treated subjects was 77 L/sec x h. Each subject's percent reduction from this mean value [(77 – AUE) / 77 x 100] was then plotted versus the corresponding FTY720-P AUC(0-72)$_b$ as shown in the figure below.

Figure 7-17 Exposure-pulmonary function response plot



Linear regression relating the percent decrease in mid-expiratory flow area under the effect curve AUE(0-78) versus FTY720-phosphate AUC(0-72). *Filled circles* designate subjects reporting chest tightness or discomfort.

- Attempts to fit an inhibitory effect Emax model to the data did not yield a more informative description than a simple linear regression: percent reduction in FEF25-75% AUE(0-78) = −0.021 x AUC(0- 72) − 2.06 (r2 = 0.272).
- Based on the sponsor, there was evidence of small airway reactivity (obstructive pattern) at higher doses of FTY720, specifically 25 mg and 40 mg, as indicated by the mid-expiratory flow (FEF25-75%) data.
- Nine subjects reported chest tightness or discomfort. The majority of subjects were in the 40 mg cohort (n = 5) and had the highest exposure to FTY720-phosphate.

- Subjects were in no acute distress, there was no indication of ischemic changes on ECG, and the chests were clear to auscultation. All events resolved spontaneously with discontinuation of exercise testing and/or rest.

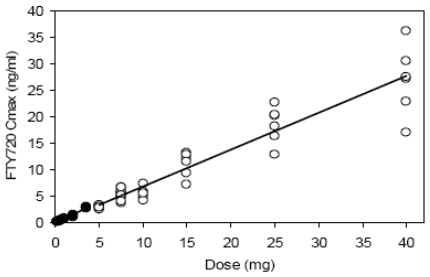
Exposure response-expanded dose range

The first-in-man study B101 was a single-dose escalation study in 22 maintenance renal transplant patients using doses of 0.25 mg (*n* = 4), 0.5 mg (*n* = 6), 0.75 mg (*n* = 3), 1 mg (*n* = 3), 2 mg (*n* = 3), and 3.5 mg (*n* = 3). The exposure, lymphocyte, and heart rate data from this study at low FTY720 doses were combined with the present data from doses of 5 mg to 40 mg for an expanded view of dose-exposure and dose-response relationships. Since the pharmacologically active analyte FTY720-P was not measured in study B101, exposure-response relationships were not evaluated over the expanded dose range.

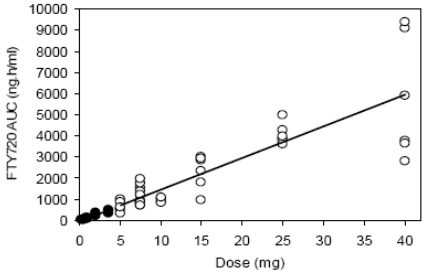
Dose-exposure relationship

The $C_{max,b}$ and AUC_b data from the two studies are shown in the figure below.

Figure 7-18 FTY720 dose-proportionality: 0.25 – 40 mg



FTY720 dose-Cmax relationship. Shown are the individual values from renal transplant patients (*filled circles*) and healthy subjects (*open circles*) and the linear regression line: Cmax = 0.69 x Dose – 0.05 (r^2 = 0.889).



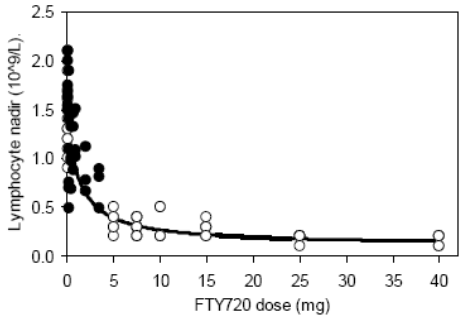
As in left panel for FTY720 dose-AUC. Regression line: AUC = 150 x Dose – 57 (r^2 = 0.719).

- The linear regressions were unchanged by the addition of the lower dose data.
- The dose-Cmax,b regression supported dose-proportionality over the extended range with a slope of 1.02 (90%CI, 0.99 – 1.06).
- A slight, +8% departure from dose-proportionality was detected for AUCb with a slope of 1.08 (90%CI, 1.03 – 1.12).

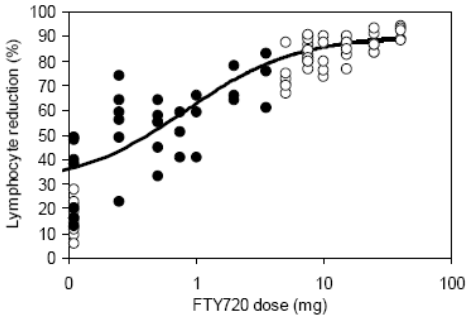
Dose-lymphocyte relationship

The relationships between FTY720 dose and lymphocyte nadir were adequately described by an inhibitory effect Emax model.

Figure 7-19 FTY720 dose-lymphocyte response: 0.25 – 40 mg



FTY720 dose vs lymphocyte nadir relationship. Shown are the individual values from renal transplant patients (*filled circles*) and healthy subjects (*open circles*) and the fit of an inhibitory effect Emax model to the data.



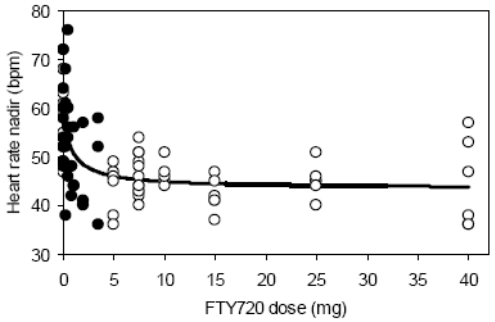
As in left panel for lymphocyte nadir expressed as percent reduction from predose count with a logarithmic dose scale (x-axis).

- When the lymphocyte nadir was expressed as the measured values (cells x 10^9/L), the model-estimated nadir in placebo was 1.39 x 10^9/L (CV, 5%), the half-maximal dose was 1.4 mg (CV, 24%), and the minimal nadir was 0.11 x 10^9/L (CV, 35%).
- When the lymphocyte nadir was expressed as percent reduction from the predose count, the model-estimated reduction in placebo was 30% (CV, 4%), the half maximal dose was 0.8 mg (CV, 22%), and the maximal reduction was 90% (CV, 14%).

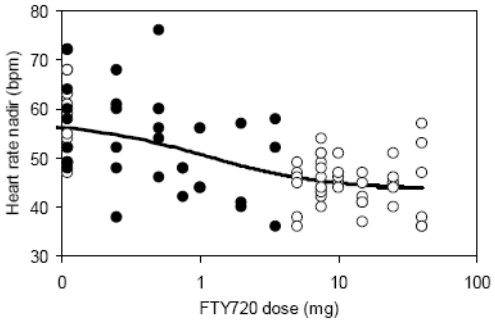
Dose-heart rate relationship

The relationships between FTY720 dose and nadir heart rate from vital signs recordings (pulse) were described by an inhibitory effect Emax model shown below.

Figure 7-20 FTY720 dose-heart rate response: 0.25 – 40 mg



FTY720 dose vs heart rate nadir relationship. Shown are the individual values from renal transplant patients (*filled circles*) and healthy subjects (*open circles*) and the fit of an inhibitory effect Emax model to the data.



As in left panel with a logarithmic dose scale.

- The model-estimated nadir in placebo was 57 bpm (CV, 4%), the half-maximal dose was 1.1 mg (CV, 70%), and the minimal nadir was 44 bpm (CV, 4%).

Conclusions:

- Single-dose FTY720 from 5 mg to 40 mg was generally well tolerated by healthy subjects based on the sponsor.
- The dose escalation was terminated at 40 mg due to changes in pulmonary function tests and reports of chest tightness or discomfort.
- There were no clinically relevant departures from dose-proportionality for FTY720 $C_{max,b}$ and AUC_b over the single-dose range 5 mg to 40 mg.
- FTY720-P $C_{max,b}$ rose in an under-proportional manner by −25% over the dose range 5 mg to 40 mg; whereas, the AUC portion measurable at all dose levels, $AUC(0\text{-}144)_b$, was dose-proportional.
- The $AUC(0\text{-}144)_b$ molar-ratio between FTY720-P/FTY720 averaged 0.38 and was independent of dose.
- Lymphocyte count nadirs were dose-dependent with reductions from baseline of 74% at 5 mg to 91% at 40 mg. A mechanistic model relating FTY720-P blood levels to lymphocyte trafficking estimated a maximum reduction from baseline of 90%. Recovery of lymphocyte counts back to baseline was dose-dependent ranging from full recovery at day 39 for 5 mg to 58% recovery at 40 mg.
- FTY720 reduced the mean morning nadir heart rate by 25%. These responses were independent of dose over the range 5 mg to 40 mg. Recovery of the mean morning heart rate back to pretreatment baseline occurred by day 5 postdose.
- Single-dose FTY720 at 25 mg and 40 mg reduced pulmonary function up to 17% for FEV_1 and up to 29% for $FEF_{25\text{-}75\%}$. At 40 mg, 5 of 6 subjects reported mild chest tightness or discomfort which was clinically consistent with small airway reactivity.

Study FTY720A 2217*:* **A study to assess the disposition and biotransformation of [14C]FTY720 and metabolites after a single oral dose to healthy male subjects**

A brief overview of some essential components of the study design is given below:

Study Design	Single-dose, open label
Study Population	N=4 enrolled, 4 completed Age: 35-45 years (mean 40 years) Gender: 4 males Weight: 75.1-85.2 kg (mean 82 kg) Race: 4 Caucasian (100 %)
Dosage and Administration	Single oral dose of 5 mg [^{14}C]FTY720 hydrochloride salt was given to male subjects with a 240-hour in-house observation period (Days 1-10). Study drug was administered as a drinking solution under fasted conditions. [^{14}C]FTY720 hydrochloride salt, crystalline solid drug, specific radioactivity 431.8 kBq/mg FTY720 hydrochloride (148.5 Bq/nmol). The radiolabeled drug was provided in individual vials containing a dose of 5 mg of drug product (4.47 mg free base). The contents of each vial was freshly dissolved in 5% aqueous glucose solution to a concentration of about 1 mg/mL and diluted with about 75 mL of tap water for preparation of a drinking solution. Diet: All subjects fasted for at least 10 hours prior to the dose of study medication on Day 1 and continued to fast for at least 4 hours thereafter. During waking hours, subjects were required to have a fluid intake of at least 200 mL every 4 hours in addition to fluid taken with the medication. Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood	Blood samples were taken at pre-dose (0-hour), 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours post-dose. Three additional blood samples were taken at 312, 480 and 816 hours post-dose (beginning of Days 14, 21 and 35 post-dose).
Sampling: Urine	All (and complete) urine was collected at 0, 0 to 6 hours, 6 to 12 hours, 12 to 24 hours, and thereafter in 24-hour sampling periods during the whole observation period from Day 2 up to the end of Day 10. In addition, complete urine was collected during Day 13 (collection period 288-312 hours), Day 20 (period 456-480 hours) and Day 34 (period 792-816 hours).
Sampling: Feces	All feces were collected before dosing and during the entire observation period (Day 1 to (and including) Day 10).

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 0.987 ng/mL FTY720: Linear range : 0.08-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 13.6 % Inter-day accuracy: < 14 % FTY720-P: Linear range : 0.987-329 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 4 % Inter-day accuracy: < 7 %
Analysis (Blood, Urine and Feces)	Method (1) Liquid scintillation counting (LSC) total ^{14}C-radioactivity in all samples The mean value (± SD) of the whole blood, urine and feces quality control sample was found to be 100.2 ± 0.4 % (99.1-100.8 %, performed at a level of 6000 dpm), 100.0 ± 1.3 % (96.9-101.9 % performed at a level of 5024 dpm) and 101.5 ± 1.5 % (98.2-103.8 % performed at a level of 5212 dpm), respectively. (2) HPLC-radiometry metabolite profiles in blood extracts Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 0.987 ng/mL FTY720: Linear range : 0.08-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 13.6% Inter-day accuracy: < 14 % FTY720-P: Linear range : 0.987-329 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 4.0% Inter-day accuracy: < 7.0 % Metabolite structures were characterized by LC-MS, LC-MS/MS, wet-chemical methods and, if possible, by comparison with reference compounds.
PK Assessment	FTY720 in blood: Cmax, tmax, AUC0-t, AUC0-tz, AUCo-∞, t½λz, CL/F, and

	Vz/F FTY720-P in blood: Cmax, tmax, AUC0-t and AUC0-tz Radioactivity in blood: Cmax, tmax, AUC0-t, AUC0-tz, AUCo-∞, t½λz Metabolites (including FTY720-P) in blood: AUC0-816 h, AUCo-∞, t½λz Radioactivity in urine and feces: cumulative excretion up to 240 hours and extrapolation to infinity FTY720 and metabolites in urine and feces: cumulative excretion up to 240 hours
Safety Assessment	Physical exam, vital signs, blood pressure, laboratory parameters and ECG were assessed. Adverse events were recorded throughout the study.

Pharmacokinetic Results:

FTY720 and FTY720-P pharmacokinetic in blood:

Mean PK parameters of FTY720 and FTY720-P are shown in the following table:

Table 7-4 Pharmacokinetic parameters of total radiolabeled components (radioactivity), FTY720 and FTY720-P in blood

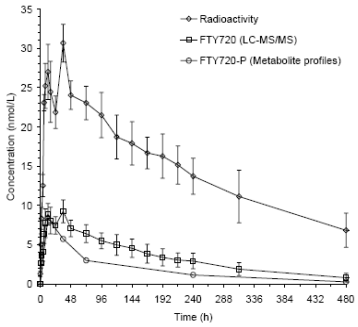
Mean ± SD (CV), n=4		Radioactivity	FTY720 [b] (Parent drug)	FTY720-P
t_{max} [a, e]	(h)	36	24	7 [b]
C_{max} [e]	(nmol/L)	33.29 ± 1.05 (3.2%)	9.53 ± 1.58 (17%)	9.10 ± 1.19 (13%) [b]
	(ng/mL)	10.24 ± 0.32 [c]	2.93 ± 0.49 [e]	3.53 ± 0.43 [b]
AUC_{0-t} [d]	(nmol·h/L)	9828 ± 1405 (14%)	1704 ± 551 (32%)	965 [d]
	(ng·h/mL)	3022 ± 432 [c]	524 ± 170	374 [d]
$AUC_{0-\infty}$	(nmol·h/L)	12725 ± 2478 (19%)	1847 ± 588 (32%)	999 [d]
	(ng·h/mL)	3913 ± 762 [c]	568 ± 181	387 [d]
$t_{½\lambda z}$	(h)	382 ± 43 (11%)	137 ± 55 (40%)	166 [d]
	(days)	15.9 ± 1.8	5.7 ± 2.3	6.9 [d]
CL/F	(L/h)	-	8.68 ± 3.65 (42%)	-
	([L/h]/kg)	-	0.106 ± 0.044 (42%)	-
	([mL/min]/kg)	-	1.77 ± 0.73	-
V_z/F	(L/kg)	-	18.5 ± 3.0 (16%)	-
	(L)		1509 ± 225 (15%)	

-: no value or not meaningful to calculate. [a]: median value. [b]: determined by LC-MS/MS method. [c]: radioactivity expressed as ng-eq/mL for C_{max} and ng-eq·h/mL for AUC. [d]: AUC_{0-816h} of FTY720-P was derived from metabolite profiles in blood. [e]: global t_{max}, C_{max}.

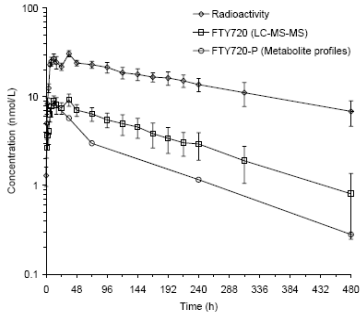
Mean FTY720 and FTY720-P blood concentration-time plots are shown below:

Figure 7-1 Pharmacokinetics of total radiolabeled components (radioactivity), FTY720 and FTY720-P in blood

Linear graph
Mean, n=4 (± SD for radioactivity and FTY720)



Semi-logarithmic graph
Mean, n=4 (± SD for radioactivity and FTY720)



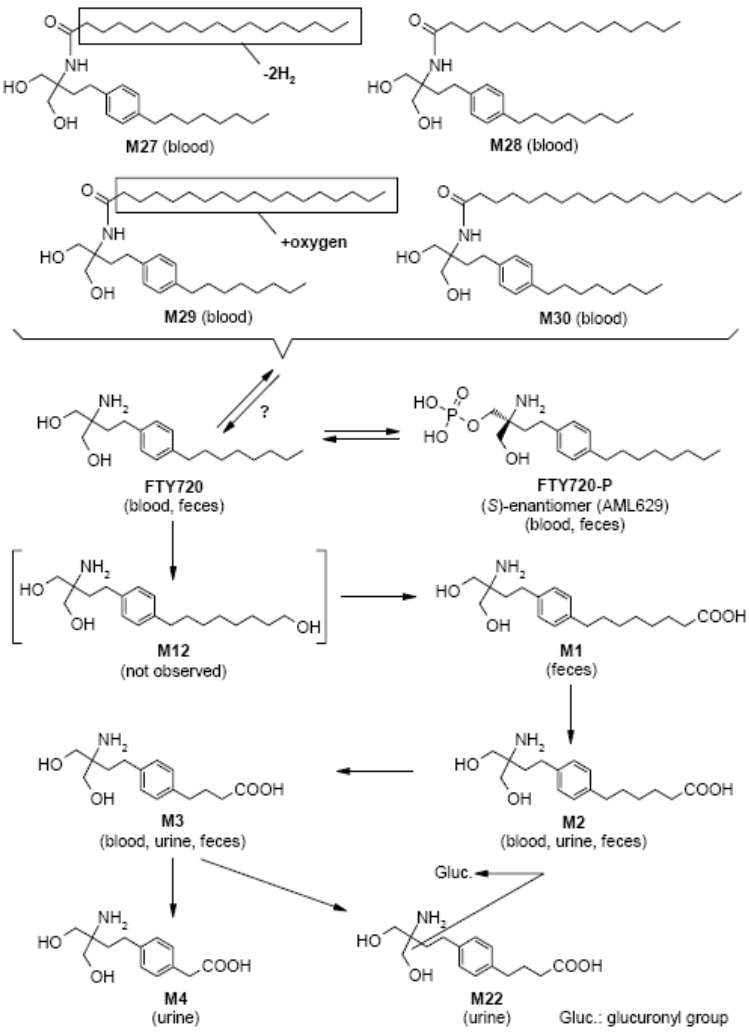
- FTY720 was slowly absorbed as indicated by the late tmax, of 8-36 hours post-dose (global tmax).
- Likewise, FTY720-related radioactivity showed a first maximum between 6 and 12 hours; the second maximum was at 36 hours in all four subjects.

- The main metabolite FTY720-P maximized at 7 hours and showed a second, smaller concentration peak at 36 hours.
- Beyond 36 hours, radioactivity, FTY720 and FTY720-P disappeared slowly from blood, as characterized by their respective long terminal half-lives of 382 hours (15.9 days), 137 hours (5.7 days) and 166 hours (6.9 days).

Metabolite identification:

The proposed metabolic pathways are summarized below:

Figure 7-2 Proposed biotransformation pathways of FTY720 in humans



The metabolites of FTY720 in blood, urine and/or feces, for which structural information was obtained in this study, were divided into three categories:

(i) metabolites M1, M2, M3, M4 and FTY720-P for which synthetic reference compounds were available,

(ii) metabolite M22 for which partial structural information could be derived from mass spectral data and wet-chemical experiments and

(iii) the nonpolar metabolites M27, M28, M29 and M30 which were assigned based on a comparison of chromatographic retention times with metabolites identified in the rat and on wet-chemical experiments.

(i) Metabolites identified by comparison with synthetic reference compounds

Methods utilized for metabolites identification were summarized below.

Table 7-5 Assignment of FTY720-related components in metabolite profiles based on comparison with synthetic reference compounds

A: Retention time under the chromatographic conditions of the metabolite profiles (Section 3.5.5) identical (within experimental variability) with that of the respective synthetic reference compound.

B: Retention time under the chromatographic conditions of the LC-MS runs (LC-MS system 1, Appendix 5.4-Section 2.5.2) identical (within experimental variability) with that of the respective synthetic reference compound.

C: Retention time under the chromatographic conditions of the nano-LC-MS/MS runs (nano-LC-MS/MS system 2, Appendix 5.4-Section 2.5.2) identical (within experimental variability) with that of the respective synthetic reference compound.

D: Exact mass of $[M+H]^+$ ion in agreement with proposed structure: difference between measured and calculated mass < 5.0 mDa (except for M3 in feces: 6.7 mDa); exact mass data from LC-MS runs of isolated metabolites.

E: Product ion spectrum of $[M+H]^+$ ion, obtained by nano-LC-MS/MS of the isolated component, identical (within experimental variability) with that of the respective synthetic reference compound.

Components listed in the order of elution.

	Blood pool of 12 h and 72 h and across all 4 subjects; polar fraction	Urine 0-240 h Subject 5103	Feces 0-240 h Subjects 5102 and 5104
M4	n.d.	A, B, C, D, E	n.d.
M3	A, B, D	A, B, C, D, E	A, B, C, D, E
M2	A, B, D	A, B, C, D, E	A, B, C, D, E
M1	n.d.	n.d.	A, B, C, D, E
FTY720	A, B, D	n.d.	A, B, C, D, E
FTY720-P	A [a]	n.d.	A, B, C, D, E

n.d.: not detected

a: identity further supported by enantioselective analysis (Section 7.5.4); in addition, metabolite FTY720-P quantified by LC-MS/MS (Section 7.4.2)

- Besides the "not detected" (n.d.) samples, most of the metabolites as well as the FTY720 and FTY720-P were able to be compared using multiple methods.
- No FTY720 and FTY720-P were detected in the urine.

Reviewer's notes:
This data suggests the identifications of these metabolites are likely to be correct since identical peaks with the synthesized compounds were observed in multiple methods.

(ii) Metabolite M22

- Metabolite M22 was identified as a glucuronide of metabolite M3.
- The structure of metabolite M3 contains four potential sites of glucuronidation: two hydroxyl groups, a primary amino group and a carboxylic group. Glucuronidation at the latter site (formation of an acyl glucuronide) could be excluded by demonstrating that isolated M22 was stable in alkaline solution (pH 10, 2 hours at room temperature). Hence, M22 is glucuronidated either at one of the two hydroxyl groups or at the primary amino group. These remaining possibilities could not be differentiated on the basis of the available data.
- In the metabolite profiles in urine, M22 appeared very shortly before M3.

(iii) Nonpolar metabolites in blood

- The structures of the nonpolar metabolites M27, M28, M29 and M30 were assigned primarily based on their retention times, in comparison with metabolites characterized in rat blood.
- All four metabolites are proposed to be products of acylations of FTY720 at its amino group with endogenous fatty acids and, hence, analogs of endogenous ceramides.

- M27 is proposed to be an amide of an octadecadienoic acid (possibly linoleic acid), M28 an amide of palmitic acid, M29 an amide of a hydroxystearic acid and M30 an amide of stearic acid.
- Attempts to confirm these assignments failed due to the low concentrations of these metabolites in the human blood samples and interferences of endogenous compounds of similar structure.
- The FTY720 in the alkaline hydrolysate of the nonpolar metabolites was formed directly from the nonpolar metabolites, not *via* FTY720-P.

Enantiomeric composition of metabolite FTY720-P

- In contrast to the parent drug and the metabolites M1-M4, metabolite FTY720-P has an asymmetric center and is therefore chiral.
- Metabolite FTY720-P in blood represented exclusively the (*S*)-enantiomer AML629 at both time points post-dose. The (*R*)-enantiomer AML627 was below the detection limit (estimated < 3% of total FTY720-P).

Metabolite profiles in blood

PK parameters of FTY720 and metabolites in blood are shown in the following table:

Table 7-7 Pharmacokinetic parameters of FTY720 and metabolites in blood (estimated from metabolite profiles)

AUC values and half-lives of FTY720 and metabolites in the blood of healthy male volunteers after a single oral dose of 5 mg [^{14}C]FTY720 hydrochloride. Data estimated from metabolite profiles. For technical reasons, polar and nonpolar blood extracts had to be analyzed separately (Section 3.5.5). The AUC values represent the sum of both fractions and are mean values of 3-4 subjects (details given in Table 11-12 to Table 11-15). Half-lives were estimated from mean concentrations across 4 subjects. Components listed in the order of elution. Chemical structures shown in Figure 7-2.

Component	$AUC_{0-816\ h}$ in nmol·h/L *(ng·h/mL in parentheses)* [a]	$AUC_{0-816\ h}$ (% AUC of total radiolabeled components) [a,b]	$AUC_{0-\infty}$ in nmol·h/L *(ng·h/mL in parentheses)* [c]	$AUC_{0-\infty}$ (% AUC of FTY720) [b,c]	$t_{½\lambda z}$ (h)	Time interval for estimation of $t_{½\lambda z}$ (h)
M3	776 *(218)*	8.3	808 *(227)*	35.9	170	72-816
M2	140 *(43)*	1.5	140 *(43)*	6.2	n.c.	
FTY720	2176 *(669)*	23.3	2247 *(691)* [d]	100	162 [d]	72-816
FTY720-P	965 *(374)*	10.3	999 *(387)*	44.4	166	72-816
M27	72 *(41)*	0.8	72 *(41)*	3.2	n.c.	
M28	46 *(25)*	0.5	46 *(25)*	2.0	n.c.	
M29	834 *(492)*	8.9	1194 *(704)*	53.1	404	480-816
M30	684 *(393)*	7.3	927 *(532)*	41.3	410	480-816
Sum of additional metabolites detected	2778	29.7 [e]	n.c.	n.c.	n.c.	
Total components detected	8471	90.6	n.c.	n.c.	n.c.	
Lost during sample processing [f]	884	9.4	n.c.	n.c.	n.c.	
Lost during HPLC	0	0.0	n.c.	n.c.	n.c.	
Total radiolabeled components in original sample	9355	100	n.c.	n.c.	n.c.	

n.c.: not calculated

[a]: AUC values calculated using the linear trapezoidal method; concentrations at time zero taken as zero

[b]: referring to molar concentrations

[c]: $AUC_{0-\infty}$ set equal to $AUC_{0-816\ h}$ if component no more detectable at 816 h

[d]: values derived from metabolite profiles, used in this table for consistency reasons; similar values were obtained by quantitative LC-MS/MS analysis (Table 7-4)

[e]: numerous individual components, none above 2.2% of $AUC_{0-816\ h}$ of total radiolabeled components or above 9.1% of $AUC_{0-816\ h}$ of FTY720

[f]: material recovered neither in polar nor in nonpolar fraction

- FTY720-related components in blood covered a very wide range of polarities and had been analyzed in two fractions: a polar fraction containing the main part and a nonpolar fraction containing a minor part of the total radiolabeled components.
- The polar fractions contained FTY720, FTY720-P and M3 as the major components.
- The nonpolar fractions contained predominantly the nonpolar ceramide analogs M27-M30.
- The decline of the concentrations of FTY720, M2, M3 and FTY7320-P occurred approximately in parallel.
- The nonpolar ceramide analogs M29 and M30 decreased very slowly with apparent half-lives of approximately 400 hours, close to the half-life of the total radiolabeled components (382 ± 43 h, mean ± SD) and longer than the half-lives of FTY720 (137 ± 55 h, mean ± SD; similar to the 162 hours estimated from the metabolite profiles), M3 (approximately 170 h) and FTY720-P (approximately 166 h). These half-lives were used to extrapolate the AUC0-816 h values to infinity.
- The two minor nonpolar ceramide analogs M27 and M28 were detected only sporadically.

- Among the trace metabolites of unknown structures, none accounted for more than 2.2% of the AUC0-816 h of total radiolabeled.
- The AUC0-∞ values of the metabolites, relative to that of FTY720 (estimated from the metabolite profiles), amounted to 36% for M3, 44% for FTY720-P, 53% for M29 and 41% for M30. The AUC0-∞ values of M2, M27 and M28 were below 10% of the AUC0-∞ of FTY720.

Metabolite profiles in excreta

Table 7-8 FTY720 and metabolites in excreta

Amounts of FTY720 and metabolites in the excreta of healthy male volunteers (mean ± SD of n=4), 0-240 hours following a single oral dose of 5 mg [^{14}C]FTY720 hydrochloride. Data derived from metabolite profiles. Components listed in the order of elution. Chemical structures shown in Figure 7-2.

Component	Urine (0-240 h)	Feces (0-240 h)	Total excretion (0-240 h)
	Excretion (% of dose)		
M4	3.1 ± 0.9	-	3.1 ± 0.9
M22	1.3 ± 0.1	-	1.3 ± 0.1
M3	36.6 ± 5.0	0.3 ± 0.1	36.8 ± 5.1
M2	7.1 ± 1.2	0.5 ± 0.2	7.6 ± 1.2
M1	-	0.2 ± 0.01	0.2 ± 0.01
FTY720	-	2.4 ± 0.5	2.4 ± 0.5
FTY720-P	-	1.7 ± 0.4	1.7 ± 0.4
Sum of additional metabolites	4.2 ± 0.5	0.7 ± 0.1	4.8 ± 0.6
Total components detected	52.3 ± 6.3	5.8 ± 0.9	58.0 ± 7.0
Lost during sample processing	0.0	3.7 ± 0.8	3.7 ± 0.8
Lost during HPLC	0.0	0.4 ± 0.1	0.4 ± 0.1
Total radiolabeled components in original sample	52.3 ± 6.3	9.9 ± 1.7	62.1 ± 7.6

-: not detected (or too minor for unequivocal assignment)

- The urinary excretion of radioactivity 0-240 hours amounted to 52.3 ± 6.3% of dose (mean ± SD) of which 100% was accounted for by the metabolite profiles.
- In urine, M3 (36.6 % of total dose) was the major metabolite, accompanied by M2 (7.1 %) and small amounts of M4 (3.1 %) and M22 (1.3 %). Other, unidentified metabolites were present in trace amounts only.
- Neither FTY720 and FTY720-P nor the nonpolar ceramide analogs M27-M30 were detected in urine.
- The interindividual variability of the metabolite profiles in urine was small.
- The fecal excretion of radioactivity 0-240 hours amounted to only 9.9 ± 1.7 of dose (mean ± SD) of which 59 ± 2% was accounted for by the metabolite profiles while approx. 40% (~ 4% of dose) could not be recovered.
- The radiolabeled material in the feces extracts consisted mainly of FTY720 (2.4 % of dose) and FTY720-P (1.7 %) with smaller contributions of M1, M2, M3 and a few unidentified metabolites of very low abundance.
- The nonpolar ceramide analogs M27-M30 were not detected.
- The inter-individual variability of the metabolite profiles in feces was small.

Excretion and mass balance in urine and feces

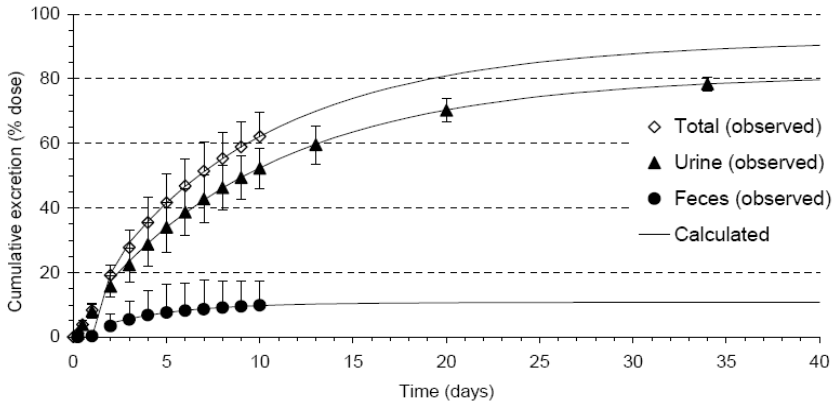
Urine and feces were collected between 0 and 240 hours post-dose. In order to monitor the slow excretion of radioactivity, additional urine fractions were collected between 288-312, 456-480 and 792-816 hours post-dose.

Table 7-9 Balance of excretion of radioactivity in urine and feces

Collection time period	Excretion (% of dose) (mean ± SD, n=4)		
	Urine	Feces	Total
0-72 h	22.3 ± 5.3	5.4 ± 1.1	27.7 ± 5.3
0-240 h	52.3 ± 6.3	9.9 ± 1.7	62.1 ± 7.6
288-312 h	2.0 ± 0.5	n.s.	-
456-480 h	1.0 ± 0.4	n.s.	-
792-816 h	0.3 ± 0.2	n.s.	-
Estimated excretion in the period of 0 to 816 h	78 ± 3	11 ± 2	89 ± 3
Estimated excretion in the period of 0 to infinity (extrapolated)	81 ± 3	11 ± 2	92 ± 2
n.s.: not sampled			

Figure 7-3 Cumulative excretion of radioactivity in urine and feces

Mean ± SD, n=4



- Radioactivity was slowly excreted in urine and feces. About one third of the administered dose was excreted within the first three days after administration.
- After 10 days, on average approximately 62% of the dose (range between 56% and 73%) were recovered in the excreta: about 52% in urine and about 10% in feces. Hence, excretion of [^{14}C]FTY720-related radioactivity occurred predominantly *via* the kidney. Fecal excretion was of minor importance.
- Small, decreasing portions of 2.0%, 1.0% and 0.3% of the dose were still recovered in urine during sample collection Days 13, 20 and 34, indicating that excretion was very slow and still continuing beyond 10 days.
- The disappearance of radioactivity from the body took place with apparent half-lives of about 7.2 days in urine and about 2.7 days in feces.
- Estimations of the excretion rate indicated that after 816 hours days, about 78% and 11% of the administered radioactivity was excreted in urine and feces, respectively.
- Based on an extrapolation to infinity, 81% and 11% of the radioactive dose are expected to be excreted in urine and feces, respectively. Hence, excretion of [^{14}C]FTY720-related radioactivity in human volunteers can be judged as being essentially complete but slow.
- The extent of absorption was estimated to be ≥ 85% of dose, based on the amount of radioactivity excreted in urine (81% of dose) and the amount of metabolites in feces (~ 4% of dose) extrapolated to infinity.

Adverse events:

Table 7-2 Frequency and severity of adverse events after a single oral dose of 5 mg [^{14}C]FTY720 hydrochloride in healthy subjects

Adverse events (AEs) by organ system MedDRA term	Intensity	Relationship to study drug	5.0 mg [^{14}C]FTY720 (n=4)
Cardiac disorders			
Sinus bradycardia	Severe	Suspected	1
Vascular disorders			
Hypotension	Severe	Suspected	1
Nervous system disorders			
Syncope	Severe	Suspected	1
Dizziness	Moderate	Suspected	1
Headache	Mild	Not suspected	1
Gastrointestinal disorders			
Flatulence	Mild	Not suspected	1
Loose stools	Mild	Not suspected	2
General disorders			
Fatigue	Moderate	Suspected	1
Fatigue	Mild	Not suspected	1
Total number of AEs			10

- Subject 5101 experienced intermittent dizziness at ~ 2 hours post-dose and severe sinus bradycardia starting at 4 hours post-dose with nadir heart rate of 26 bpm at 6 hours post-dose. The subject received 0.5 mg atropine intravenously twice and his heart rate recovered to baseline conditions approximately 24 hours post-dose. Sinus bradycardia was associated with temporary severe hypotension (supine blood pressure of 86/45, at 5 hours post-dose) and syncope. These events were suspected to be attributable to the study medication by the investigator.
- Subject 5103 experienced moderate fatigue of 8.5 hours duration on Day 1, which was considered attributable to the study drug by the investigator and no concomitant medication was administered.

Reviewer's notes:
There were three (out of ten) AEs reported to be severe in intensity and were suspected to be related to the study drug; however, the sponsor stated that there were no serious adverse events. This seems to be controversy. The clinical significance of the safety of the study drug will be carefully reviewed by medical officers.

Conclusions:

- [14C]FTY720 was slowly absorbed (tmax of 8-36 hours). The extent of absorption was high (≥ 85% of dose). The apparent volume of distribution of FTY720 was large (mean: Vz/F = 1509 L).
- FTY720 and its metabolites disappeared slowly from the blood with mean terminal half-lives of 137 hours for FTY720, 166 hours for FTY720-P and 382 hours for radioactivity. Inter-individual differences in pharmacokinetics and metabolism were small to moderate.
- The biotransformation of FTY720 involved (i) reversible phosphorylation to FTY720-P, (ii) hydroxylation at the terminal methyl group of the octyl chain, followed by rapid further oxidation (M1) and subsequent β-oxidation (M2, M3, M4) and (iii) formation

of nonpolar ceramide analogs (M27-M30). FTY720-P was observed in blood only in the form of its (*S*)-enantiomer (AML629; active principle).

- The subjects were systemically exposed mainly to FTY720, FTY720-P, M3, M29 and M30 with minor contributions of M2, M27 and M28.
- The clearance of FTY720 was low (mean CL/F = 8.68 L/h). Elimination of FTY720 occurred predominantly by oxidative metabolism. FTY720-P was eliminated mainly by dephosphorylation back to FTY720.
- [14C]FTY720-related radioactivity was excreted very slowly, predominantly *via* the kidney in the form of metabolite M3. FTY720 and FTY720-P were not detected in urine. Fecal excretion was minor (11% of dose on average) and represented mainly FTY720 and FTY720-P. Excretion of radioactivity in urine and feces was still incomplete after 10 days (62% of dose on average) but continued beyond this time point, reaching 89% of dose by Day 34.

Reviewer's note:
No information regarding the pharmacological activity of the metabolites M3, M29 and M30 were provided. These three metabolites were found to be ~7-8 % each of the total dose in the circulation which might be of clinical importance if they have pharmacological activities.

4.1-3. HUMAN PK STUDIES
4.1-3.2 Patient PK

Study FTY720A B101:

A randomized, double-blind, placebo-controlled, time-lagged, multicenter, ascending, single oral dose pharmacokinetic, safety and tolerability study of FTY 720 in stable renal transplant patients

A brief overview of some essential components of the study design is given below:

<table>
<tr><td>Study Design</td><td>Ascending single-dose, randomized, double-blind, placebo-controlled</td></tr>
<tr><td>Study Population</td><td>N=32, (20 enrolled, 12 re-enrolled)
Age: 29-60 years (mean 45 years)
Gender: 19 males (95%), 1 female (5%)
Weight: 57.5-116 kg (mean 84.67 kg)
Race: 20 Caucasian (100%)</td></tr>
<tr><td>Dosage and Administration</td><td>
Table 3.1-1.: Study design
<table>
<tr><th>Screening</th><th>Baseline 1</th><th colspan="2">Treatment 1</th><th>Treatment Completion Evaluation</th><th>Washout</th><th>Baseline 2</th><th colspan="2">Treatment 2</th><th>Treatment Completion Evaluation</th></tr>
<tr><td>Day -30 to -2</td><td>Day -1</td><td>Day 1
FTY 720 single dose</td><td>Day 2-4
PKPD</td><td>Day 4*</td><td>21 days</td><td>Day -1</td><td>Day 1
FTY 720 single dose</td><td>Day 2-4
PKPD</td><td>Day 4*</td></tr>
<tr><td>Clinic</td><td>Inpatient</td><td colspan="2">Inpatient</td><td>Inpatient</td><td>Home</td><td>Inpatient</td><td colspan="2">Inpatient</td><td>Inpatient</td></tr>
</table>
Study drugs were given under fasted state with 200 mL of water.
Table 3.1-2.: Dose per cohort and dose escalation
<table>
<tr><th>Dosing Cohort</th><th>Dose</th><th>N Total</th><th>N Treatment</th><th>N Placebo</th></tr>
<tr><td>I</td><td>0.25 mg</td><td>8</td><td>6</td><td>2</td></tr>
<tr><td>II</td><td>0.5 mg</td><td>8</td><td>6</td><td>2</td></tr>
<tr><td>III</td><td>0.75 mg</td><td>4</td><td>3</td><td>1</td></tr>
<tr><td>IV</td><td>1.0 mg</td><td>4</td><td>3</td><td>1</td></tr>
<tr><td>V</td><td>2.0 mg</td><td>4</td><td>3</td><td>1</td></tr>
<tr><td>VI</td><td>3.5 mg</td><td>4</td><td>3</td><td>1</td></tr>
<tr><td>Total</td><td></td><td>32</td><td>24</td><td>8</td></tr>
</table>
Diet:
Subjects fasted for approximately 10 hours before dosing and 4 hours after dose.

All subjects drank 200 mL of water for dosing. Subjects had to consume an average of 120 mL of water per hour during the baseline period and for 24 hours post dosing.
Alcohol was prohibited for 72 hours prior to dosing until study completion.
</td></tr>
<tr><td>PK Sampling: Blood</td><td>At predose (0 hour), and 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, 60, 72, 84 and 96 hours. The samples were analyzed for blood concentrations of FTY720.</td></tr>
<tr><td>Sampling: Urine</td><td>At (-24)-0 and 0-24 hours.</td></tr>
<tr><td>PD Sampling:</td><td>Lymphocyte counts: at screening, baseline, -1 hours predose, 0, 0.5, 1, 2, 6, 12</td></tr>
</table>

Blood	and 24 hour post dose. **Lymphocyte subsets:** at screening, baseline, 12, 24, 48 and 96 hour post dose
Analysis (Blood)	Method HPLC/MS/MS Lower Limits of Quantitation Blood FTY720 0.063 ng/mL FTY720: Linear range : 0.063-178.01 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 15% Inter-day accuracy: < 9.6 %
PK Assessment	FTY720 in blood: Cmax, Tmax, T ½, AUC, AUCinf, λz, CL_T/F and Vz/F
Safety Assessment	Assessment of physical examination findings, vital signs, electrocardiogram (ECG), adverse events (AEs), pulmonary function, cardiopulmonary testing, hematology, serum chemistry, and urinalysis laboratory results.

Pharmacokinetic Results:

FTY720 pharmacokinetic in blood:

Mean FTY720 PK parameters after 0.25 mg to 3.5 mg of FTY720 single dose administration are shown in the following table:

Table 7.4.2-1.: Mean (CV%) pharmacokinetic parameters of FTY720

Dose mg	N	t_{max}** (hr)	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng*hr/mL)	$t_{1/2}$ (hr)	V_z/F (L)	Cl_T/F (mL/min)
0.25	4*	18 (12-36)	0.158 (12)	28 (26)	119 (43)	1489 (30)	155 (23)
0.5	6	18 (8-36)	0.282 (10)	42 (12)	89 (18)	1526 (13)	200 (12)
0.75	3	36 (12-36)	0.520 (21)	91 (17)	93 (21)	1116 (17)	140 (18)
1.0	3	36 (12-36)	0.713 (9)	120 (16)	104 (17)	1264 (7)	142 (18)
2.0	3	36 (12-36)	1.195 (22)	275 (33)	157 (12)	1737 (24)	130 (31)
3.5	3	12 (12-36)	2.823 (6)	434 (20)	100 (24)	1162 (7)	139 (22)
Overal	22	24 (8-36)	---	---	108 (30)	1407 (23)	158 (24)

*6 Subjects were dosed in this dose group. However, most of the FTY 720 blood concentrations of two of the subjects (#4 and #104) were below limit of quantitation. Due to this reason PK parameters of these two subjects were not calculated.

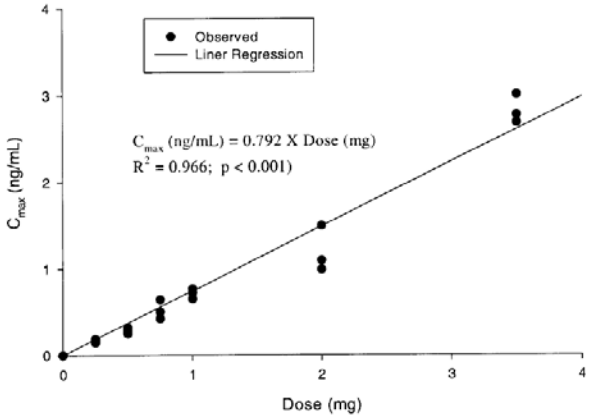
** Median (range)

- FTY720 was absorbed slowly with a lag time of 0.5 to 2 hours.
- Median Tmax ranged between 12 and 36 hours.
- FTY720 blood concentration declined monoexponentially and slowly with t1/2 ranging from 89 to 157 hours.
- T1/2 was found to be independent of dose.

Dose proportionality:

Relationship of FTY720 dose levels versus FTY720 Cmax is shown in the figure below (Similar results were observed for AUC therefore plot not shown):

Figure 7.4.2-2.: C_{max} versus Dose profile for FTY720



- Cmax and AUC increase proportionately with dose indication linear PK over 0.25 to 3.5 mg after single dose of FTY720.

Pharmacodynamics:

Table 7.5-1.: Summary of lymphocyte counts: average baseline (baseline, -1 hr, predose) and nadir.

Dose (mg)	n	Baseline Lymphocyte Count (cells / μL^3) Mean (Min,Max) SD	Nadir Lymphocyte Count (cells / μL^3) Mean (Min,Max) SD	Nadir Lymphocyte Count as Percent of Baseline Mean (Min,Max) SD
Placebo	8	2730 (1870,3417) 585	1728 (1500, 2100) 215	66 (51,87) 15
0.25	6	2284 (1477,3453) 690	1057 (490,1400) 524	48 (26,77) 15
0.5	6	2307 (1516,2977) 585	1127 (680,1500) 298	61 (36,67) 11
0.75	3	2457 (2163,2957) 435	1220 (880,1460) 303	53 (41,49) 9
1.0	3	2760 (2500,3213) 393	1203 (1020,1510) 267	44 (34,59) 13
2.0	3	2844 (2150,3323) 616	853 (660, 1120) 239	31 (22, 36) 8
3.5	3	3002 (2010, 4717) 1491	730 (490, 890) 212	27 (17, 39) 11

- After the dosing the lymphocyte count of placebo group dropped to become 66% of the baseline.
- All treated groups manifested a more pronounced decrease with a mean nadir ranged approximately 500-1000 cells/uL^3.

- At doses of 1 to 3.5 mg, a dose response became apparent.
- The maximal decrease in lymphocyte count was seen in the 3.5 mg treatment group in which the mean lymphocyte count dropped from 302 to 730.

Similar results were observed for lymphocyte subsets therefore data not shown.

Table 7.5-3. Mean percentage of cells by leukocyte subset, time post dose and treatment.

		Time				
Treatment	Data	-12	12	24	48	96
Placebo	T-memory	742	773	838	877	893
	T-naive	644	523	615	645	640
	NK-cells	173	213	266	203	198
	Monocytes	526	533	576	604	429
	Granulocyte	5106	5059	4795	4324	4774
0.25 mg	T-memory	541	442	478	581	683
	T-naive	531	298	495	592	580
	NK-cells	169	165	133	147	139
	Monocytes	464	531	431	480	416
	Granulocyte	4100	4248	2813	3510	3420
0.5 mg	T-memory	737	460	597	587	672
	T-naive	506	169	348	370	443
	NK-cells	229	138	198	165	166
	Monocytes	493	399	406	390	353
	Granulocyte	3752	4135	3977	3517	3760
0.75 mg	T-memory	687	312	520	571	579
	T-naive	559	138	429	496	433
	NK-cells	116	109	168	143	169
	Monocytes	603	400	477	520	503
	Granulocyte	5233	7257	5003	5457	5067
1.0 mg	T-memory	716	449	599	654	661
	T-naive	684	230	434	439	604
	NK-cells	202	129	280	294	252
	Monocytes	327	407	600	670	390
	Granulocyte	3957	5633	4283	3813	3767
2.0 mg	T-memory	703	550	648	620	723
	T-naive	554	109	292	371	408
	NK-cells	140	241	215	231	197
	Monocytes	413	367	400	623	497
	Granulocyte	5653	6613	4697	5113	4640
3.5 mg	T-memory	1504	255	282	333	347
	T-naive	1551	122	149	188	199
	NK-cells	376	132	136	309	219
	Monocytes	780	270	297	397	357
	Granulocyte	5710	4250	3653	3440	3933

Note: N=3-6 for all cells except the -12 hr / 3.5 mg cell, in which N=1 (due to missing data)

- Monocyte remained stable and within normal range in all groups.

Conclusions:

- FTY720 manifested unique PK with a slow and broad absorption, a high volume of distribution and a long half-life.
- Both Cmax and AUC were linear over the doses of 0.25mg to 3.5 mg single dose.
- Single FTY720 dose resulted in a reduction of lymphocyte count and was found to be dose dependent with the maximal reduction to 27% of the baseline at the 3.5 mg group.
- The lymphocyte effect was first measured at 6 hours after the dose and lasted 24 to 72 hours.
- Monocyte counts were not affected by FTY720.

Study CFTY720A B102*:* **A multicenter, randomized, double-blind, placebo-controlled, time-lagged, ascending, multiple oral dose pharmacokinetic, safety, and tolerability study of FTY720 capsules in stable renal transplant patients**

A brief overview of some essential components of the study design is given below:

<table>
<tr><td>Study Design</td><td>randomized, double-blind, ascending, multiple oral dose, placebo-controlled study</td></tr>
<tr><td>Study Population</td><td>N=65 enrolled, 11 re-enrolled
Table 7-3. Patient demographics (safety population)
<table>
<tr><th>Demographic variable</th><th>Total FTY720 (N=61)</th><th>Placebo (N=15)</th></tr>
<tr><td>Age (years)</td><td></td><td></td></tr>
<tr><td>Mean ± SD</td><td>47.4 ± 11.20</td><td>42.5 ± 11.82</td></tr>
<tr><td>Median</td><td>50.0</td><td>44.0</td></tr>
<tr><td>Range</td><td>20 – 63</td><td>27 – 63</td></tr>
<tr><td>Sex, n (%)</td><td></td><td></td></tr>
<tr><td>Male</td><td>35 (57.4%)</td><td>9 (60.0%)</td></tr>
<tr><td>Female</td><td>26 (42.6%)</td><td>6 (40.0%)</td></tr>
<tr><td>Race, n (%)</td><td></td><td></td></tr>
<tr><td>Caucasian</td><td>34 (55.7%)</td><td>5 (33.3%)</td></tr>
<tr><td>Black</td><td>6 (9.8%)</td><td>4 (26.7%)</td></tr>
<tr><td>Oriental</td><td>0</td><td>1 (6.7%)</td></tr>
<tr><td>Other</td><td>21 (34.4%)</td><td>5 (33.3%)</td></tr>
<tr><td>Weight (kg)</td><td></td><td></td></tr>
<tr><td>Mean ± SD</td><td>80.68 ± 13.508</td><td>74.45 ± 15.819</td></tr>
<tr><td>Median</td><td>80.00</td><td>73.30</td></tr>
<tr><td>Range</td><td>55.5 – 126.4</td><td>47.9 – 106.7</td></tr>
<tr><td>Height (cm) [1]</td><td></td><td></td></tr>
<tr><td>Mean ± SD</td><td>167.96 ± 10.857</td><td>165.87 ± 12.761</td></tr>
<tr><td>Median</td><td>168.50</td><td>172.00</td></tr>
<tr><td>Range</td><td>137.0 – 185.0</td><td>147.0 – 181.0</td></tr>
</table>
Source: Post-text table 7.4-1.
[1] Data are not available for one patient in the total FTY720 group.</td></tr>
<tr><td>Dosage and Administration</td><td>Maintenance kidney transplant patients on an immunosuppressive regimen of cyclosporine and prednisone received once daily oral administration of FTY720 for 28 days at dose levels of 0.125, 0.25, 0.5, 1, 2.5 and 5 mg/day or placebo.

Study drugs were given each morning 30 to 60 minutes prior to food intake on days 1 through 28.
<table>
<tr><th>Study medication</th><th>Formulation no.</th><th>Batch no.</th></tr>
<tr><td>FTY720 0.125 mg capsules</td><td>3754553.00.001</td><td>X167 0998</td></tr>
<tr><td>FTY720 0.25 mg capsules</td><td>3753912.00.001</td><td>X135 0898
X081 0498</td></tr>
<tr><td>FTY720 1.0 mg capsules</td><td>3754538.00.001</td><td>X168 0998</td></tr>
<tr><td>FTY720 2.5 mg capsules</td><td>3752938.00.001</td><td>X137 0898
X082 0498</td></tr>
<tr><td>Placebo</td><td>3704898.00.002</td><td>X017 0396
X134 0898
X080 0498</td></tr>
</table></td></tr>
<tr><td>Sampling: Blood</td><td>Pharmacokinetic visits took place on days 0, 1, 2, 3, 4, 7, 10, 14, 17, 21, 24, and 28. On days 1 and 28 a 24-hour AUC profile for FTY720 was obtained consisting of blood samples predose and then 2, 4, 6, 8, 12, 16, and 24 hours postdose.</td></tr>
</table>

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.025 ng/mL M2 and M3 0.3 ng/mL FTY720: Linear range : 0.025-15 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 8.2% Inter-day accuracy: < 4 % M2: Linear range : 0.3-20 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 10% Inter-day accuracy: < 7.4 % M3: Linear range : 0.3-20 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 14.9% Inter-day accuracy: < 1.8 %
PK Assessment	FTY720, M2, M3 and cyclosporine in blood: C0b, tmax , Cmax,b, AUCτ,b , Cavg,b , PTF, CLb/F, R, t1/2
PD Assessment	Predose count, Nadir count , Time of nadir, AUE and Recovery rate
Safety Assessment	**Safety:** Safety assessments consisted of monitoring all adverse events, laboratory assessments (hematology, blood chemistry and urine), vital signs, physical examinations, pulmonary function tests, exercise oximetry, chest X-ray, electrocardiograms (ECGs), echocardiograms, ophthalmological examinations and blood pressure and pulse monitoring.

Pharmacokinetic Results:

FTY720 pharmacokinetic in blood:

First-dose exposure

Mean FTY720 PK parameters after first dose of 0.125 mg to 5 mg FTY720 are shown in the following table:

Table 6-1 FTY720 pharmacokinetics: first-dose

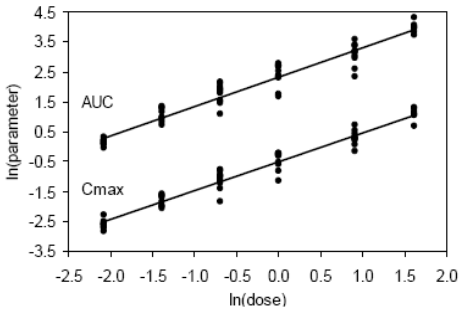
Parameter	0.125 mg	0.25 mg	0.5 mg	1 mg	2.5 mg	5 mg
N	9	8	12	10	10	7
t_{max} (h)	12 (6-24)	12 (8-24)	14 (6-24)	20 (6-25)	12 (4-23)	12 (4-16)
$C_{max,b}$ (ng/ml)	0.08 ± 0.01	0.17 ± 0.03	0.35 ± 0.09	0.65 ± 0.17	1.37 ± 0.33	3.02 ± 0.58
$C_{max,b}$/dose (ng/ml/mg)	0.60 ± 0.10	0.66 ± 0.12	0.70 ± 0.18	0.65 ± 0.17	0.55 ± 0.13	0.60 ± 0.12
$AUC_{\tau,b}$ (ng.h/ml)	1.2 ± 0.1	3.0 ± 0.7	6.1 ± 1.7	11.5 ± 3.8	23.0 ± 7.5	54.4 ± 10.8
$AUC_{\tau,b}$/dose (ng.h/ml/mg)	9.2 ± 0.9	11.9 ± 2.7	12.2 ± 3.5	11.5 ± 3.8	9.2 ± 3.0	10.9 ± 2.2

Values are mean ± sd except for tmax which is median (range).
Data source: Post-text Table 1, Table 2.

- Blood concentrations rose over the first 12 hours postdose and thereafter entered a plateau-like region up to 24 hours before the next dose was administered.

Dose proportionality:

Relationship of FTY720 dose levels versus FTY720 PK parameters after single dose is shown in the figure below:



FTY720 dose versus first-dose Cmax and AUC in natural logarithmic (ln) coordinates. Shown are the linear regression lines. Additional plots are in Post-text Figure 1 and regression statistics are in Post-text Table 3.

Linear regression for dose proportionality testing after single dose is shown below:

FTY720 dose-proportionality testing

Table 3 (Part 1 of 4)

Estimate of the slope for the linear regression between FTY720 log-pk parameter and log-dose: day 1

Parameter	Slope estimate	Lower 90% confidence limit	Upper 90% confidence limit	Dose proportionality across the whole dose range?*
AUC(0-24) [h.ng/ml]	0.985	0.928	1.043	yes
Cmax [ng/ml]	0.969	0.922	1.015	yes

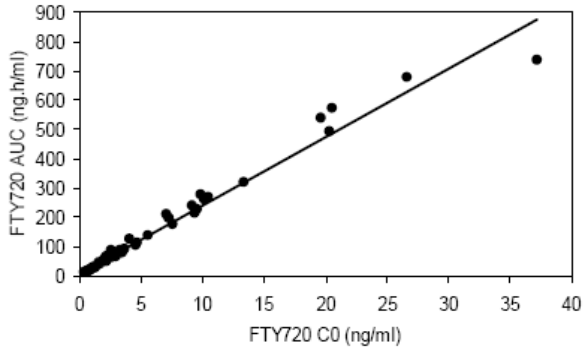
* dose range = ratio highest to lowest dose = 4

The critical region for the 90% confidence interval for the slope in order to conclude dose-proportionality across the dose range considered is 0.839, 1.161.

- Cmax,b rose in a dose proportional manner as indicated by the power model slope (90%CI) of 0.969 (0.922 - 1.015) that was close to unity.
- Intersubject variability in Cmax,b/dose across all patients was moderate at 23 percent.
- AUCτ,b also rose in a dose-proportional manner with a slope (90%CI) from the power model of 0.985 (0.928 – 1.043) near unity.
- Intersubject variability in AUCτ,b/dose was moderate at 28 percent.

Trough levels: accumulation, steady state, and exposure-marker

Relationship of FTY720 C0,ss versus FTY720 AUC on day 28 is shown in the figure below:



Linear regression of steady-state FTY720 C0 versus AUC in 49 evaluable patients on day 28. Shown is the linear regression line for which the equation is AUC = 23.2 x C0 + 10.3 (r^2 = 0.974).

Steady-state C0 and C24 over the dose range of 0.125 mg to 5 mg are shown in the figure below:

Table 6-2 FTY720 pharmacokinetics: accumulation and reaching steady state

Parameter	0.125 mg	0.25 mg	0.5 mg	1 mg	2.5 mg	5 mg
N	9	8	9	9	9	5
R	15.2 ± 3.0	11.4 ± 3.0	10.9 ± 2.7	10.5 ± 2.8	9.8 ± 4.9	9.8 ± 1.9
$C0_b^{55}$ (ng/ml)	0.7 ± 0.2	1.4 ± 0.4	2.9 ± 0.8	5.2 ± 2.4	9.3 ± 5.1	23.5 ± 9.0
$C24_b^{55}$ (ng/ml)	0.7 ± 0.2	1.4 ± 0.4	3.1 ± 1.0	5.2 ± 2.6	9.8 ± 4.5	24.4 ± 7.3

Values are mean ± sd. Data source: Post-text Table 2, Table 4.

- The accumulation index based on the AUCτ,b on day 1 versus day 28 appeared dose-independent and averaged 11.4 ± 3.7 across doses.
- The FTY720 concentrations at the beginning (C0b) and end (C24b) of the dose interval on day 28 were very similar indicating ~ 90% of steady state exposure has been reached.xr14i
- C0b, ss was well correlated with AUCτ,b, ss on day 28.
- C0b,ss ranged from 0.35 to 37.18 ng/ml and yielded a coefficient of determination (r2) to the AUCτ,b ss of 0.974.

Day 28 exposure

FTY720 exposure in blood:

Mean FTY720 steady-state PK parameters are shown in the following table:

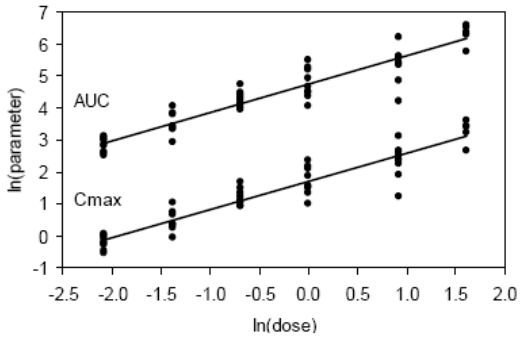
Table 6-3 FTY720 pharmacokinetics: day 28

Parameter	0.125 mg	0.25 mg	0.5 mg	1 mg	2.5 mg	5 mg
N	9	8	9	9	9	5
$C0_b^{ss}$ (ng/ml)	0.7 ± 0.2	1.4 ± 0.4	2.9 ± 0.8	5.2 ± 2.4	9.3 ± 5.1	23.5 ± 9.0
t_{max} (h)	6 (1-24)	8 (1-12)	6 (3-24)	9 (6-13)	7 (2-8)	8 (2-12)
$C_{max,b}^{ss}$ (ng/ml)	0.8 ± 0.2	1.7 ± 0.6	3.4 ± 1.0	6.0 ± 2.6	11.6 ± 5.5	27.5 ± 8.6
$C_{max,b}^{ss}$/dose (ng.h/ml/mg)	6.7 ± 1.4	6.7 ± 2.3	6.9 ± 1.9	6.0 ± 2.6	4.6 ± 2.2	5.5 ± 1.7
$AUC_{\tau,b}^{ss}$ (ng.h/ml)	18 ± 4	35 ± 13	73 ± 19	130 ± 62	238 ± 117	568 ± 160
$AUC_{\tau,b}^{ss}$/dose (ng.h/ml/mg)	140 ± 30	142 ± 51	147 ± 39	130 ± 62	95 ± 47	114 ± 32
$C_{avg,b}^{ss}$ (ng/ml)	0.7 ± 0.2	1.5 ± 0.5	3.1 ± 0.8	5.4 ± 2.6	9.9 ± 4.9	23.7 ± 6.7
PTF (%)	24 ± 19	21 ± 12	19 ± 13	17 ± 9	27 ± 14	18 ± 16
CL_b^{ss}/F (L/h)	7.5 ± 1.7	7.9 ± 2.9	7.2 ± 1.7	9.3 ± 4.3	13.8 ± 9.5	9.6 ± 3.6
$t_{1/2}$ (days)	7.1 ± 1.5	7.7 ± 1.5	9.0 ± 3.1	8.0 ± 2.2	9.4 ± 3.4	9.3 ± 2.6

Values are mean ± sd except for tmax which is median (range).

Data source: Post-text Table 2, Table 5.

Relationship of FTY720 dose levels versus FTY720 PK parameters at steady-state is shown in the figure below:



FTY720 dose versus steady-state Cmax and AUC. Shown are the linear regression lines. Additional plots are in Post-text Figure 1 and regression statistics in Post-text Table 3.

Linear regression for dose proportionality testing at steady-state is shown below:

Estimate of the slope for the linear regression between FTY720 log-pk parameter and log-dose: day 28

Parameter	Slope estimate	Lower 90% confidence limit	Upper 90% confidence limit	Dose proportionality across the whole dose range?*
AUC(0-24) [h.ng/ml]	0.881	0.804	0.958	no
C0 [ng/ml]	0.890	0.803	0.977	no
Cmax [ng/ml]	0.885	0.810	0.960	no

* dose range = ratio highest to lowest dose = 4

The critical region for the 90% confidence interval for the slope in order toconclude dose-proportionality across the dose range considered is 0.839, 1.161.

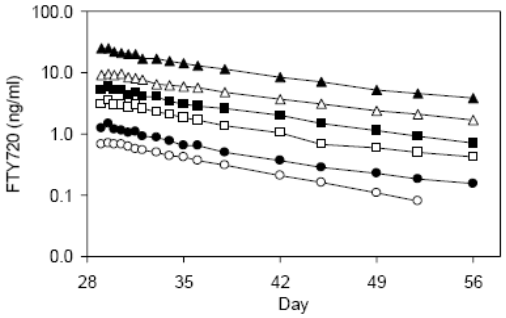
- Predose trough blood levels showed a -11 percent deviation from dose-proportionality based on the power model slope of 0.890 (90%CI, 0.803 – 0.977).
- Blood concentrations over the 24-hour dose interval were very flat giving rise to a shallow peak-trough fluctuation averaging 20 percent.
- Cmax,b,ss was underproportional by -12 percent based on the power model exponent of 0.885 (90%CI, 0.810 – 0.960).

- AUCτ,b exhibited an under-proportionality with dose by -12 percent based on the power model exponent: 0.881 (90%CI, 0.804 – 0.958).
- Apparent oral clearance averaged 9.2 ± 5.2 L/h.

Washout:

Mean FTY720 concentrations during the washout period are shown below:

Figure 6-4 FTY720 concentrations during the washout period



Mean FTY720 concentrations during the washout period after treatment with 0.125 mg/day (*open circles*), 0.25 mg/day (*filled circles*), 0.5 mg/day (*open squares*), 1 mg/day (*filled squares*), 2.5 mg/day (*open triangles*), and 5 mg/day (*filled triangles*).

- The elimination half-life appeared dose-independent and, when averaged across all dose levels, was 8.4 ± 2.6 days corresponding to 202 ± 62 hours.

FTY720 metabolites:

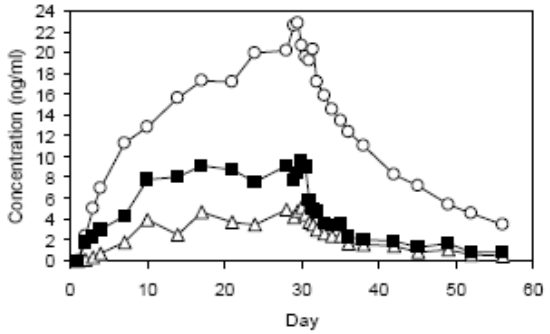
Mean PK parameters of FTY720 metabolites are shown in the following table:

Table 6-4 FTY720 metabolite pharmacokinetics

Parameter/analyte	0.5 mg/day	1 mg/day	2.5 mg/day	5 mg/day
N	4	3	3	3
$AUC_{\tau,b}$ (ng.h/ml)				
FTY720	77 ± 26	204 ± 31	219 ± 79	522 ± 184
M2	--	--	40 ± 21	113 ± 110
M3	20 ± 8	35 ± 7	106 ± 87	237 ± 74
$AUC_{\tau,b}$-ratio				
M2/FTY720	--	--	0.15 ± 0.08	0.21 ± 0.18
M3/FTY720	0.28 ± 0.13	0.17 ± 0.04	0.47 ± 0.29	0.52 ± 0.30
$t_{1/2}$ (days)				
FTY720	7.9 ± 2.7	10.8 ± 0.4	7.6 ± 1.2	9.5 ± 2.7
M2	--	--	12.4 ± 7.0	7.3 ± 4.0
M3	--	12.2 ± 4.7	10.0 ± 2.6	8.1 ± 3.1

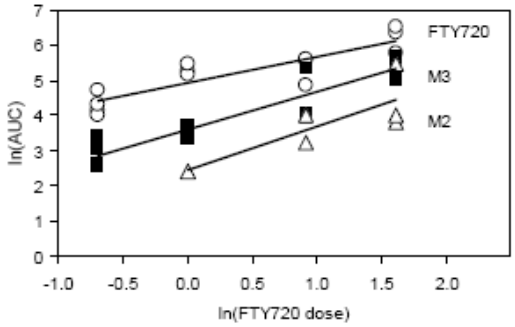
Data are mean ± sd. Data source: Post-text Table 6, Table 7.

Mean FTY720, M2 and M3 concentration-time profiles are shown in the figure below:



Mean morning concentrations of FTY720 (*open circles*), metabolite 3 (*filled squares*), and metabolite 2 (*open triangles*) during treatment with 5 mg/day FTY720 in 3 patients from day 1 to 28. Plots at all dose levels are in Post-text Figure 3.

Relationships between FTY720 dose versus AUC of FTY720, M2 and M3 are shown in the figure below:



Dose versus AUC relationships for FTY720 and metabolites 2 and 3 on day 28. Shown are the linear regression lines.

Hexanoic acid metabolite (M2)

- M2 was not quantifiable at doses of 0.125, 0.25, or 0.5 mg/day. At 1 and 2.5 mg/day, concentrations were quantifiable from week 2 through 4 but not consistently across patients in the washout phase. At 5 mg/day concentrations were quantifiable throughout the study duration.
- The M2 AUCτ,b,ss on day 28 rose with dose over the range 1 to 5 mg/day roughly in parallel with the dose-AUC relationship of FTY720.
- Based on the steady-state M2/FTY720 AUCratio, exposure to the M2 was about 20 percent of the corresponding exposure to FTY720.
- The terminal decline in M2 blood concentrations paralleled that of FTY720 yielding a similar half-life.

Butanoic acid metabolite (M3)

- M3 was not quantifiable at 0.125 or 0.25 mg/day. It was quantifiable at 0.5 mg/day only between week 2 and 4. At higher doses it was quantifiable from week 1 through the washout phase. At 5 mg/day concentrations were quantifiable throughout the full study duration.
- The M3 AUCτ,b,ss on day 28 rose with dose over the range 0.5 to 5 mg/day in parallel with the dose-AUC relationship of FTY720.
- Based on the steady-state M3/FTY720 AUC-ratio at the higher dose levels, exposure to the M3 was about 50 percent of the corresponding exposure to FTY720.

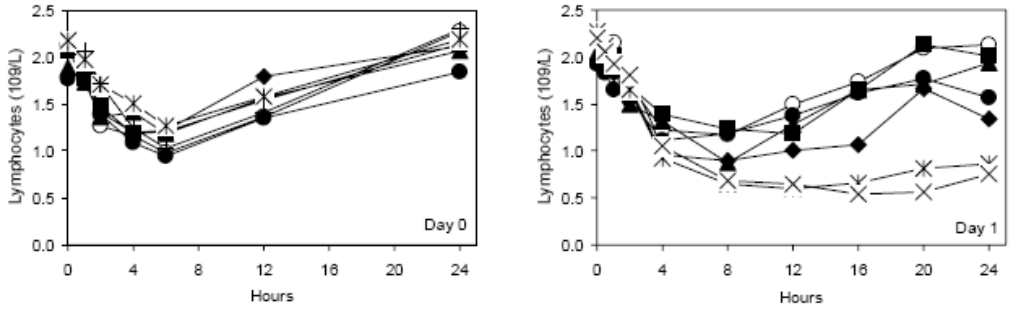
- The terminal decline in M3e blood concentrations paralleled that of FTY720 yielding a similar half-life.

Pharmacodynamics results:

Lymphocyte response: Baseline and Acute response

Mean lymphocyte trajectories are shown in the figure below:

Figure 6-6 Lymphocyte trajectories on days 0 and 1



Mean lymphocyte counts over 24 hours on day 0 after a placebo dose and on day 1 after the first dose of placebo (*open circles*) or FTY720 0.125 mg (*filled circles*), 0.25 mg (*filled squares*), 0.5 mg (*filled triangles*), 1 mg (*filled diamonds*), 2.5 mg (*stars*), 5 mg (*X*).

Baseline.

- In placebo, lymphocytes displayed the conventional circadian rhythm with an average 45% decline in the morning from a predose count of 1.63 x 10^9/L to a nadir at around noon (6 hours postdose) of 0.88 x 10^9/L. In the afternoon and evening there was a rise in lymphocyte counts. The same temporal pattern with similar magnitudes was seen in all FTY720 groups

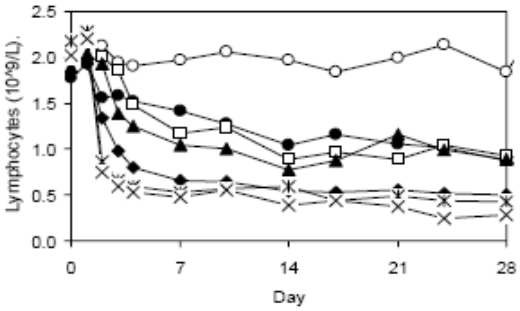
Acute response.

- On day 1, mean predose lymphocyte counts were similar across groups.
- Mean lymphocyte counts in placebo-treated patients demonstrated the same circadian pattern as on day 0.
- Mean lymphocyte counts in patients receiving FTY720 at doses of 0.125 and 0.25 mg/day did not notably differ from placebo.
- At higher doses the nadir count decreased from baseline in a dose-dependent manner by 52% at 0.5 mg/day, 57% at 1 mg/day, 72% at 2.5 mg/day, and 75% at 5 mg/day.
- The nadir in these dose groups also tended to occur progressively later on day 1 with increasing dose.

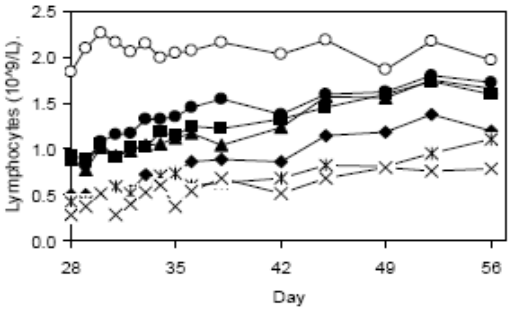
Chronic response and recovery

Mean lymphocyte counts during the treatment period and post-treatment are shown in the following figure:

Figure 6-7 Chronic lymphocyte responses and posttreatment recovery



Mean lymphocyte trajectories over the 28-day treatment period. Symbols as in Figure 6-6.



Mean lymphocyte counts during the month after treatment. Symbols as in Figure 6-6.

Chronic response.

- During maintenance daily dosing there was a continuing decrease in lymphocyte counts in all treatment arms by about 20% from the nadir observed on day 1.
- By day 29 the morning mean lymphocyte count decrease from the day 1 baseline was 50% at 0.125 and 0.25 mg/day, 57% at 0.5 mg/day, 70% at 1 mg/day, 77% at 2.5 mg/day, and 79% at 5 mg/day.

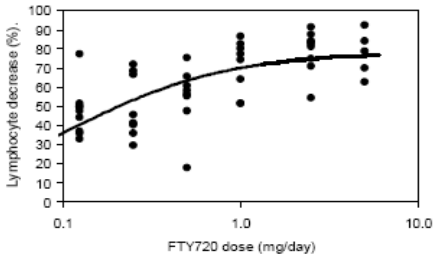
Recovery.

- The return of lymphocytes to blood was evident within a few days of stopping FTY720 treatment at all dose levels.
- The recovery rate was estimated using linear regression on the mean trajectory at each dose level from day 29 to 56.
- At doses between 0.125 and 1 mg/day, the rates were similar at about 0.030 x 10^9/L per day but progressively slower at higher doses with rates of 0.021 and 0.16 x 10^9/L per day at 2.5 and 5 mg/day, respectively.
- Return to baseline was evident by week 7 or 8 at doses of 0.5 mg/day and lower but baseline was not achieved over the follow up period at dose levels between 1 and 5 mg/day.
- At these doses, mean lymphocyte counts were 31% (1 mg/day), 39% (2.5 mg/day), and 56% (5 mg/day) compared with day 1 baseline means.

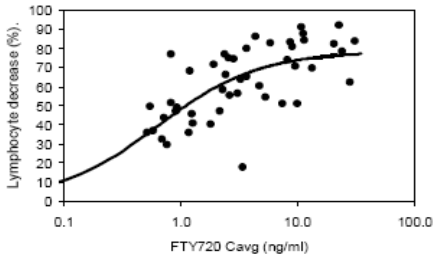
Dose- and exposure-response relationships

The relationships between FTY720 dose and the percent decrease from baseline in lymphocyte count on day 29 were described with a simple Emax model.

Figure 6-8 FTY720 dose and exposure versus lymphocyte responses

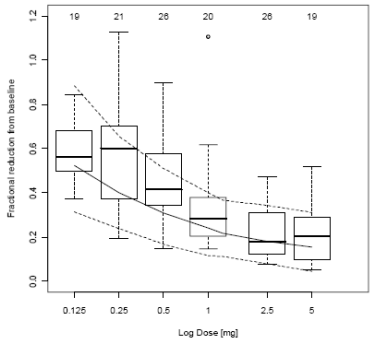


Scatterplot of FTY720 dose versus perecent decrease from baseline in lymphocyte count at day 29. The line is the fit of an Emax model to the data.



As in the left panel for FTY720 average concentration on day 29.

Figure 5-5 **Predicted dose-response superimposed on the distribution of lymphocyte response data at each dose level in study B102**



Post-text Figure 9.2.22; The box plots characterize the distribution of the lymphocyte responses in each cohort in study FTY270A-B102. The numbers are the top are the sizes of the respective cohorts. The solid line is the predicted median dose-response based on a simulation of 500 healthy volunteers at each dose level; the dashed lines are the 5th and 95th percentiles

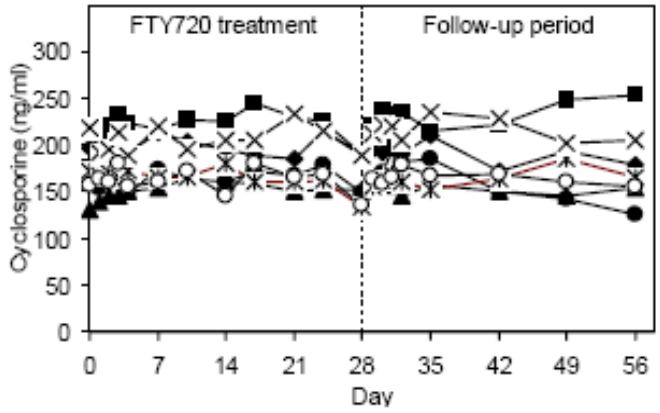
- EC_{50} was 0.12 ± 0.03 mg/day and the maximum lymphocyte decrease from baseline (E_{max}) was 78 ± 4 percent.
- The corresponding relationship for exposure using FTY720 $C_{avg,b,\ ss}$ yielded the parameters: EC_{50} 0.63 ± 0.16 ng/ml and E_{max} 79 ± 4 percent.

Cyclosporine Pharmacokinetics:

Dosing and trough blood levels

Mean cyclosporine trough levels are shown in the following figure:

Figure 6-9 Cyclosporine trough blood levels



Mean cyclosporine trough blood levels over the study duration in patients receiving placebo (*open circles*) or FTY720 0.125 mg (*filled circles*), 0.25 mg (*filled squares*), 0.5 mg (*filled triangles*), 1 mg (*filled diamonds*), 2.5 mg (*stars*), 5 mg (*X*).

- Cyclosporine doses were unchanged during the study.
- Patients provided an average of 17.5 ± 2.5 trough blood samples from the 19 protocol scheduled visits.

- Mean trough blood levels remained stable in patients receiving placebo and at all FTY720 dose levels over the 4-week treatment period (days 0 to 28) and the 4-week washout period (days 29 to 56).

AUC profiles:

Ratios of geometric means and 90% confidence intervals for cyclosporine are shown below:

Ratios of geometric means and 90% confidence intervals for cyclosporine

Parameter	Day	Geometric mean	Ratio of geometric means	Lower 90%CI	Upper 90%CI
AUC(0-12)[h.ng/ml]	0	3728.9	0.967	0.898	1.041
	28	3855.5			
C0 [ng/ml]	0	133.09	0.978	0.888	1.076
	28	136.13			
C2 [ng/ml]	0	645.28	0.908	0.795	1.036
	28	710.87			
Cmax [ng/ml]	0	836.39	0.957	0.866	1.059
	28	873.54			

- Steady-state cyclosporine pharmacokinetics were unchanged after 28 days of FTY720 exposure compared with the pretreatment baseline.
- For the primary parameters, the ratio of the geometric means and 90% confidence intervals were: C0b,ss 0.978 (0.888 - 1.076); Cmax,b,ss 0.957 (0.866 - 1.059); C2b,ss 0.908 (0.795 - 1.036); and AUCτ,b,ss 0.967 (0.898 - 1.041).
- All tested parameters satisfied bioequivalence criteria with the exception of C2b,ss which slightly transgressed the lower 90% confidence bound.
- Regardless of the FTY720 dose level, cyclosporine day 28 to day 0 AUC-ratios were randomly distributed around unity.

Conclusions:

- FTY720 exposure after the first dose did not deviate to a clinically relevant extent from dose proportionality over the range 0.125 to 5 mg/day.
- Steady state exposure was reached after 1 month of daily dosing with an 11.4-fold accumulation.
- The elimination half-life averaged 8.4 days.
- Mean lymphocyte counts on day 28 exhibited a dose dependent decrease from baseline by 49% at doses of 0.125 and 0.25 mg/day to 79% at a dose of 5 mg/day.
- Cyclosporine pharmacokinetics were unaffected by steady-state coadministration of FTY720.

Reviewer's note:

The sponsor concluded that FTY720 exposure at steady state did not deviate to a clinically relevant extent from dose proportionality over the range 0.125 to 5 mg/day. This is controversy with their observation mentioned previously regarding dose proportionality after multiple doses where the sponsor stated that trough blood levels, C0,ss and AUC,ss showed a -11 to -12 percent deviation from dose-proportionality. In addition, when compare the dose normalized Cmax or AUC, there appears to be an under proportionality after multiple doses at doses greater than 0.5 mg.

Study CFTY720A0115*:* **A multicenter, open-label study to assess the pharmacokinetics, safety, and tolerability of single-dose FTY720 in pediatric stable renal transplant recipients**

The chosen dose was a mg-per-kg scaling of a 5-mg dose used in previous adult studies (5 mg/70 kg = 0.07 mg/kg).

A brief overview of some essential components of the study design is given below:

Study Design	Single-dose, open-label
Study Population	N=7 Age: 11-16 years (mean 14 years) Gender: 5 boys, 2 girls Weight: 37-67 kg (mean 53.8 kg) Race: 7 White (100%)
Dosage and Administration	All 7 patients received a single dose of 0.07 mg/kg FTY720. The dose was administered from the glass mixing vial or with an oral syringe followed by drinking 2 water-rinse cycles of the vial or syringe and an additional 50-200 mL of water. Patients fasted for 2 hours before dosing and a light breakfast was served 1 hour postdose. FTY720 0.5 mg capsules (FMI): KN 3752920.002, batch CSUS/2002-1257, bulk H-05930
Sampling: Blood	At predose (0 hour), and 2, 4, 6, 8, 12, 24 (day 2), 48 (day 3), 96 (day 5), 144 (day 7), 312 (day 14), 480 (day 21), and 648 hours (day 28) postdose
PD Sampling	**Lymphocytes** Blood samples for absolute lymphocyte counts were collected at screening, day 1 (0, 6, 12 hours), and days 2, 5, 7, 14, 21, and 28. Lymphocyte subsets were determined by flow cytometry on day 1 (0, 12 hours) and day 14. **Heart rate** Continuous 24-hour Holter monitoring was performed at the baseline and on day 1. The monitoring on day 1 began 2 hours before FTY720 administration until 24 hours postdose. Standard 12-lead ECGs were performed and acquired in digital format at screening, 6 hours postdose on day 1, and at end-of-study. An echocardiogram was performed predose (before Holter placement) and at 4 hours postdose on day 1.
Analysis (Blood)	Method: HPLC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 9.9% Inter-day accuracy: < 8.8 % FTY720-P: Linear range : 1-50 ng/mL in blood

	Inter-day Precision (%CV for Quality Controls) : < 4.5% Inter-day accuracy: < 4.5 %
PK Assessment	FTY720 in blood: Tmax, Cmax, AUC(0-tlast), AUC(0-inf), t1/2, CL/F and Vz/F FTY720-P in blood: Tlag, tmax, Cmax, and AUC(0-tlast)
PD Assessment	**Lymphocyte responses** The predose lymphocyte count, nadir count, time of nadir, and area under the effect-time curve from day 1-2 [AUEC(1-2 days)] and day 1-28 [AUEC(1-28 days)] were calculated. **Heart rate responses** The predose heart rate, nadir rate, time of nadir rate, and area under the effect-time curve from 0-4 hours [AUEC(0-4h)] and 0-24 hours [AUEC(0-24h)] were calculated.
Safety Assessment	Physical examinations, information on adverse events, standard laboratory parameters, vital signs, electrocardiography, Holter monitoring, and echocardiography

Pharmacokinetic Results:

FTY720 and FTY720-P pharmacokinetics in blood:

Descriptive statistics for FTY720 and FTY720-P blood PK parameters after single dose of 0.07 mg/kg FTY720 administration are shown in the following table:

Pharmacokinetic parameters

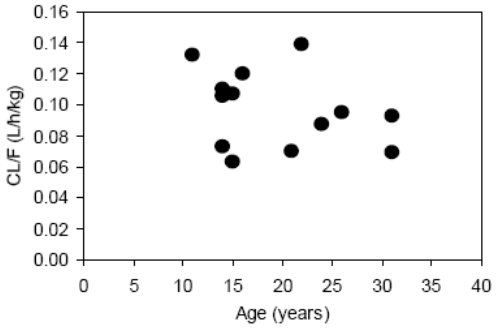
Parameter	Fingolimod		Fingolimod-phosphate	
	Adolescents	Adults	Adolescents	Adults
tmax (h)	8 (4-24)	12 (12-16)	6 (4-24)	12 (6-12)
Cmax (ng/mL)	3.6 ± 0.6	4.4 ± 0.9	3.2 ± 1.4	3.6 ± 0.8
AUC(0-tlast) (ng.h/mL)	675 ± 194	794 ± 250	100 ± 69	142 ± 72
AUC(0-inf) (ng.h/mL)	731 ± 240	861 ± 302	--	--
CL/F (L/h/kg)	0.10 ± 0.02	0.09 ± 0.03	--	--
Vz/F (L/kg)	22 ± 6	20 ± 5	--	--
t1/2 (days)	6.5 ± 1.9	6.7 ± 2.8	--	--

Data are mean ± sd except for tmax which is median (range)
Adult data (n = 6) are from study 2304 in which a fixed 5-mg dose was given averaging 0.07 ± 0.02 mg/kg.

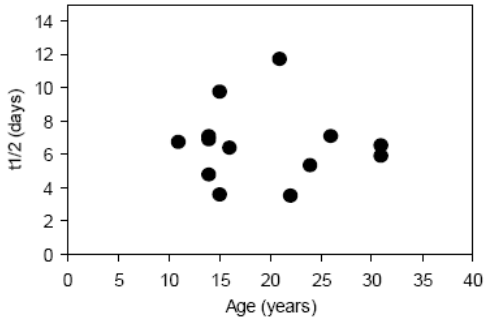
- The dose averaged 3.7 ± 0.8 mg (range, 2.5 – 4.5 mg).
- The adolescent pharmacokinetic data from this study were compared with data from study 2304 in which 6 healthy adults received a single 5 mg dose of FTY720.
- With a first postdose blood sample at 2 hours in adolescents and 1 hour in adults, no lag-time was observed for fingolimod appearance in blood.
- Mean fingolimod concentrations rose similarly in the two groups to 6 hours postdose.
- Peak concentrations occurred generally between 8 and 12 hours, but appeared slightly blunted in adolescents, averaging 19% lower than adults.
- AUC(0-inf), t1/2, CL/F per kg, and V/F per kg were similar in adolescents and adults.
- The lag-time for fingolimod-phosphate reflects the higher assay quantification limit for this analyte.
- Time to peak was generally earlier and peak concentrations were lower for fingolimod-P compared with fingolimod in both age groups.

- Overall exposure over the time period when fingolimod-P was quantifiable (to day 5) and similar in adolescents and adults.

Figure 7-2 Age-exposure relationships



Scatter plot of age versus weight-normalized apparent clearance of fingolimod.



As in left panel for fingolimod half-life.

- Once CL/F was scaled for body weight (L/h/kg), there was no age-dependence for this parameter in adolescents and adults (regression slope -0.001 L/h/kg per year; p = 0.3066).
- Lack of age-dependency for t1/2 (p = 0.9012) and Vz/F (p = 0.2606) were also observed .

Pharmacodynamics results:

Lymphocyte responses

Table 7-3 Lymphocyte response parameters

Response	Adolescents	Adults
Acute response:		
Predose count (10^9/L)	2.64 ± 1.04	1.62 ± 0.33
Nadir time (days)	2 (1.25 – 7)	18 (8-24)
Nadir count (10^9/L)	0.37 ± 0.17	0.41 ± 0.09
Nadir count (% predose)	15 ± 7	26 ± 5
AUEC(1-2 day) (10^9/L x day)	0.9 ± 0.3	0.7 ± 0.1
Recovery:		
Day 14 count (10^9/L)	1.10 ± 0.37	1.25 ± 0.45
Day 21 count (10^9/L)	1.67 ± 1.03	1.51 ± 0.28
Day 28 count (10^9/L)	2.34 ± 0.83	1.69 ± 0.43
AUEC(1-28 day) (10^9/L x day)	35 ± 14	33 ± 7

Data are mean ± sd except for nadir time which is median (range).
AUEC is the area under the effect-time curve.
Source data: Appendix 3 Table 3.3.3.5 and Appendix 4 Table 3.

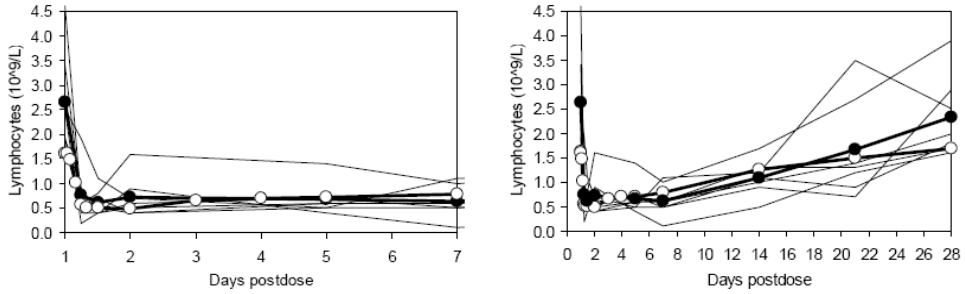
Table 6.3: Mean lymphocyte counts and percent reduction change from baseline

Visit	Absolute Lymphocyte Count		% Reduction	
	Mean (±SD)	%CV	Mean (±SD)	%CV
Baseline [1]				
DAY 1	2.6 (±1.04)	39.2		
Treatment				
DAY 1	0.7 (±0.42)	61.6	72.1 (±17.81)	24.7
DAY 2	0.7 (±0.42)	58.1	72.0 (±10.98)	15.2
DAY 5	0.7 (±0.34)	49.9	74.1 (±7.41)	10
DAY 7	0.6 (±0.33)	52.8	75.4 (±13.20)	17.5
DAY 14	1.1 (±0.37)	33.6	55.9 (±15.82)	28.3
DAY 21	1.7 (±1.03)	62	33.2 (±38.25)	115.2
DAY 28	2.3 (±0.83)	35.4	9.5 (±14.55)	152.9

[1] The absolute lymphocyte count at scheduled timepoint 0 was used for the baseline.

Mean lymphocyte trajectories are shown in the figure below:

Figure 7-3 Lymphocyte trajectories



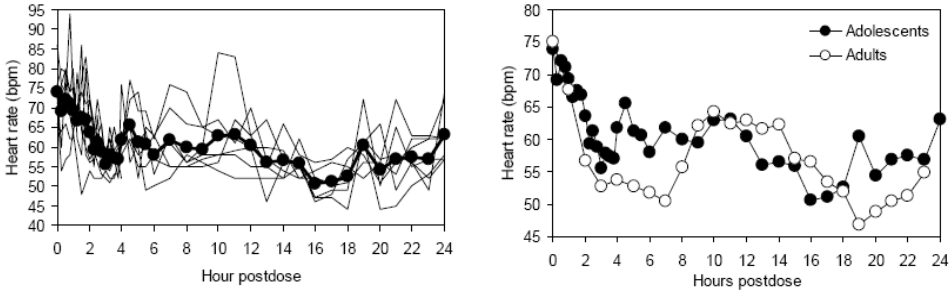
Lymphocyte trajectories to day 7 in individual adolescents (*thin lines*) with an overlay of the mean trajectories in adolescents (*filled circles*) and adults (*open circles*).

As in left panel showing the data over the full study course of 28 days.

- The nadir count was generally reached by 6 hours postdose (the first postdose blood sampling time point) and represented an average 85% decrease from baseline in adolescents versus 74% decrease in adults.
- Lymphocyte recovery was similar in adolescents and adults.
- A similar temporal pattern is evident for all subsets with decreased counts at 6 hours postdose and values indicative of recovery on day 14.
- There was no particular temporal pattern noted for monocytes (CD14+).

Heart rate responses

Figure 7-4 Heart rate trajectories



Individual heart rate trajectories in adolescents (*thin lines*) with an overlay of the mean trajectory (*filled circles*).

Mean heart rate trajectories in adolescents (*filled circles*) and adults (*open circles*).

- The heart rate trajectory for adolescents was slightly higher during the day but similar at night to adults; however there were no substantial differences in response parameters between 2 age groups.
- Mean heart rates in adolescents measured in the morning on day 7 was still slightly depressed (65 ± 4 bpm) compared with predose on day 1 (74 ± 6 bpm), but exhibited full recovery by the next visit on day 14 (75 ± 9 bpm).

Conclusions:

- Administration of an FTY720 dose scaled for body weight (0.07 mg per kg) yielded similar blood level exposure to fingolimod and fingolimod-phosphate in adolescents compared with adults who received 5 mg in a previous study.
- Apparent clearance and distribution volumes (both scaled for body weight per kg) and elimination half-life were similar between adolescents and adults.
- Decreases in lymphocyte counts and heart rate were similar in adolescents and adults as was their recovery back to baseline.

4.1-3. HUMAN PK STUDIES
4.1-3.3 Intrinsic factors

Study CFTY720A0112*:* **An open-label, single-dose, parallel-group study to compare the pharmacokinetics of FTY720 in subjects with mild, moderate and severe hepatic impairment with that in matched healthy control subjects**

No severe patients in this study via a protocol amendment.

A brief overview of some essential components of the study design is given below:

Study Design	Single-dose, open-label
Study Population	N=32 enrolled, 32 completed **Table 7-1 Demographic and clinical characteristics**

Characteristic	Mild impairment		Moderate impairment	
	Controls	Impaired	Controls	Impaired
Demographics:				
Sex	3M / 5F	3M / 5F	8M	8M
Age (yrs)	52.1 ± 7.9	53.3 ± 8.7	53.5 ± 9.0	52.5 ± 7.9
Weight (kg)	73.5 ± 16.1	73.9 ± 17.6	82.3 ± 7.6	83.9 ± 9.0
Height (cm)	162 ± 10	166 ± 11	173 ± 6	174 ± 6
Ethnicity	1W, 7O	4W, 1B, 3O	1W, 7O	1W, 7O
Hepatic function:				
Child-Pugh score (*n*)	--	5 (*2*), 6 (*6*)	--	7 (*6*), 8 (*1*), 9 (*1*)
Bilirubin (mg/dl)	0.5 ± 0.1	0.9 ± 0.3	0.7 ± 0.2	2.4 ± 1.3
Albumin (g/dl)	4.1 ± 0.1	4.3 ± 0.3	4.4 ± 0.2	3.6 ± 0.4
Prothrombin time (s)	9.8 ± 0.5	10.0 ± 0.3	10.2 ± 0.4	12.0 ± 1.1
Renal function:				
CLcr (ml/min/1.73m^2)	77 ± 15	69 ± 15	79 ± 20	80 ± 29

Values are mean ± sd.
Clinical laboratory parameters are derived from each subject's mean from screening and baseline.
Sex: *M* = male, *F* = female; Ethnicity: *W* = white, *B* = black, *O* = other.
CLcr = measured creatinine clearance from day 0
Source data: Appendix 3, Tables 1-1, 2-3, and 2-4; Appendix 6, Table 1.

Dosage and Administration	A total of 32 subjects were enrolled consisting of 8 subjects with mild hepatic impairment and their 8 matched controls and 8 subjects with moderate hepatic impairment and their 8 matched controls. All subjects received a single 1 mg oral dose of FTY720 after which safety assessments and pharmacokinetic blood and urine samples were collected up to 5 days postdose. Subjects were discharged from the study center and returned on days 7, 11, and 15 for safety and pharmacokinetic evaluations. The end-ofstudy evaluation was performed on day 15. Study drugs were given under fasted state with 200 mL of water. **Table 3.2. Study Groups and Treatments**

Group	Number of Males	Number of Females	First Dose Date	XP13512 Dose (mg)	Second Dose Date	Neurontin® Dose (mg)
1	4	4	11/8/03	350	11/15/03	200
	1	1	11/8/03	0	11/15/03	200
2	4	4	11/15/03	700	11/22/03	400
	1	1	11/15/03	0	11/22/03	400
3	4	4	11/22/03	1400	11/29/03	800
	1	1	11/22/03	0	11/29/03	800
4	3	5	11/29/03	2100	12/6/03	1200
	1	1	11/29/03	0	12/6/03	1200
5	4	4	12/6/03	2800	12/13/03	1400
	1	1	12/6/03	0	12/13/03	1400

	Lot No. FTY720 1 mg capsules: Batch X1680998, KN 3754538.00.001 Diet: Subjects fasted for approximately 10 hours before dosing and 4 hours after dose. No grapefruit or grapefruit juice could be consumed during the 15-day study duration. Consumption of xanthene containing food or beverages was to be discontinued 10 hours before dosing. Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood	At predose (0 hour), and 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 192, 240, and 336 hours.
Sampling: Urine	Daily urine collections were performed on days 1-4, 8, 11, and 14.
PD measurement	Peripheral blood lymphocyte counts were obtained determined from morning blood samples drawn at baseline and days 1, 2, 3, 4, 5, 7, 11, and 15. Supine heart rate was recorded at baseline, before FTY720 administration, and 1, 2, 4, 8, 12, 24, 48, 72, and 96 hours postdose.
Analysis (Blood)	Method : LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.05 ng/mL M2 0.1 ng/mL M3 0.1 ng/mL FTY720: Linear range : 0.05-15 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 9.5% Inter-day accuracy: < 4.6 % M2: Linear range : 0.1-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 10.5 % Inter-day accuracy: < 8.6 % M3: Linear range : 0.1-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 12.6% Inter-day accuracy: < 3.8 %
Analysis (Urine)	Method: LC/MS/MS Lower Limits of Quantitation Urine FTY720 0.1 ng/mL M2 0.1 ng/mL M3 0.1 ng/mL FTY720: Linear range : 0.1-50 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 8.4 % Inter-day accuracy: < 17.5 % M2:

	Linear range : 0.1-25 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 10 % Inter-day accuracy: < 5.2 % <u>M3:</u> Linear range : 0.1-25 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 10.6% Inter-day accuracy: < 10.7 %
PK Assessment	FTY720, M2 and M3 in blood and urine: Tmax, Cmax,b, Cap,b, Tap, Tdur, AUC(0-tz)b, AUCb, CLb/F, Vz,b/F, Vz,b/F, t1/2, Ae(0-t), Ae(0-inf) and CLR.
PD Assessment	Derived response parameters for peripheral blood lymphocyte counts and supine heart rate included the nadir value, time to reach the nadir, and area-under-the-effect curve.
Safety Assessment	Assessment of physical examination, vital signs, ECG, clinical laboratory parameters (biochemistry, hematology, urinalysis), lymphocyte counts, pregnancy test for women, and adverse events.

Pharmacokinetic Results:

<u>FTY720 pharmacokinetic in blood and urine:</u>

Mean FTY720 pharmacokinetic parameters are summarized in the following table:

Table 7-4 FTY720 pharmacokinetic parameters

Parameter	Mild impairment		Moderate impairment	
	Controls	Impaired	Controls	Impaired
Peak exposure:				
$C_{max,b}$ (ng/ml)	0.70 ± 0.20	0.65 ± 0.12	0.57 ± 0.10	0.57 ± 0.10
t_{max} (h)	12 (8 – 36)	12 (6 – 36)	12 (8 – 48)	24 (8 – 48)
$C_{ap,b}$ (ng/ml)	0.64 ± 0.19	0.59 ± 0.11	0.53 ± 0.09	0.52 ± 0.09
t_{ap} (h)	12 (10 – 24)	12 (10 – 24)	18 (10 – 24)	24 (18 - 48)
$t_{ap,dur}$ (h)	28 (16 – 30)	29 (16 – 40)	28 (24 – 42)	40 (12 – 60)
Total exposure:				
$AUC(0\text{-}tz)_b$ (ng.h/ml)	83 ± 25	86 ± 27	75 ± 16	94 ± 21
AUC_b (ng.h/ml)	93 ± 29	105 ± 39	89 ± 22	131 ± 45
CL_b/F (L/h)	11.9 ± 4.4	10.6 ± 3.4	11.9 ± 2.9	8.5 ± 3.4
$V_{z,b}/F$ (L)	1564 ± 484	1667 ± 348	1789 ± 557	1794 ± 351
$t_{1/2}$ (days)	4.0 ± 1.1	4.9 ± 1.7	4.6 ± 1.8	6.7 ± 2.5
Protein binding:				
Fraction unbound (%)	0.156 ± 0.024	0.171 ± 0.025	0.144 ± 0.016	0.187 ± 0.019
Fraction bound (%)	99.84 ± 0.02	99.83 ± 0.03	99.86 ± 0.02	99.81 ± 0.02
Urinary excretion:				
Ae(0-t) (mcg)	0	0	0	0

Data are mean ± sd except for time parameters which are median (range).
Source data: Appendix 4, Tables 1, 2, 3 and Appendix 6, Table 1.

Statistics for the PK parameter comparisons are shown below:

Table 1 Effect of hepatic impairment on PK and PD responses to FTY720 dosing

Parameters	Comparison Test (N)	Comparison Ref. (N)	Arithmetic Means Test (STD)	Arithmetic Means Ref. (STD)	p-value	Ratio of geometric means	90% CI for ratio
FTY Cmax (ng/mL)	A (8)	C (8)	0.7 (0.1)	0.7 (0.2)	0.526	0.95	(0.83, 1.09)
	B (8)	D (8)	0.6 (0.1)	0.6 (0.1)	0.938	1.01	(0.87, 1.16)
FTY AUC(0-t) (ng.h/mL)	A (8)	C (8)	85.6 (27.2)	82.6 (25.4)	0.751	1.04	(0.84, 1.29)
	B (8)	D (8)	93.9 (20.6)	75.3 (16.2)	0.099	1.24	(1.00, 1.54)
FTY AUC(0-inf) (ng.h/mL)	A (8)	C (8)	104.9 (38.7)	93.4 (29.1)	0.493	1.12	(0.85, 1.47)
	B (8)	D (8)	131.3 (44.5)	88.5 (22.1)	0.034	1.44	(1.10, 1.90)
FTY Volume (L)	A (8)	C (8)	1667.1 (347.9)	1564.0 (484.2)	0.397	1.10	(0.91, 1.34)
	B (8)	D (8)	1794.1 (351.2)	1789.0 (557.0)	0.776	1.03	(0.85, 1.26)
FTY Clearance (L/h)	A (8)	C (8)	10.6 (3.4)	11.9 (4.4)	0.502	0.90	(0.68, 1.18)
	B (8)	D (8)	8.5 (3.4)	11.9 (2.9)	0.034	0.69	(0.53, 0.91)
FTY t1/2 (h)	A (8)	C (8)	118.4 (41.5)	95.0 (27.7)	0.298	1.23	(0.88, 1.73)
	B (8)	D (8)	160.1 (59.0)	110.5 (43.4)	0.059	1.49	(1.06, 2.09)
FTY unbound predose plsm%	A (8)	C (8)	0.2 (0.0)	0.2 (0.0)	0.761	1.02	(0.91, 1.14)
	B (8)	D (8)	0.2 (0.0)	0.2 (0.0)	0.022	1.18	(1.05, 1.32)

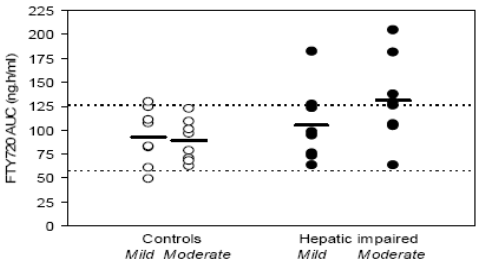
Note:
1. C = Matching control to Mild impairment (A), and D = Matching control to Moderate impairment (B).
2. p-value, geometric means, ratio of geometric means, and 90% confidence interval for ratio of population means are determined from an ANOVA model for the log transformed values with Group and Pair as model factors.

- For impaired subjects, Tap was 28 hours (range, 16 to 42 hours) emphasizing the broadness of the plateau whererus Tap was 12 and 18 hours in the two control groups and close to tmax of 12 hours.
- Cap,b was 0.64 ng/ml (mild impairment controls) and 0.53 ng/ml (moderate impairment controls) which were close to the respective Cmax,b. Apical concentrations were similar to controls for both mild and moderate hepatic impaired subjects.
- Compared with control subjects, mild hepatic impaired subjects had a 12 percent higher AUCb (p = 0.493) and moderate hepatic impaired subjects had a 44 percent higher AUCb (p = 0.034).
- CL/F was reduced on average by 10 and 31 percent for mild and moderate hepatic impaired subjects compared with the respective controls.
- T1/2 averaged 4.2 days across all control subjects. It was similar for mild hepatic impaired subjects (p = 0.298) but prolonged 49 percent in the moderate hepatic impaired group (p = 0.059).
- The V/F was similar across all groups.

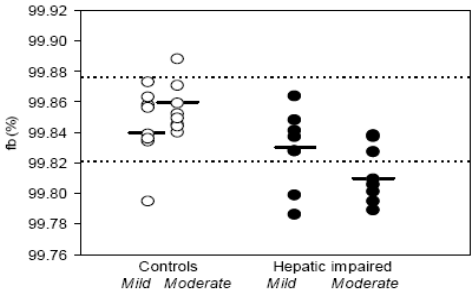
FTY720 plasma protein binding

AUC and protein binding across groups are shown below:

Figure 7-2 Comparison of FTY720 AUC and protein binding across groups



Distribution of individual FTY720 AUCs in control and hepatic impaired groups. *Bars* represent group mean. *Dashed lines* demarcate the 10 to 90 percentile AUC bounds for healthy controls.

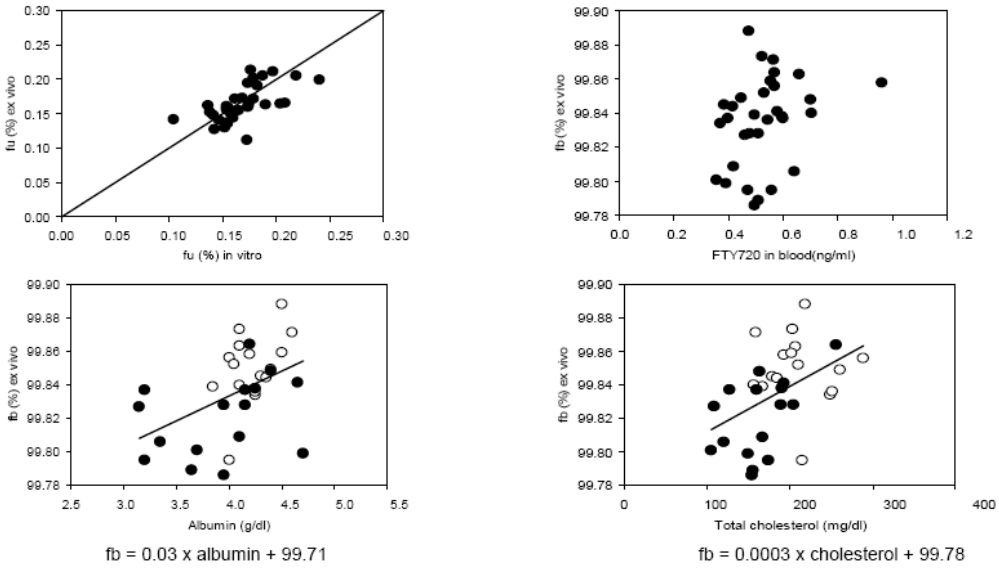


As in left panel for the fraction of FTY720 bound to plasma proteins (f_b) *ex vivo*.

Protein binding covariates are shown in the plots below:

Protein binding covariates

Figure 2
Scatterplots of FTY720 protein binding



- The free fraction of FTY720 in postdose plasma was similar in controls and mild hepatic impairment subjects ($p = 0.115$) but significantly increased in moderate hepatic impairment subjects: 0.144% versus 0.187% ($p < 0.001$).
- The free fractions were similar in postdose plasma samples compared with predose spiked plasma samples (paired t-test, $p = 0.283$) indicating that *in vitro* and *ex vivo* measurement yielded similar results.
- The linear regression demonstrated that the postdose fraction bound was independent of the concurrent blood concentration of FTY720 (r2 = 0.089, $p = 0.098$). Serum levels of the main binding proteins of FTY720, however, were significantly correlated with the fraction bound: r2 = 0.217, $p = 0.007$ for albumin and r2 = 0.213, $p = 0.007$ for total cholesterol.

<u>Metabolites pharmacokinetic in blood and urine:</u>

Mean M2 and M3 pharmacokinetic parameters are summarized in the following table:

Table 7-5 Metabolite pharmacokinetic parameters

Parameter	Mild impairment		Moderate impairment	
	Controls	Impaired	Controls	Impaired
Metabolite 2:				
Subjects with blood concs	5	4	3	1
Blood conc range (ng/ml)	0.10 – 0.17	0.10 – 0.21	0.10 – 0.13	0.11 – 0.15
Urine Ae(0-96) (mcg)	18.8 ± 11.5	18.5 ± 21.8	17.9 ± 7.1	6.6 ± 4.9
Urine Ae(0-inf) (mcg)	30.4 ± 21.3	25.8 ± 27.3	31.2 ± 13.6	17.2 ± 22.4
Metabolite 3:				
Subjects with blood concs	7	8	7	6
$C_{max,b}$ (ng/ml)	0.28 ± 0.18	0.42 ± 0.33	0.20 ± 0.11	0.24 ± 0.25
t_{max} (h)	8 (6 – 36)	12 (6 – 24)	8 (6 – 48)	10 (6 – 12)
AUC(0-t_z)$_b$ (ng.h/ml)	20 ± 24	27 ± 31	7 ± 7	7 ± 10
Metabolite/parent AUC_b-ratio	0.24 ± 0.28	0.39 ± 0.48	0.09 ± 0.09	0.05 ± 0.07
Urine Ae(0-96) (mcg)	84 ± 36	60 ± 52	83 ± 32	29 ± 15
Urine Ae(0-inf) (mcg)	157 ± 66	117 ± 61	177 ± 62	93 ± 63

Data are mean ± sd except for time parameters which are median (range).

Source data in Appendix 4, Tables 4 - 8 and Appendix 6, Table 1.

Statistics for the PK parameter comparisons are shown below:

Table 1 Effect of hepatic impairment on PK and PD responses to FTY720 dosing

Parameters	Comparison Test (N)	Comparison Ref. (N)	Arithmetic Means Test (STD)	Arithmetic Means Ref. (STD)	p-value	Ratio of geometric means	90% CI for ratio
FTY unbound postdose plsm%	A (8)	C (8)	0.2 (0.0)	0.2 (0.0)	0.115	1.10	(1.00, 1.21)
	B (8)	D (8)	0.2 (0.0)	0.1 (0.0)	<0.001	1.30	(1.18, 1.44)
M2 excreted in urine (mcg)	A (8)	C (8)	18.5 (21.7)	18.8 (11.5)	0.137	0.53	(0.26, 1.08)
	B (8)	D (8)	6.6 (4.9)	17.8 (7.1)	0.010	0.30	(0.15, 0.62)
M3 Cmax (ng/ml)	A (8)	C (8)	0.4 (0.3)	0.3 (0.2)	0.651	1.17	(0.64, 2.12)
	B (8)	D (8)	0.2 (0.2)	0.2 (0.1)	0.803	1.10	(0.58, 2.09)
M3 AUCt (ng.h/ml)	A (8)	C (8)	27.3 (30.8)	20.2 (23.5)	0.762	0.80	(0.21, 2.99)
	B (8)	D (8)	7.1 (9.9)	6.9 (7.0)	0.505	0.58	(0.14, 2.41)
AUCs M3/FTY	A (8)	C (8)	0.4 (0.5)	0.2 (0.3)	0.826	0.83	(0.19, 3.59)
	B (8)	D (8)	0.1 (0.1)	0.1 (0.1)	0.352	0.43	(0.09, 2.06)
M3 excreted in urine (mcg)	A (8)	C (8)	60.0 (51.9)	83.5 (35.6)	0.043	0.53	(0.32, 0.88)
	B (8)	D (8)	29.1 (14.6)	82.6 (31.8)	0.001	0.32	(0.20, 0.53)
Mean Bilirubin	A (8)	C (8)	0.9 (0.3)	0.5 (0.1)	<0.001	1.84	(1.42, 2.39)
	B (8)	D (8)	2.5 (1.3)	0.7 (0.2)	<0.001	3.25	(2.51, 4.21)

Metabolite 2 (M2) in blood.

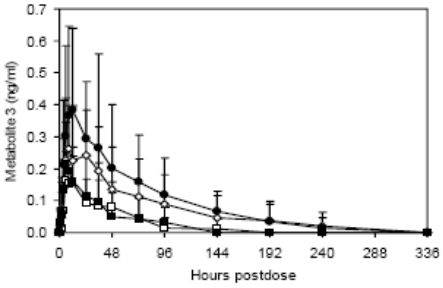
- Blood concentrations of M2 were detectable in half of the 16 control subjects and were generally near the assay quantification limit. This was similar for subjects with mild hepatic impairment. M2 was not detected in blood in moderate hepatic impaired subjects with a single exception.
- Blood concentrations were generally quantifiable between 2 and 24 hours postdose as shown in the mean profiles above.

Metabolite 2 urinary excretion.

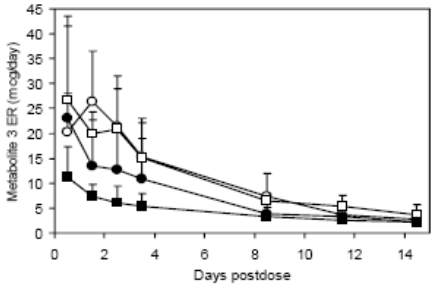
- The profiles were generally superimposable in controls and mild hepatic impairment subjects and notably lower in moderate hepatic impaired subjects.
- The Ae(0-96) range across individuals was 7-44 mcg in control subjects, 2-60 mcg in mild hepatic impaired subjects, and 1-15 mcg in moderate hepatic impaired subjects. Ae(0-96) was not significantly affected by mild hepatic impairment (p = 0.137) but was significantly reduced by an average 70 percent in moderate hepatic impairment (p = 0.010).

Mean M3 concentration-time profiles are shown in the following figure:

Figure 7-5 Metabolite 3 blood and urine profiles



Mean metabolite 3 concentration profiles in control and mild hepatic impaired subjects (*open and filled circles*) and in control and moderate hepatic impaired subjects (*open and filled squares*). *Bars* represent 95% confidence intervals. Individual subject plots are in Appendix 4, Figure 1.



Mean excretion rates (ER) of metabolite 3 on days 1, 2, 3, 4, 8, 11, and 14. Data are plotted at midpoint of daily urine collection interval. *Symbols* are defined in the legend in the left panel. *Bars* represent 95% confidence intervals.

Metabolite 3 in blood.

- Blood concentrations of M3 were detectable in nearly all subjects and generally quantifiable between 2 and 192 hours postdose.
- Peak and total exposure to M3 and the M3/parent AUCb-ratios were not different between control and hepatic impaired subjects due, in part, to high intersubject variability ($p > 0.05$ for all).

Metabolite 3 urinary excretion.

- The mean profiles showed a progressive decrease with degree of hepatic impairment. The Ae(0-96) range across individuals was 45-140 mcg in control subjects, 12-150 mcg in mild hepatic impaired subjects, and 8-54 mcg in moderate hepatic impaired subjects.
- Ae(0-96) was reduced on average by 47 percent in mild hepatic impairment ($p = 0.043$) and by an average 68 percent in moderate hepatic impairment ($p = 0.001$).

Reviewer's note:

Why the AUC of the control in the moderate impairment group is about~ 30% of the control in the mild impairment group? As healthy matching controls, they should be similar. No explanation was provided by the sponsor.

Individual M3 PK parameters are shown in the table below:

Metabolite 3 blood pharmacokinetic parameters in mild hepatic impairment

Pair	Subject		tmax (h)		Cmax (ng/ml)		AUC(0-t)		Met/parent ratio	
	Healthy	Hepatic	Healthy	Hepatic	Healthy	Hepatic	Healthy	Hepatic	Healthy	Hepatic
1	19	1	36	12	0.44	0.51	50.8	18.8	0.45	0.22
2	20	2	12	12	0.55	0.20	61.8	3.4	0.82	0.03
3	27	9	.	6	0.00	0.26	0.0	19.5	0.00	0.22
4	28	10	8	12	0.14	0.47	3.8	36.6	0.04	0.52
5	29	11	8	8	0.23	1.08	6.2	91.8	0.06	1.36
6	30	12	6	24	0.36	0.64	16.7	45.5	0.30	0.79
7	31	13	8	24	0.34	0.12	19.3	1.9	0.25	0.01
8	33	15	6	24	0.17	0.12	2.7	0.7	0.02	0.01
N			7	8	8	8	8	8	8	8
Mean			12	15	0.28	0.42	20.2	27.3	0.24	0.39
SD			11	8	0.18	0.33	23.5	30.8	0.28	0.48
Min			6	6	0.00	0.12	0.0	0.7	0.00	0.01
Median			8	12	0.29	0.37	11.4	19.1	0.16	0.22
Max			36	24	0.55	1.08	61.8	91.8	0.82	1.36

Metabolite 3 blood pharmacokinetic parameters in moderate hepatic impairment

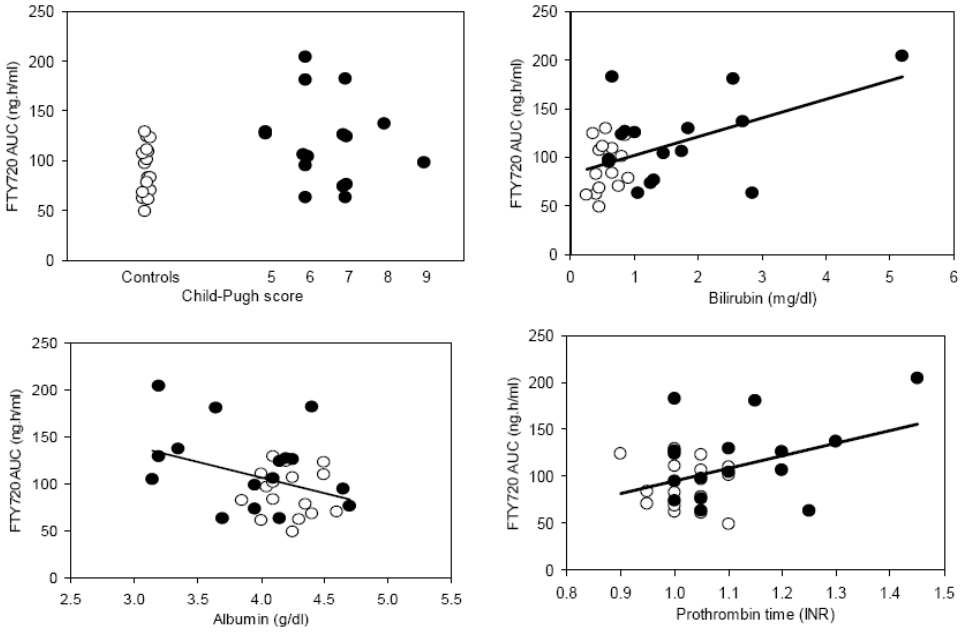
Pair	Subject		tmax (h)		Cmax (ng/ml)		AUC(0-t)		Met/parent ratio	
	Healthy	Hepatic	Healthy	Hepatic	Healthy	Hepatic	Healthy	Hepatic	Healthy	Hepatic
9	21	3	8	12	0.23	0.12	9.1	1.5	0.14	0.01
10	22	4	48	8	0.24	0.45	21.0	25.6	0.24	0.15
11	23	5	.	.	0.00	0.00	0.0	0.0	0.00	0.00
12	24	6	36	12	0.25	0.13	9.6	0.8	0.10	0.01
13	25	7	8	12	0.15	0.13	2.2	0.8	0.02	0.01
14	26	8	8	8	0.22	0.50	2.9	18.5	0.03	0.15
15	32	14	6	.	0.13	0.00	0.8	0.0	0.01	0.00
16	34	16	6	6	0.37	0.64	9.6	9.2	0.15	0.08
N			7	6	8	8	8	8	8	8
Mean			17	10	0.20	0.24	6.9	7.1	0.09	0.05
SD			17	3	0.11	0.25	7.0	9.9	0.09	0.07
Min			6	6	0.00	0.00	0.0	0.0	0.00	0.00
Median			8	10	0.22	0.13	6.0	1.2	0.06	0.01
Max			48	12	0.37	0.64	21.0	25.6	0.24	0.15

Reviewer's note:
There are very wide variations in both mild and moderate groups, but the big difference between controls is still very questionable. Within all control subjects, #19 and 20 seem to have very high AUC.

Protein binding:

The three laboratory parameters that are components of the Child-Pugh score were assessed. The regressions are plotted and are shown in the figure below:

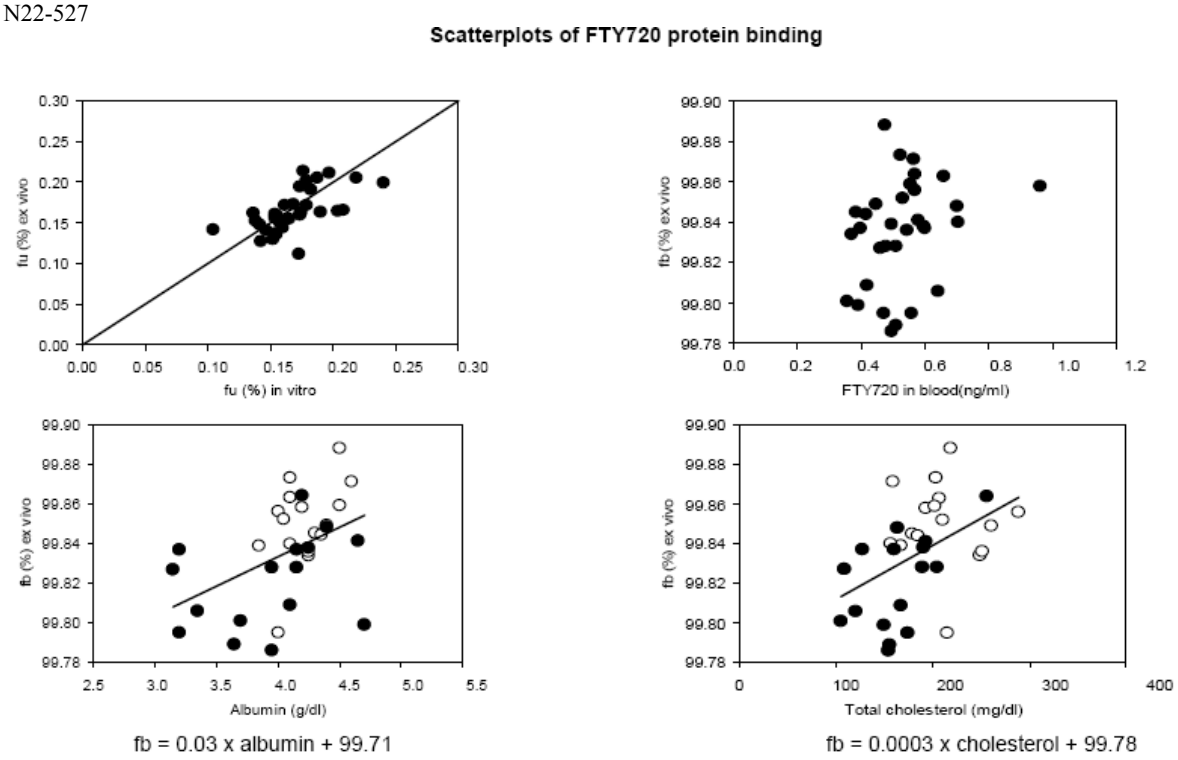
Figure 7-6 FTY720 AUC versus hepatic impairment markers



Relationship between FTY720 AUC and Child-Pugh score and linear regression of FTY720 AUC versus total bilirubin levels, albumin levels, and prothrombin time. Shown are data from healthy controls (*open circles*) and subjects with mild to moderate hepatic impairment (*filled circles*).

- A scatterplot of the Child-Pugh score versus FTY720 AUCb did not reveal any apparent relationship.
- **Bilirubin.** Total bilirubin ranged from 0.6 to 5.2 mg/dl in hepatic impaired subjects compared with 0.3 to 0.9 mg/dl in matched controls. FTY720 AUCb was positively correlated with bilirubin: AUCb = 19x + 84 (p = 0.0002, r = 0.520).
- **Albumin.** Albumin concentrations ranged from 3.2 to 4.7 g/dl in hepatic impaired subjects compared with 3.9 to 4.6 g/dl in matched controls. FTY720 AUCb was negatively correlated with albumin concentrations: AUCb = -34x + 242 (p = 0.037, r = 0.371).
- **Prothrombin time.** Prothrombin times ranged from 1.0 to 1.5 INR (9.7 to 14.0 sec) in hepatic impaired subjects compared with 0.9 to 1.1 (9.0 to 10.7 sec) in matched controls. FTY720 AUCb was positively correlated with prothrombin time: AUCb = 134x -39 (p = 0.022, r = 0.404) when expressed as INR and AUCb = 13.4x -35.8 (p = 0.024, r = 0.380) when expressed in sec.

Reviewer's note:
Patients have higher bilirubin, prothrombin time and lower albumin, but in term of their relationship to the FTY720 AUC, if not considering the one with AUC of ~ 200 (#3 moderate), there is barely a trend (may be a little for albumin). No definite conclusion could be drawn based on this data.

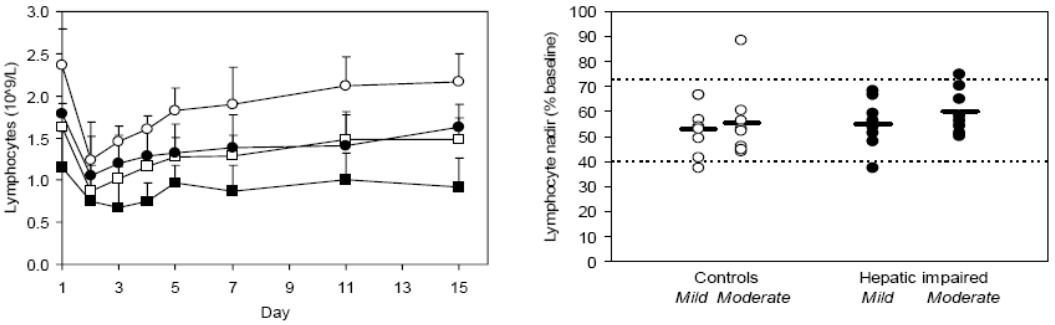


- FTY720 in blood vs fb(%) ex vivo have no clear trend. FTY720 in blood is consistent disregard to the fb(%) ex vivo.
- Albumin and cholesterol levels are correlated with fb(%) ex vivo.
- Since patients have lower albumin and they have consistent blood FTY720 levels, the free fraction in these patients are expected to be higher which is shown in the data shown previously.

Effect of FTY720 on lymphocytes

Mean lymphocyte trajectories are shown in the figure below:

Figure 7-7 Lymphocyte trajectories and nadirs



Mean total blood lymphocyte counts in control and mild hepatic impaired subjects (*open and filled circles*) and in control and moderate hepatic impaired subjects (*open and filled squares*). *Bars* represent 95% confidence intervals. Synoptic plots by subject group are in Appendix 4, Figure 3.

Distribution of individual lymphocyte nadirs (as percent of baseline value) in control and hepatic impaired groups. *Bars* represent group means. *Dashed lines* demarcate the 10 to 90 percentile bounds for healthy controls.

- The predose lymphocyte count was borderline lower in mild hepatic impaired subjects ($p = 0.086$) and significantly lower in moderate hepatic impaired subjects ($p = 0.027$) compared to controls.

- Nadir lymphocyte counts were generally observed at the 24- hour postdose blood collection. Both mild and moderate hepatic impaired subjects had significantly lower nadir counts and AUEs compared with controls ($p < 0.05$ for all). These differences were likely due to the lower predose values in the hepatic impairment groups since the percent decrease from baseline to nadir was similar for all groups ($p = 0.742$ and 0.383).
- The nadir as percent of baseline had a 10 to 90 percentile range of 40 to 73 percent in control subjects. The values for hepatic impaired subjects also fell within this range. Hence, independent of group, a 1 mg single dose of FTY720 reduced lymphocytes by an average 44 percent from baseline.
- Lymphocyte counts gradually recovered in all groups over the 2-week follow-up period. By day 15 counts were on average 90 percent of those at study entry in controls and mild hepatic impaired subjects with a slightly lower recovery in moderate hepatic impairment (84 % of baseline).

Reviewer's note:
Predose baselines in mild and moderate hepatic impairment patients are significantly lower than control. No reasonable explanations were provided. In addition, even two control groups seem to show significantly different baselines. This finding could be due to insufficient subject numbers or possibility of inadequate study conduct.

Effect of FTY720 on heart rate

Heart rate response parameters are summarized in the table below:

Table 7-7 Heart rate response parameters

Parameter	Mild impairment		Moderate impairment	
	Controls	Impaired	Controls	Impaired
Baseline:				
Predose rate (bpm)	69 ± 7	68 ± 7	68 ± 6	72 ± 7
Daytime:				
Nadir rate (bpm)	62 ± 3	60 ± 8	60 ± 2	66 ± 6
Nadir rate (% predose)	-12 ± 14	-16 ± 13	-14 ± 9	-11 ± 9
Time of nadir (h)	3 (1 – 8)	3 (1 – 8)	4 (1 – 8)	2 (1 – 4)
AUE(0-8) (bpm · h)	523 ± 21	513 ± 57	517 ± 21	549 ± 52
Nadir period:				
4h rate (bpm)	65 ± 3	63 ± 7	64 ± 6	67 ± 6
4h rate (% predose)	-7 ± 11	-10 ± 13	-7 ± 9	-8 ± 10
AUE(0-4) (bpm · h)	261 ± 9	261 ± 29	262 ± 10	278 ± 27
Recovery:				
24h rate (bpm)	70 ± 8	61 ± 5	67 ± 6	68 ± 8
48h rate (bpm)	68 ± 3	66 ± 12	67 ± 6	73 ± 9

Values are mean ± standard deviation. *AUE* is area-under-the-effect curve.
Source data: Appendix 3, Table 5-3; Appendix 4, Table 10; Appendix 6, Tables 1 and 2.

- The mean nadir heart rate was around 60 bpm in all groups which corresponded to an average 13 percent decrease from predose values and occurred generally between 2 and 4 hours postdose.
- The effect of FTY720 on heart rate was highly consistent in control subjects as noted from the low standard deviations and slightly more variable in hepatic impaired subjects.

- None of the daytime response parameters was significantly different among groups. This was also the case when focusing on the nadir region in the first 4 hours postdose ($p > 0.05$ for all comparisons).
- The subsequent mean morning heart rate recovered to predose values in the control groups by 24 hours postdose and in hepatic impaired subjects by 48 hours postdose.

Conclusions:

- CL/F was reduced by 10% in mild and 31% in moderate hepatic impairment yielding increases in AUC by 12% and 44%, respectively. Plasma protein binding decreased slightly with increasing hepatic impairment. Intact FTY720 was not detected in urine.
- There were no clear differences in M2 or M3 blood concentrations in hepatic impaired versus healthy subjects. Urinary excretion of M2 was decreased by 70% in moderate hepatic impaired. Urinary excretion of M3 was reduced by 47% and 68% in mild and moderate hepatic impaired subjects.
- Based on the sponsor, there were significant positive correlations between FTY720 AUC versus bilirubin and prothrombin time and a significant negative correlation versus albumin.
- Mean lymphocyte counts decreased by approximately 44 percent in all subject groups by 24h postdose. Mean lymphocyte counts returned to 90% of baseline in control and mild hepatic impaired subjects and to 84% of baseline in moderate hepatic impaired subjects by 2 weeks postdose.
- The morning mean supine heart rate transiently decreased approximately 13% at 2 to 4 hours postdose in all groups independent of hepatic impairment.
- While hepatic impairment elicited changes in the disposition of FTY720, the magnitude of these changes suggests that the FTY720 dose does not need to be adjusted in mild or moderate hepatic impaired patients.

Reviewer's Comment:
The sponsor stated the increased exposure with increasing hepatic impairment is not considered clinical significant and not suggesting dose adjustment. However, in light of the single dose design in the present study and knowing the ~11 fold accumulation in exposure after multiple doses of FTY720, the possibility of an excessive increased exposure in hepatic impair patients after long term use of the drug is very likely. Dose adjustment in these patients is therefore necessary. In addition, with a non-linear PK behavior (although underproportional) after multiple doses, the magnitude of the possible increase in exposure in these patient populations is not predictable. Additional studies might be needed to explore this issue. FTY720 should not be used in these patient populations until more data regarding long term use of FTY720 become available to ensure safely use of the drug.

Study FTY720A 2204*:* **An open-label, single-dose, parallel-group study to compare the pharmacokinetics of FTY720 in subjects with severe hepatic impairment with that in matched healthy control subjects**

A brief overview of some essential components of the study design is given below:

Study Design	Open-label, single-dose, parallel-group study
Study Population	**Table 7-1 Demographic and clinical characteristics** **Characteristic** / **Controls** / **Heaptic impaired** ***Demographics:*** Sex: 2 males / 4 females / 2 males / 4 females Age (yrs): 49.0 ± 6.4 / 49.7 ± 5.0 Weight (kg): 70.2 ± 17.2 / 69.1 ± 19.8 Height (cm): 168 ± 6 / 166 ± 6 ***Hepatic function:*** Child-Pugh score (n): -- / 10 (4), 12 (2) Bilirubin (mg/dl): 0.7 ± 0.2 / 2.8 ± 0.9 Albumin (g/dl): 3.1 ± 0.5 / 4.0 ± 0.3 Prothrombin time (INR): Not measured / 1.4 ± 0.1 ***Renal function:*** CrCL (ml/min): 93 ± 18 / 97 ± 26
Dosage and Administration	A total of 12 subjects were enrolled into two groups. Group 1 consisted of 6 subjects with severe hepatic impairment with a Child-Pugh score of ≥10 (Child-Pugh class C). Group 2 consisted of 6 healthy subjects matched by sex, age, lean body mass, and smoking status to group 1 subjects. Subjects received a 5-mg oral dose of FTY720 after which safety assessments, pharmacodynamic assessments, and pharmacokinetic blood and urine samples were collected for three weeks postdose. Subjects were domiciled at the study center until the morning of day 2 and returned on days 3, 4, 5, 7, 9, 11, 15, and 22 for study evaluations. The end-of-study visit was on day 22. All subjects had to fast for at least 10 hours before dosing and continue to fast for at least 4 hours thereafter. Lot No. FTY720 1.25 mg capsule (FMI): batch US02029 CSUS/2003-0461 Diet: No fluid intake apart from the fluid given with the drug was allowed from 2 hours before until 2 hours after dosing. During waking hours on day 1, subjects were required to have a fluid intake of at least 200 ml every 4 hours in addition to fluid taken with the medication. Intake of xanthine-containing food or beverages had to be discontinued 48 hours before dosing. Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood	At predose (0 hour), and 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 192, 240, 336, and 504 hours postdose.
Sampling: Urine	Daily urine collections were performed on days 1-4, 8, 10, and 14.
PD measurement	Peripheral blood lymphocyte counts were obtained at baseline; predose; 2, 4, 6,

	and 12 hours postdose on day 1; and in the morning on days 2, 3, 4, 5, 7, 9, 11 ,15, and 22. Cardiac monitoring was performed by telemetry for 24 hours postdose. Supine heart rate was recorded with vital signs predose, at 1, 2, 4, 8, 12 hours on day 1, and each morning from day 2 to 5.
Analysis (Blood)	Method: LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 1.5 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 12% Inter-day accuracy: < 4.8 % FTY720-P: Linear range : 1.5-500 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 9.9% Inter-day accuracy: < 4.5 %
Analysis (Urine)	Method: LC/MS/MS Lower Limits of Quantitation Urine M2 2 ng/mL M3 5 ng/mL M2: Linear range : 2-800 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 33.6% Inter-day accuracy: < 17.5 % M3: Linear range : 5-2000 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 12.9% Inter-day accuracy: < 9 %
PK Assessment	FTY720 in blood: Tmax, Cmax,b, Cap,b, Tap, Tdur, AUC(0-tz)b, AUCb, CLb/F, Vz,b/F, Vz,b/F, t1/2, Ae(0-t), Ae(0-inf) and CLR. FTY720-P in blood: tlag, Cmax,b, tmax, AUC(0-96)b, and AUC(0-t)b and the ratio of FTY720-phosphate to FTY720 AUC(0-96). M2 and M3 in urine: Ae(0-96) and Ae(0-inf).
PD Assessment	Response parameters included predose value, nadir value, and area under the effect-time curve.
Safety Assessment	Assessment of physical examination, vital signs, ECG, clinical laboratory parameters (biochemistry, hematology, urinalysis), pregnancy test for women, and adverse event monitoring.

Pharmacokinetic Results:

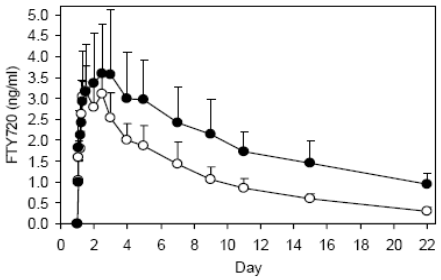
FTY720 pharmacokinetic in blood:

Mean FTY720 PK parameters and FTY720 concentration-time profiles are shown below:

Table 7-5 Pharmacokinetics: FTY720

Parameter	Healthy controls	Hepatic impaired
$C_{max,b}$ (ng/ml)	3.4 ± 0.5	3.7 ± 1.1
t_{max} (h)	12 (8 – 36)	36 (36-96)
$C_{ap,b}$ (ng/ml)	3.1 ± 0.4	3.4 ± 1.0
t_{ap} (h)	12 (10 – 24)	36 (24 – 42)
$t_{ap,dur}$ (h)	29 (28 – 40)	60 (40 – 88)
AUC(0-t_z)$_b$ (ng.h/ml)	563 ± 107	985 ± 308
AUC_b (ng.h/ml)	639 ± 105	1326 ± 389
CL_b/F (L/h)	8.0 ± 1.5	4.0 ± 1.1
$V_{z,b}$/F (L)	2026 ± 756	1494 ± 437
$t_{1/2}$ (days)	7.3 ± 2.3	10.7 ± 1.8
Fraction free (%)	0.35 ± 0.08	0.36 ± 0.16
Fraction protein bound (%)	99.65 ± 0.08	99.64 ± 0.16
FTY720 Ae(0-96) (mcg)	0	0

Values are mean ± sd except for time parameters which are median (range).
Data source: Appendix 4 Table 1 and Appendix 4, Table 2



FTY720 mean concentration profiles in control subjects (*open circles*) and in severe hepatic impaired subjects (*filled circles*). *Bars* represent the 95% confidence intervals. Individual and synoptic plots in Appendix 4, Figure 1.

- FTY720 appearance in blood was slower in hepatic impaired subjects as evidenced by a median 24-hour delay in tmax and a doubling in the duration over which apical concentrations were present (tap,dur).
- Peak blood levels were similar in hepatic impaired and control subjects with a ratio (90%CI) of 1.07 (0.81 – 1.42).
- Overall exposure based on AUCb was doubled in severe hepatic impairment with a ratio of 2.03 (1.62 – 2.54) and apparent clearance was halved with a ratio of 0.49 (0.39 – 0.62).
- The elimination half-life was prolonged by 50 percent in severe hepatic impairment.
- Protein binding was similar in both groups.

FTY720-P pharmacokinetic in blood:

Mean FTY720-P PK parameters after administration of 5 mg FTY720 are shown in the following table:

Table 7-6 Pharmacokinetics: FTY720-phosphate and metabolites

Parameter	Healthy controls	Hepatic impaired
Blood: FTY720-phosphate		
t_{lag} (h)	2 (1 – 4)	2 (2 – 4)
C_{max} (ng/ml)	5.4 ± 1.4	4.5 ± 1.9
t_{max} (h)	8 (6 – 12)	10 (6 – 12)
AUC(0-t_z)$_b$ (ng.h/ml)	191 ± 112	179 ± 133
FTY720-P/FTY720 AUC-ratio	0.83 ± 0.36	0.55 ± 0.36
Protein binding (%)	99.2[a]	98.9 ± 0.10
Urine: metabolites		
M2 Ae(0-96) (mcg)	106 ± 117	50 ± 22
M2 Ae(0-inf) (mcg)	200 ± 181	189 ± 109
M3 Ae(0-96) (mcg)	745 ± 579	258 ± 161
M3 Ae(0-inf) (mcg)	1404 ± 681	914 ± 575

Values are mean ± sd except for time parameters which are median (range).
(a) n = 1. Data source: Appendix 4,Table 3, Appendix 4, Table 4, Appendix 4, Table 5, Appendix 4, Table 6.

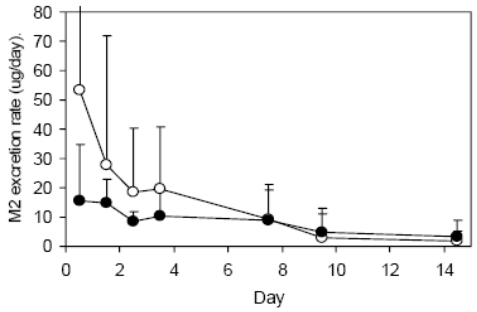
- FTY720-phosphate was quantifiable in blood by 2 hours postdose in both groups.
- FTY720-phosphate reached peak blood levels earlier than FTY720 in both groups with a 21 percent lower Cmax in hepatic impaired subjects: ratio (90%CI), 0.78 (0.53 – 1.15).

- Concentrations were quantifiable generally up to day 5 postdose and then declined below the assay quantification limit.
- Exposure based on AUC(0-tz)b was 29 percent lower in hepatic impaired subjects: ratio 0.71 (0.28 – 1.76).

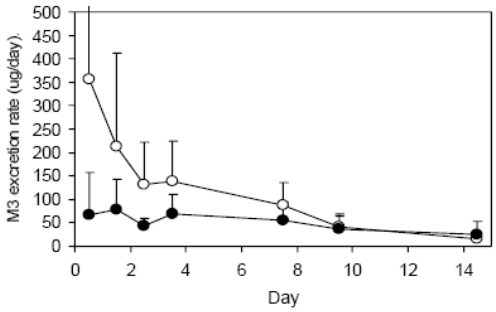
Metabolites pharmacokinetic in urine:

Mean M2 and M3 urinary recovery profiles are shown in the following:

Figure 7-2 Metabolite excretion profiles



Excretion rates of the hexanoic acid metabolite M2 in control subjects (*open circles*) and in severe hepatic impaired subjects (*filled circles*). *Bars* represent the 95% confidence intervals.



As in left panel for the butanoic acid metabolite M3.

- Calculated creatinine clearance were similar between groups.
- Recovery of the hexanoic (M2) and butanoic (M3) acid metabolites in urine to day 5 based on Ae(0-96) was reduced in severe hepatic impairment to one-half and one-third the corresponding recoveries in control subjects, respectively. Differences between groups remained after extrapolation to time infinity but were of a lesser magnitude.

Lymphocyte responses

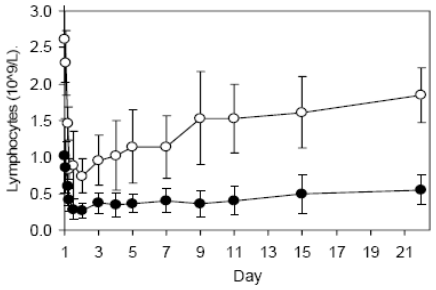
Table 7-7 Lymphocyte response parameters

Parameter	Healthy controls	Hepatic impaired
Baseline		
Predose count (10^9/L)	2.61 ± 0.56	1.02 ± 0.48
Reduction		
Time to nadir (h)	18 (12 – 48)	20 (12 – 72)
Nadir count (10^9/L)	0.62 ± 0.15	0.21 ± 0.06
Nadir count (%predose)	24 ± 5	23 ± 8
Recovery		
Day 15 count (10^9/L)	1.61 ± 0.46	0.49 ± 0.25
Day 22 count (10^9/L)	1.85 ± 0.36	0.56 ± 0.20
Rate (10^9/L per day)	0.047 ± 0.015	0.011 ± 0.005
AUE(0-504) (10^9/L x hr)	749 ± 192	228 ± 88

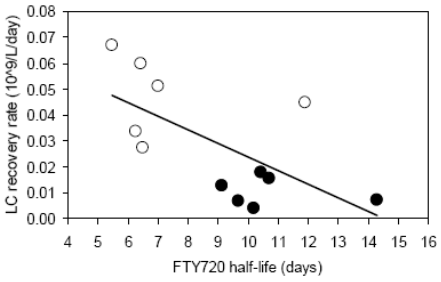
Values are mean ± sd except for time parameters which are median (range).
Data source: Appendix 3, Table 3.3.4.1 and Appendix 3, Table 5.2.1 and Appendix 4, Table 7.

Lymphocyte trajectories and recovery rate are shown in the figure below:

Figure 7-3 Lymphocyte trajectories and recovery rates



Blood lymphocyte trajectories over the 3-week study in control subjects (*open circles*) and in severe hepatic impaired subjects (*filled circles*). *Bars* represent the 95% confidence intervals. Synoptic plots in Appendix 4, Figure 2.



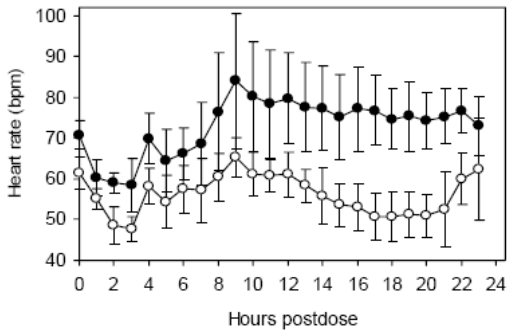
Regression of FTY720 eliminiation half-life versus lymphocyte (LC) recovery rate across control subjects (*open circles*) and hepatic impaired subjects (*filled circles*).

- Predose blood lymphocyte counts were lower in hepatic impaired subjects.
- The nadir count was reached generally by 12 to 24 hours postdose.
- Although the absolute nadir count was lower in the hepatic impaired, this was a consequence of their lower predose lymphocyte count. The lymphocyte responses were similar when expressed as reduction from baseline to nadir with average decreases of 76 and 77 percent in the two groups, respectively.
- The return of lymphocytes to blood was evident by day 3 in control subjects; whereas the nadir was maintained for a few to several days followed by a slower recovery in the hepatic impaired. The rate of lymphocyte reappearance in blood was fourfold slower in hepatic impaired versus control subjects. Across all subjects, the recovery rate was negatively correlated with the elimination half-life of FTY720 ($r = 0.645$, $p = 0.02$).
- Since FTY720-P is the active moiety driving the lymphocyte response, these recovery rates may serve as a marker indicating that the FTY720-Phalf-life is prolonged in severe hepatic impairment likely in parallel to that observed for FTY720.

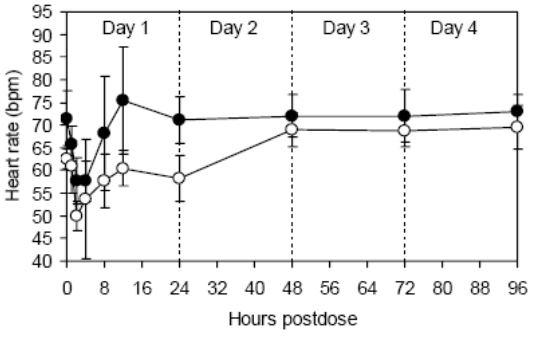
Heart rate

Heart rate trajectories are shown in the figure below:

Figure 7-4 Heart rate trajectories



Mean heart rate by telemetry over 24 hours postdose in control subjects (*open circles*) and in severe hepatic impaired subjects (*filled circles*). *Bars* represent the 95% confidence intervals. Synoptic plots in Appendix 4, Figure 3.



As in the left panel for supine heart rate to the morning of day 5 postdose from vital signs measurements. Synoptic plots in Appendix 4, Figure 3.

- The mean initial heart rate was higher in hepatic impaired subjects.
- The mean heart rate subsequently decreased on average by 22 percent in both groups to a daytime nadir generally at 3 to 4 hours postdose.

- The mean nighttime nadir was similar to the daytime nadir in healthy controls. Due to a dampened circadian rhythm in hepatic impaired subjects, a nighttime nadir was not clearly observed in this group.
- The mean predose heart rate was slightly elevated and more variable in the hepatic impaired versus control subjects: 72 ± 6 versus 63 ± 2 bpm. The mean rate at 4 hours postdose was 58 ± 4 versus 54 ± 13 bpm. By day 3 (48 hours postdose) the mean morning heart rate had returned to baseline: 72 ± 5 versus 69 ± 4 bpm.

Comparison of mild, moderate and severe patients

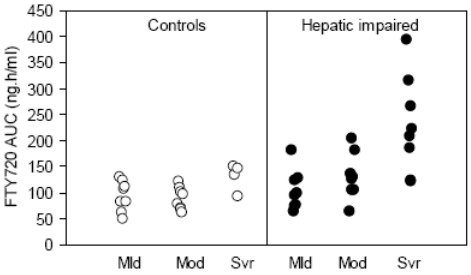
Table 7-9 FTY720 pharmacokinetics in hepatic impairment

Parameter	Controls	Hepatic impaired		
	Healthy (n = 22)	Mild (n = 8)	Moderate (n = 8)	Severe (n = 6)
t_{max} (h)	12 (8 - 48)	12 (6 – 36)	24 (8 – 48)	36 (36 – 96)
$C_{max,b}$ (ng/ml)	0.65 ± 0.15	0.65 ± 0.12	0.57 ± 0.10	0.75 ± 0.22
AUC_b (ng.h/ml)	101 ± 29	105 ± 39	131 ± 45	265 ± 78
$t_{1/2}$ (days)	5.1 ± 2.2	4.9 ± 1.7	6.7 ± 2.5	10.7 ± 1.8
CL_b/F (L/h)	10.8 ± 3.6	10.6 ± 3.4	8.5 ± 3.4	4.0 ± 1.1
$V_{z,b}/F$ (L)	1772 ± 594	1667 ± 348	1794 ± 351	1494 ± 437
Lymphocyte recovery (10^9/L/day)	0.046 ± 0.018	0.031 ± 0.032	0.028 ± 0.025	0.011 ± 0.005

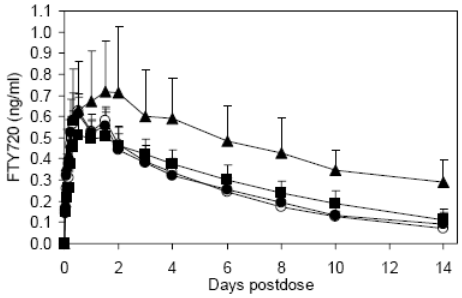
Values are mean ± sd except for time parameters which are median (range).
Cmax and AUC are scaled to a 1-mg dose.

The mean FTY720 concentration-time profiles in healthy controls and all hepatic impaired patients are shown in the figure below:

Figure 7-5 FTY720 exposure across hepatic impairment categories



Comparison of individual FTY720 AUC values in mild (Mld), moderate (Mod) and severe (Svr) hepatic impaired subjects (*filled circles*) and matched healthy controls (*open circles*). Data adjusted for a 1-mg dose. Additional plots in Appendix 4, Figure 4.

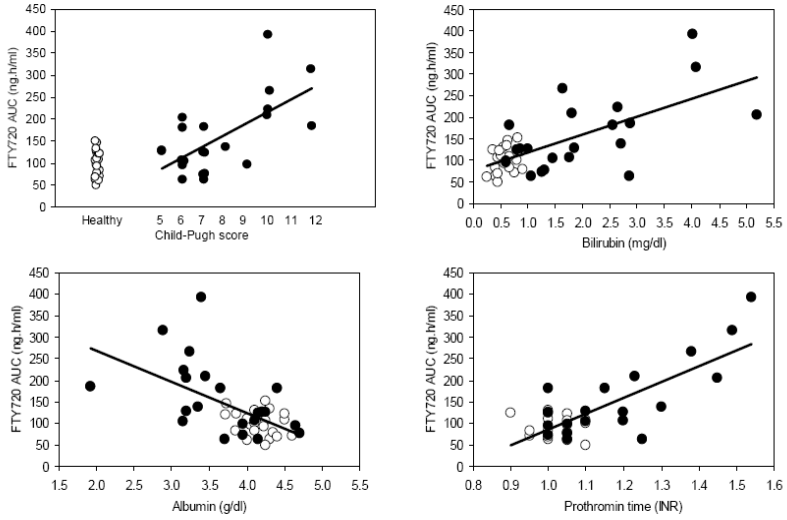


Mean FTY720 concentration profiles in healthy subjects (*open circles*) and subjects with mild (*filled circles*), moderate (*filled squares*), and severe (*filled triangles*) hepatic impairment. *Bars* represent 95% confidence intervals. Data adjusted to a 1-mg dose.

- Relatively minor changes were evident for mild and moderate hepatic impairment.
- Severe impairment was associated with clinically notable changes in exposure.

Clinical markers of hepatic impairment

Figure 7-6 FTY720 exposure and clinical markers of hepatic impairment



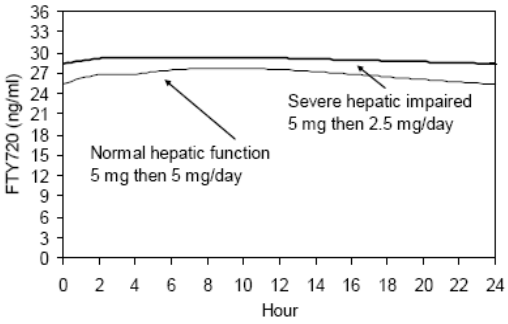
Correlations between Child-Pugh score or laboratory components thereof and FTY720 AUC. Shown are the data from healthy control subjects (*open circles*) and hepatic impaired subjects (*filled circles*) and the regression lines.

- Bilirubin ranged from 0.3 to 0.9 mg/dl in controls and from 0.6 to 5.2 in hepatic impaired subjects. There was a significant positive correlation to exposure: $p < 0.0001$.
- Albumin ranged from 3.7 to 4.6 g/dl in controls and from 1.9 to 4.7 in hepatic impaired subjects. There was a significant negative correlation to exposure: $p = 0.0001$.
- Prothrombin time ranged from 0.9 to 1.1 INR in controls and from 1.0 to 1.54 in hepatic impaired. There was a significant positive correlation to exposure: $p < 0.0001$.

Dose Simulation

A dosing simulation was made based on the mean concentration-time data of the two groups in this study.

Figure 7-7 FTY720 dosing simulation



Predicted steady-state FTY720 concentration profiles by nonparametric superposition from the mean healthy subject data and the mean severe hepatic impaired data in study 2204.

- The dose simulation began with an identical 5-mg first dose followed by 5 mg/day in non-hepatic impaired and 2.5 mg/day in the severe hepatic impaired. The predicted steady-state predose blood level was 25 and 28 ng/ml in the non-impaired and hepatic impaired. The corresponding Cmax,b,ss was 28 and 29 ng/ml, respectively.

Reviewer's note:
The PK of FTY720 is not proportional after MD at doses higher than 0.5 mg QD. This approach assuming linear PK and therefore the adequacy is questionable.

Conclusions:

- Compared with healthy control subjects, severe hepatic impaired subjects had a similar Cmax, a doubled AUC, and 50 percent prolonged elimination half-life for FTY720 and a 29 percent reduced AUC(0-96) for FTY720-P.
- The decrease in heart rate was similar in both groups. One healthy control subject had first degree atrioventricular block that resolved spontaneously. There were no pulmonary-related adverse events.
- The FTY720-related decrease in lymphocyte counts was similar between groups but the lymphocyte recovery rate was slower in subjects with severe hepatic impairment.
- For patients with severe hepatic impairment, a standard first dose could be given followed by a maintenance dose that is reduced by half from the normal maintenance dose.

Reviewer's note:
The sponsor is suggesting dose adjustment in the severe hepatic impaired patients in this study report; however, the sponsor did not recommend any dose adjustment in this specific patient population in the clinical pharmacology summary and proposed labeling. Since this study report was completed before the summary and labeling, one would think the sponsor decided no dose adjustment is required in this population. Nevertheless, based on the magnitude of exposure change after single dose of FTY720, and the expected further increase in exposure after multiple doses, a reasonable dose adjustment is necessary. Dose of FTY720 should be reduced by half in the severe hepatic impaired patients. In addition, with a non-linear PK behavior (although underproportional) after multiple doses, the magnitude of the possible increase in exposure in these patient populations is not predictable. More studies might be needed to explore this issue.

Study CFTY720D2108*:* **An open-label, single-dose, parallel-group study to compare the pharmacokinetics of FTY720 and metabolites in subjects with severe renal impairment with that in matched healthy control subjects**

Sponsor's rationale for only the severe renal impaired patients were evaluated: Data obtained from the renal transplant experience tend to show that CLcr had little or no influence on FTY720, FTY720-P, M2 and M3 pharmacokinetics. However, these observations were made in subjects having CLcr greater than 30 mL/min. This study was therefore conducted to investigate the pharmacokinetics of FTY720, FTY720-P, M2 and M3 in severe renal impaired patients (CLcr <30 mL/min).

A brief overview of some essential components of the study design is given below:

Study Design	open-label, single-dose, parallel-group study
Study Population	**Table 11-2 Summary of demographics and baseline characteristics by group (safety population)** Demographic variable / Renal impaired N=9 / Healthy controls N=9 / All subjects N=18 Age (years) Mean (SD) 47.9 (10.01) 47.1 (10.23) 47.5 (9.82) Height (cm) Mean (SD) 164.9 (8.78) 171.2 (9.19) 168.1 (9.31) Weight (kg) Mean (SD) 67.58 (16.764) 69.33 (16.416) 68.46 (16.120) Body mass index (kg/m^2) Mean (SD) 24.5 (3.73) 23.4 (3.36) 24.0 (3.49) Sex Male 4 (44.4 %) 4 (44.4 %) 8 (44.4 %) Female 5 (55.6 %) 5 (55.6 %) 10 (55.6 %) Predominant race Caucasian 9 (100 %) 9 (100 %) 18 (100 %) Serum creatinine (µmol/L) Mean (SD) 477.9 (172.33) 68.6 (7.20) - Glomerular filtration rate according to Cockcroft-Gault (mL/min) Mean (SD) 16.24 (7.112) 100.94 (16.836) - Source: Post-text Table 14.1-3.1 and Post-text Table 14.1-3.2
Dosage and Administration	9 patients with severe renal impairment and 9 matching healthy controls were enrolled. Patients with severe renal impairment and healthy subjects were matched by ethnicity, smoking status, gender, age and weight. All patients/subjects received a 1.25 mg oral dose of FTY720 with 240 mL of water immediately after modest breakfast after an overnight fast. Lot No. FTY720 1.25 mg capsule (FMI): batch number H378BD <u>Diet:</u> No fluid intake apart from the fluid given at the time of drug intake and breakfast was allowed from 2 h before until 2 h after dosing. Intake of xanthine-containing food or beverages and grapefruit or grapefruit juice had to be discontinued 48 hours before dosing. Alcohol was prohibited for 48 hours prior to dosing until study completion.
Sampling: Blood	At predose (0 hour), and 1, 2, 4, 6, 8, 10, 12, 16, 24, 36, 48, 72, 120, 192 (±24), 264 (±24), 360 (±24), 480h (±24) post-dose.
Sampling: Urine	At pre-dose, 0-6, 6-12, 12-24 (Day 1), 24-48 (Day 2), 48-72 (Day 3), 108-120, 180-192 (±24), 252-264 (±24), 348-360 (±24) and 468-480h (±24) post-dose.
Sampling: PD	Lymphocyte count: pre-dose, 4, 8, 12 and 24 h post-dose

	Complete blood count: 6 h post-dose
Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 0.1 ng/mL M2 0.1 ng/mL M3 0.1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 3.4% Inter-day accuracy: < 9.6 % FTY720-P: Linear range : 0.1-20 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 10.7% Inter-day accuracy: < 8.7 % M2: Linear range : 0.1-10 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 13.3% Inter-day accuracy: < 8 % M3: Linear range : 0.1-10 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 33.1% Inter-day accuracy: < 8.7 %
Analysis (Urine)	Method LC/MS/MS Lower Limits of Quantitation Urine FTY720 1.0 ng/mL M2 1.0 ng/mL M3 1.0 ng/mL FTY720: Linear range : 1-200 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 7% Inter-day accuracy: < 3 % M2: Linear range : 1-200 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 8.4% Inter-day accuracy: < 5 % M3: Linear range : 1-200 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 7.3% Inter-day accuracy: < 10.8 %
PK	FTY720, FTY720-P, M2 and M3 in blood:

Assessment	PK parameters, AUC0-t, AUC0-∞,Cmax, Clast, tmax, tlast, t1/2, V, will be determined.
Safety Assessment	Adverse event (AE) monitoring, regular monitoring of hematology, blood chemistry and urine and regular assessments of vital signs, physical condition and body weight.

Pharmacokinetic Results:

FTY720 pharmacokinetic in blood:

Descriptive statistics for FTY720 and FTY720-P blood PK parameters after single dose of 1.25 mg FTY720 administration are shown in the following table:

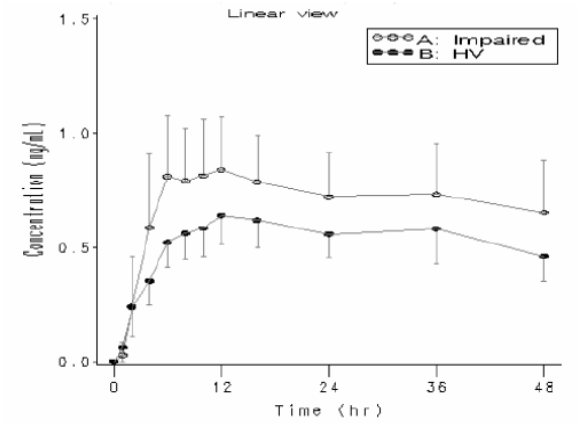
Table 11-4 FTY720 and FTY720-P pharmacokinetics in renal impaired patients and in matched healthy volunteers after a 1.25 mg dose (n=9 in each group)

PK parameters	FTY720		FTY720-P	
	Renal impaired	Healthy subjects	Renal impaired	Healthy subjects
t_{lag} * (h)	1.00 (0.00- 2.00)	1.00 (0.00- 2.00)	2.00 (1.00- 2.03)	1.00 (1.00- 2.00)
t_{max} * (h)	12.00 (6.00- 48.00)	12.00 (10.00- 36.00)	8.00 (6.00- 12.00)	8.00 (6.00- 10.00)
$C_{max,b}$ # (ng/mL)	0.878 ± 0.256 [0.844; 31]	0.653 ± 0.138 [0.639; 24]	1.13 ± 0.293 [1.097; 25]	0.904 ± 0.229 [0.876; 28]
$AUC_{(0-72),b}$ # (ng/mL.h)	47.7 ± 13.9 [45.6; 34]	35.3 ± 8.00 [34.4; 26]	30.5 ± 6.801 [29.7; 26]	27.3 ± 6.12 [26.6; 25]
$AUC_{(0-tz),b}$ # (ng/mL.h)	109.7 ± 72.4 [91.2; 72]	67.4 ± 33.6 [61.6; 46]	42.9 ± 24.5 [37.7; 58]	37.1 ± 18.2 [33.9; 47]
AUC_b # (ng/mL.h)	131 ± 90.7 [109; 68]	82.3 ± 36.9 [76.7; 39]	75.5 ± 33.6 + [70.2; 44]	65.9 ± 30.6 + [61.7; 39]
CL_b/F # (L/h)	13.3 ± 7.22 [11.4; 68]	17.3 ± 6.03 [16.3; 39]		
Vz_b/F # (L)	1480 ± 534 [1406; 35]	2000 ± 532 [1950; 23]		
$t_{1/2}$ # (h)	94 ± 53 [85; 45]	85 ± 25 [83; 24]	95.1 ± 50.4 + [85.1; 56]	100.7 ± 46.1 + [94.4; 39]
$C_{max,b}$ ratio # FTY720-P/FTY720			1.075 ± 0.351 [1.032; 30]	1.097 ± 0.157 [1.088; 14]
$AUC_{(0-tz),b}$ ratio # FTY720-P/FTY720			0.337 ± 0.0808 [0.328; 26]	0.444 ± 0.0855 [0.437; 20]
Fraction unbound (%)	0.098 ± 0.015	0.106 ± 0.014	0.532 ± 0.059	0.494 ± 0.046
$Ae_{(0-72)}$ (% of dose)	a	b	NA	NA
$AURC_\infty$ (% of dose)	a	b	NA	NA

#: arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation];
*: median (minimum-maximum); NA: Not applicable as FTY720-P not measured in the urine;
+: n=5
a: no subject with concentrations >LLOQ; b: statistics not reported as only 1 subject with concentrations >LLOQ
(Source: Post-text Table 14.2-1.2, Post-text Table 14.2-1.3, Post-text Table 14.2-1.4, Post-text Table 14.2-1.7, Post-text Table 14.2-1.8, and report DMPK R0800403)

FTY720 concentration-time profiles of FTY720 after single dose of 1.25 mg FTY720 administration are shown in the following figure:

Figure 11-1 **Arithmetic mean (SD) FTY720 concentration-time profiles in renal impaired patients and in matched healthy volunteers after a 1.25 mg dose (n=9 in each group) – First 48 hours**



Geometric mean ratio and 90% CIs for FTY720 PK parameters are shown below:

Table 11-6 **Geometric mean ratio (test/reference) and 90% confidence intervals for FTY720 PK parameters in blood**

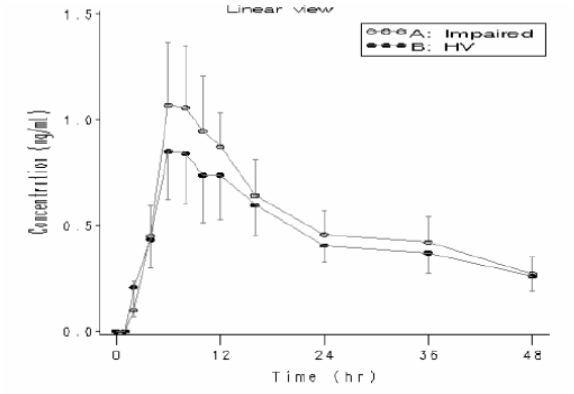
	Adjusted geo-mean*		Geo-mean ratio*		
Parameter (Unit)	**Impaired**	**Control**	**Estimate**	**Lower 90% CL**	**Upper 90% CL**
$C_{max,b}$ (ng/mL)	0.844	0.639	1.32	1.06	1.65
$AUC_{(0-tz),b}$ (h*ng/mL)	91.226	61.576	1.48	0.94	2.33
AUC_b (h*ng/mL)	109.440	76.723	1.43	0.94	2.18
$AUC_{(0-72),b}$ (h*ng/mL)	45.638	34.379	1.33	1.04	1.69

* back-transformed from log scale; geo-mean=geometric mean.
Source: Post-text Table 14.2-1.1

- FTY720 appeared in blood and reached maximum concentration at the same time in renal impaired patients and control subjects.
- Peak blood levels were significantly greater in severe renal impaired patients than in control subject with a geometric mean ratio (90%CI) of 1.32 (1.06 – 1.65).
- Overall exposure based on AUCb was also greater in severe renal impairment with a geometric mean ratio of 1.43, but without reaching statistical significance as indicated by a 90% CI (0.94 – 2.18) including 1 due to a large CV%.
- The apparent elimination half-life was comparable between the two groups.
- Protein binding was similar in both groups.

FTY720-P concentration-time profiles of FTY720 after single dose of 1.25 mg FTY720 administration are shown in the following figure:

Figure 11-2 **Arithmetic mean (SD) FTY720-P concentration-time profiles in renal impaired patients and in matched healthy volunteers after a 1.25 mg dose (n=9 in each group)- First 48 hours**



Geometric mean ratio and 90% confidence intervals for FTY720-P PK parameters are shown below:

Table 11-7 Geometric mean ratio (test/reference) and 90% confidence intervals for FTY720-P PK parameters in blood

	Adjusted geo-mean*		Geo-mean ratio*		
Parameter (Unit)	Impaired	Control	Estimate	Lower 90% CL	Upper 90% CL
$C_{max,b}$ (ng/mL)	1.097	0.876	1.25	1.01	1.56
$AUC_{(0\text{-}tz),b}$ (h*ng/mL)	37.661	33.923	1.11	0.74	1.67
AUC_b (h*ng/mL)	70.198	61.695	1.14	0.71	1.82
$AUC_{(0\text{-}72),b}$ (h*ng/mL)	29.658	26.572	1.12	0.91	1.37

* back-transformed from log scale; geo-mean=geometric mean.
Source Post-text Table 14.2-1.1

- FTY720-P appeared in blood at the same time in both groups. FTY720-P reached peak blood levels at the same time in both groups and earlier than FTY720.
- Cmax,b was significantly greater in severe renal impaired patients than in control subjects with a geometric mean ratio (90%CI) of 1.25 (1.01 – 1.56).
- AUCs were greater in severe renal impaired patients, but in a smaller extent than Cmax,b and without reaching statistical significance, with a geometric mean ratio (90%CI) of 1.14 (0.71 –1.82) for AUCb.
- The apparent elimination half-life was comparable between the two groups .
- Protein binding was similar in both groups.

Metabolite, M3, pharmacokinetic in blood:

Descriptive statistics for M2 and M3 blood PK parameters after single dose of 1.25 mg FTY720 administration are shown in the following table:

Table 11-5 M2 and M3 pharmacokinetics in renal impaired patients and in matched healthy volunteers after a 1.25 mg dose (n=9 in each group)

PK parameters	M2		M3	
	Renal impaired	Healthy subjects	Renal impaired	Healthy subjects
t_{lag} * (h)	2.00 (0.00- 4.00)	c	2.00 (1.00- 4.00)	2.00 (1.00- 6.00)
t_{max} * (h)	12.00 (6.00- 36.00)	c	48.00 (36.00- 120.00)	12.00 (10.00- 48.00)
$C_{max,b}$ # (ng/mL)	0.303 ± 0.125 [0.279; 48]	c	5.76 ± 3.70 [5.082; 52]	0.676 ± 0.231 [0.631; 45]
$AUC_{(0-72),b}$ # (ng/mL.h)	11.1 ± 5.72 [9.43; 76]	c	290 ± 142 [267; 42]	31.4 ± 11.7 [29.1; 46]
$AUC_{(0-tz),b}$ # (ng/mL.h)	11.9 ± 8.49 [9.03; 103]	c	1092 ± 699 [906; 72]	46.2 ± 21.8 [40.9; 61]
AUC_b # (ng/mL.h)	a	c	1230 ± 802 [1003; 79]	76.4 ± 20.1 + [74.0; 29]
$t_{1/2}$ # (h)	a	c	116 ± 63 [104; 48]	95 ± 54 + [85; 53]
$C_{max,b}$ ratio # Metabolite/FTY720	0.369 ± 0.183 [0.328; 56]	c	6.98 ± 2.62 [6.58; 37]	1.20 ± 0.459 [1.080; 58]
$AUC_{(0-tz),b}$ ratio # Metabolite/FTY720	0.154 ± 0.118 [0.098; 178]	c	12.8 ± 7.64 [10.9; 70]	0.869 ± 0.466 [0.73; 80]
$Ae_{(0-72)}$ (% of dose)	b	3.62 ± 2.20 [3.03; 74]	11.5 ± 12.2 [7.3; 132]	25.3 ± 12.5 [22.1; 64]
$AURC_{\infty}$ (% of dose)	b	6.79 ± 3.97 ^ [5.94; 66]	43.0 ± 29.2 ^^ [31.5; 133]	60.0 ± 25.8 + [53.6; 62]

#: arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation];
*: median (minimum-maximum);
+: n=6
^: n=4
^^: n=7
a: Parameter not reliably estimated, b: only 1 subject with estimated parameter; c: no subject with concentrations >LLOQ
(Source: Post-text Table 14.2-1.2, Post-text Table 14.2-1.3, Post-text Table 14.2-1.4, Post-text Table 14.2-1.7, and Post-text Table 14.2-1.8)

Geometric mean ratio and 90% CIs for M3 PK parameters are shown below:

Table 11-8 Geometric mean ratio (test/reference) and 90% confidence intervals for M3 PK parameters in blood

	Adjusted geo-mean*		Geo-mean ratio*		
Parameter (Unit)	Impaired	Control	Estimate	Lower 90% CL	Upper 90% CL
$C_{max,b}$ (ng/mL)	5.082	0.631	8.05	5.53	11.72
$AUC_{(0-tz),b}$ (h*ng/mL)	905.959	40.928	22.14	13.43	36.49
AUC_b (h*ng/mL)	1002.995	73.979	13.56	7.95	23.14
$AUC_{(0-72),b}$ (h*ng/mL)	267.001	29.060	9.19	6.50	12.99

* back-transformed from log scale; geo-mean=geometric mean.
Source: Post-text Table 14.2-1.1

- M2 blood concentrations were all below the LLOQ in the healthy control subjects, but were quantifiable in all severe renal impaired patients.
- M2 blood concentrations reached peak levels at the same time as FTY720. M2 blood concentrations then declined quickly below the LLOQ and the median tlast was 48 hours.
- M3 blood concentrations were quantifiable in all healthy subjects and severe renal impaired patients.
- M3 reached peak blood levels later in severe renal impaired patients (median tmax of 48 hours) than in healthy subjects (median tmax of 12 hours).
- Cmax,b was significantly greater in severe renal impaired patients than in control subjects with a geometric mean ratio (90%CI) of 8.05 (5.53 – 11.72).
- AUC-metrics were also significantly greater in severe renal impaired patients, with a geometric mean ratio (90%CI) of 13.6 (7.95 – 23.1) for AUCb.

- The apparent elimination half-life was slightly greater in severe renal impaired patients (geometric mean of 104 hours) than in control subjects (geometric mean of 85 hours, similar to that of FTY720).
- The mean M3/FTY720 molar ratio were smaller for Cmax,b and for AUC(0-tz),b in healthy subjects than in severe renal impaired patients.
- Recovery of M3 in urine to day 3 based on Ae(0-72) was reduced in severe renal impaired patients to one-third the corresponding recovery in control subjects.

Reviewer's note:
The sponsor provided the justification stating the increase of metabolite exposure is comparable to the animal low toxic effect level (LTEL).

M2 and M3 are two inactive metabolites. The human exposure to M3 in severe renal impaired patients is comparable to that observed at the low toxic effect level (LTEL) in dog (AUC(0-24),b = 1210 ng/mL.h; 1 mg/kg for 26 weeks) and 30% smaller than that observed at the LTEL in cynomolgus monkey (AUC(0-24),b = 1702 ng/mL.h; 1 mg/kg for 52 weeks). For M2, the human exposure is 15 times smaller that that observed at the low toxic effect level (LTEL) in dog (AUC(0-24),b = 186 ng/mL.h; 1 mg/kg for 26 weeks) and 41 times smaller than that observed at the LTEL in cynomolgus monkey (AUC(0-24),b = 489 ng/mL.h; 1 mg/kg for 52 weeks). Therefore, the increase in exposure of this two metabolites observed in severe renal impaired patients is unlikely to be of clinical relevance.

The present study showed ~13.6 fold increase in M3 AUC after single dose, however, there is high likelihood of further accumulation after multiple doses and the magnitude of the increased exposure is not predictable due to the non-linear PK of FTY720 after multiple doses at doses higher than 0.5 mg. The comparable exposure after single dose in human to the steady-state exposure in animal toxicity studies doesn't assure the safety after multiple doses in human.

Conclusions:

- Compared with healthy control subjects, severe renal impaired patients had increased FTY720 and FTY720-P exposure to a maximum of an average 1.48-fold and 1.25-fold, respectively, with an unchanged apparent elimination half-life.
- Compared with healthy control subjects, severe renal impaired patients had increased M3 exposure to a maximum of an average 13.56-fold, with a slightly prolonged apparent elimination half-life.
- M2 exposure was also increased in severe renal impaired patients by at least three-fold.

Reviewer's note:

- *The sponsor concluded dose adjustment is not necessary in the severe renal impaired patients. However, although metabolites, M2 and M3 are not pharmacologically active to the intended pharmacological target, this doesn't mean they don't exhibit any toxicity through other unknown targets/pathways. As mentioned above, comparing the exposure after single dose in human to the levels after multiple doses in animal doesn't resolve the concerns especially knowing accumulation occurs after multiple doses.*
- *Further information regarding the safety profiles of M2 and M3 will be required for consideration. This is pending from the pharmacology/Toxicology review.*
- *In addition, the 43% increase in FTY720 exposure after single dose will need to be addressed. Similarly to the hepatic impaired study, the exposure change after single dose of FTY720, and the expected further increase in exposure after multiple doses, a reasonable dose adjustment is necessary. More studies might be needed to explore this issue. FTY720 should not be used in these patient populations until more data regarding long term use of FTY720 become available to ensure safely use of the drug.*

Study FTY720A 2304*:* **A double-blind, parallel-group, placebo-controlled study to compare pharmacokinetics, pharmacodynamics and safety/tolerability between healthy Caucasian and Japanese volunteers after single and multiple doses of FTY720**

A brief overview of some essential components of the study design is given below:

Study Design	double-blind, parallel-group, randomized, placebo-controlled study
Study Population	N=71 enrolled, 69 completed **Table 7-1 Demographics** Age, height, and weight are mean ± sd. Source data: Appendix 3, Tables 2.2.1 and 2.2.2.
Dosage and Administration	***Phase 1*** planned to enroll a total of 48 subjects as 24 matched ethnic pairs. They were randomized into three sequential dose cohorts of 8 pairs each. Subjects received 1.25mg, 2.5mg, or 5mg single doses of FTY720 (n = 6 pairs per dose cohort) or placebo (n = 2 pairs per dose cohort). ***Phase 2*** planned to enroll a total of 18 subjects as 9 matched ethnic pairs to receive 5mg/day FTY720 (n = 6 pairs) or placebo (n = 3 pairs) for 7 days. All treatments were given with 180 mL of water after a 10-hour fast and continue to fast for at least 4 hours thereafter on days -1 and 1 in Phase 1. In Phase 2, subjects were given the study medication within 10 minutes of having completed breakfast. Lot No. FTY720 1.25 mg capsule (FMI): US02029, KN 3761319.003 Placebo 1.25mg capsule: batch AEUS/2002-0136, KN3752962.002 FTY720 2.5 mg capsule (FMI): US02030, KN 3752938.005 Placebo 2.5mg capsule: batch AEUS/2002-0137, KN3752961.003 Diet: No fluid intake apart from the fluid given at the time of drug intake was allowed from 2 hours before until 2 hours after dosing. Otherwise, subjects should have had a fluid intake of at least 200 ml every 4 hours during waking hours on day 1 in addition to fluid taken with meals and medication. Alcohol-containing foods and beverages were prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood	At predose (0 hour), and 1, 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 144, 312, 480, and 648 hours postdose in phase 1. In phase 2, blood samples were drawn before and 1, 2, 4, 6, 8, 12, 16, 24 hours postdose after the first and last dose.
Sampling: Urine	A predose urine sample was collected in both phases of the study. 24-hour urine collections were obtained on days1 and 2 in phase1 and on days 1 and 7 in phase 2.

Variable	1.25 mg	2.5 mg	5 mg	5 mg/day	Placebo
Total number of subjects	14	15	12	12	18
Evaluable ethnic pairs	6	7	6	6	9
Age (years)	34.3 ± 8.5	33.5 ± 8.6	29.0 ± 6.2	35.6 ± 7.8	37.5 ± 9.3
Sex (male/female)	12 / 2	11 / 4	8 / 4	6 / 6	12 / 6
Ethnicity (white/Asian)	7 / 7	8 / 7	6 / 6	6 / 6	9 / 9
Height (cm)	170 ± 9	173± 9	171 ± 12	169 ± 8	168 ± 8
Weight (kg)	69.1 ± 10.5	72.0 ± 11.8	66.5 ± 12.6	68.3 ± 16.1	69.7 ± 8.3

PD sampling	**Cardiac monitoring.** On days –1, 1, and 2 of phase 1, 24-hour continuous ECGs were recorded via an analog Holter monitor. Standard 12-lead ECGs were also obtained on days –1 and 1 before dosing and at 4, 6, 12, and 24 hours postdose as well as on days 3 and 28 in the morning. On days –1, 1, 2 and 7 of phase 2, 24-hour continuous ECGs were recorded via Holter monitor. Standard 12-lead ECGs were obtained during day –1, 1, and 7 before dosing and at 4, 6, and 12 hours postdose as well as on days 2, 4, 6, 8 and 63 in the morning. **Lymphocyte counts.** In phase 1, absolute lymphocyte counts were obtained at screening, on day –1 (predose), on day 1 (predose and 1, 2, 4, 6, 8, 12, 24, 48 hours postdose), and on days 4, 5, 7, 14, 21 and 28 in the morning. In phase 2, lymphocyte counts were obtained at screening, predose on days –1, 1, 2, 3, 4, 5, 6 and 7, and on days 8, 10, 14, 28, 49, and 63 in the morning.
Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 12.7% Inter-day accuracy: <8.4 % FTY720-P: Linear range : 0.987-329 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 6.2% Inter-day accuracy: <1.3 %
Analysis (Urine)	Method LC/MS/MS Lower Limits of Quantitation Urine FTY720 1 ng/mL M2 1 ng/mL M3 1 ng/mL FTY720: Linear range : 1-400 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 55.5% Inter-day accuracy: 37 % M2: Linear range : 1-400 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 22.2% Inter-day accuracy: <11 % M3: Linear range : 3-1000 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 16.3% Inter-day accuracy: <12.5 %
PK Assessment	**FTY720 in blood and urine:** tmax , C0b , Cmax,b, Cap,b , tap , tdur , Cavg,b , AUC(0-tz)b , AUCb , AUCτ,b,

	CLb/F, Vz,b/F, t1/2, PTF, R, Ae(0-t) **FTY720-P in blood:** Cmax,b, tmax, AUC(0-t)b, C0b, Cmax,b, tmax, AUCτ,b, Cavg,b, PTF, t1/2 . **Metabolites M2 and M3 in urine:** [Ae(0-t)]
PD Assessment	Lymphocyte and heart rate response parameters included predose value, nadir value, and area under the effect time curve (AUE).
Safety Assessment	Assessment of physical examination, vital signs, ECG, clinical laboratory parameters (biochemistry, hematology, urinalysis), pregnancy test, and adverse event monitoring

Pharmacokinetic Results:

FTY720 single dose pharmacokinetics in blood:

Mean FTY720 PK parameters after 1.25 mg to 5 mg FTY720 single dose administration are shown in the following table:

Table 7-6 Single-dose pharmacokinetics: FTY720

Parameter	1.25 mg		2.5 mg		5 mg	
	Asian (n=6)	White (n=6)	Asian (n=7)	White (n=7)	Asian (n=6)	White (n=6)
Peak exposure:						
t_{max} (h)	16 (16-36)	20 (12-36)	16 (16-36)	16 (12-16)	16 (12-36)	12 (12-16)
$C_{max,b}$ (ng/ml)	1.1 ± 0.2	1.0 ± 0.4	1.9 ± 0.3	1.9 ± 0.4	3.5 ± 1.2	4.4 ± 0.9
$C_{max,b}$/dose (ng/ml)	0.85 ± 0.16	0.77 ± 0.34	0.76 ± 0.12	0.78 ± 0.14	0.69 ± 0.24	0.88 ± 0.19
t_{dur} (h)	32 (20-40)	24 (4-60)	24 (24-60)	40 (24-40)	50 (24-300)	30 (0-40)
t_{ap} (h)	22 (16-26)	20 (14-30)	20 (16-30)	20 (16-24)	30 (20-42)	18 (12-24)
$C_{ap,b}$ (ng/ml)	1.0 ± 0.2	0.9 ± 0.4	1.8 ± 0.3	1.8 ± 0.3	3.2 ± 1.0	4.1 ± 1.0
Overall exposure:						
AUC(0-t_z)$_b$ (ng.h/ml)	168 ± 27	131 ± 42	353 ± 87	373 ± 130	823 ± 292	794 ± 250
AUC_b (ng.h/ml)	205 ± 47	150 ± 43	381 ± 95	455 ± 195	932 ± 402	861 ± 302
AUC_b/dose (ng.h/ml)	164 ± 38	120 ± 34	153 ± 38	182 ± 78	186 ± 80	172 ± 60
CL_b/F (L/h)	6.4 ± 1.5	9.1 ± 3.4	7.0 ± 2.0	6.3 ± 2.3	6.2 ± 2.5	6.4 ± 1.9
$V_{z,b}$/F (L)	1230 ± 350	1504 ± 450	1319 ± 147	1548 ± 293	1470 ± 346	1354 ± 373
$t_{1/2}$ (days)	5.9 ± 2.4	5.2 ± 2.2	5.8 ± 1.5	8.2 ± 4.2	7.6 ± 3.4	6.7 ± 2.8
Renal excretion:						
Ae(0-48) (mcg)	0	0	0	0	0	0

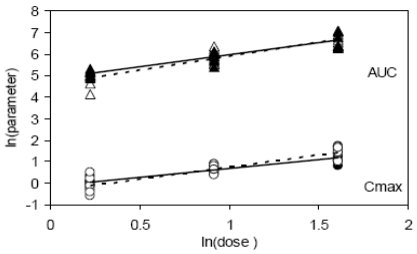
Values are mean ± sd except for temporal parameters which are median (range).
Data source: Appendix 4 Table 1 and Table 2.

- Intersubject coefficients of variability for Cmax,b were 23% in Asians and 28% in whites and for AUCb were 32% in Asians and 40% in whites.
- The Asian/white parameter ratio (90%CI) for Cmax,b/dose was 0.96 (0.85, 1.09) and for AUCb/dose was 1.07 (0.92, 1.25).
- There were no apparent differences or trends in peak, total exposure or elimination half-life (0.98 (0.79, 1.21)) between the ethnic groups

Dose proportionality:

Relationships of FTY720 dose levels versus FTY720 Cmax and AUC are shown in the figure below:

Figure 7-3 FTY720 dose-exposure relationships: ln-transformed data



Relationship between FTY720 dose versus Cmax and AUC in logarithmic coordinates for white subjects (*open symbols*) and Asian subjects (*filled symbols*). Shown are the corresponding regression lines (*dashed* and *solid*, respectively).

Regression of FTY720 dose levels versus FTY720 Cmax and AUC are shown below:

Table 7-7 Dose-exposure regressions: FTY720

Parameter	Ethnicity	Regression equation (untransformed)	Slope (90%CI) (ln transformed)*
$C_{max,b}$	Asian	$C_{max,b}$ = 0.641 x Dose + 0.271	0.84 (0.66, 1.01)
	White	$C_{max,b}$ = 0.924 x Dose – 0.270	1.13 (0.96, 1.31)
$AUC(0\text{-}t_z)_b$	Asian	$AUC(0\text{-}t_z)_b$ = 177 x Dose – 68	1.12 (0.90, 1.33)
	White	$AUC(0\text{-}t_z)_b$ = 175 x Dose – 78	1.31 (1.10, 1.53)
AUC_b	Asian	AUC_b = 198 x Dose – 74	1.05 (0.82, 1.29)
	White	AUC_b = 185 x Dose – 49	1.26 (1.02, 1.50)

* Dose-proportionality over the full dose range 0.125 – 5 mg concluded if 90%CI for slope was contained in the interval (0.84, 1.16).

- For both ethnicities, the dose-Cmax,b relationship deviated from strict dose-proportionality by −16% for Asians and +13% for whites (based on the slopes).
- While there was no significant interethnic difference in intercepts [0.23 (-0.03, 0.50) ng/ml], there was a borderline significant difference in slopes [−0.30 (−0.55, −0.05) ng/ml/mg].
- For both ethnicities, the dose-AUC,b relationship deviated from strict dose proportionality by +5% for Asians and +26% for whites.
- There was no interethnic difference in intercepts [0.26 (-0.11, 0.62) ng.h/ml] or slopes [−0.20 (−0.54, 0.14) ng.h/ml/mg].

Metabolites pharmacokinetics in blood after FTY720 single dose of 1.25 mg to 5 mg:

Mean metabolites PK parameters after 1.25 mg to 5 mg FTY720 single dose administration are shown in the following table:

Table 7-8 Single-dose pharmacokinetics: metabolites

Parameter	1.25 mg		2.5 mg		5 mg	
	Asian (n=5)	White (n=4)	Asian (n=7)	White (n=7)	Asian (n=6)	White (n=6)
FTY720-phosphate:						
t_{max} (h)	6 (6-12)	6 (6-12)	12 (6-12)	6 (6-16)	12 (6-16)	12 (6-12)
$C_{max,b}$ (ng/ml)	1.3 ± 0.2	1.5 ± 0.1	2.2 ± 0.3	2.3 ± 0.6	3.6 ± 1.1	3.6 ± 0.8
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	8 ± 5	16 ± 1	37 ± 16	41 ± 29	158 ± 76	142 ± 72
Hexanoic acid metabolite:						
Ae(0-48) (mcg)	68 ± 24	95 ± 34	140 ± 74	239 ± 115	317 ± 44	414 ± 125
Butanoic acid metabolite:						
Ae(0-48) (mcg)	355 ± 154	406 ± 160	963 ± 474	861 ± 348	1738 ± 615	1411 ± 427

Values are mean ± sd except for temporal parameters which are median (range).
Data source: Appendix 4 Table 3, Table 4, Table 5.

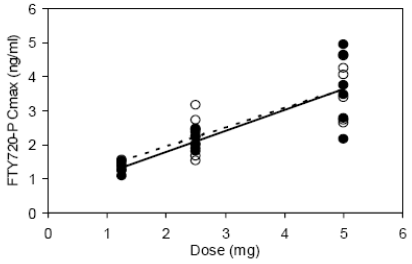
FTY720-P

- At the lowest dose, concentrations were sporadically quantifiable between 6 and 16 hours in some subjects. Pharmacokinetic parameters were difficult to interpret and compare between ethnicities.
- The mean FTY720-P profiles demonstrate a slightly earlier and higher peak for FTY720-P than for FTY720. The decline in concentrations after 24 hours was difficult to interpret due to few quantifiable levels.
- Generally robust peaks and truncated AUC(0-tz)b's could be derived at 2.5mg and appeared to be similar between ethnicities based on the Asian/white parameter ratios but with generally wide 90% confidence intervals: 0.97 (0.85, 1.11) for Cmax,b and 1.05 (0.74, 1.50) for AUC(0-tz)b.
- FTY720-P concentrations peaked around the same time as FTY720 but were slightly lower in magnitude.
- From 24 hours postdose, concentrations of both analytes appeared to decline in parallel with FTY720-P concentrations around half the respective FTY720 concentrations.
- There were no apparent ethnic differences in Cmax,b or AUC(0-tz)b with Asian/white ratios of 0.99 (0.85, 1.14) and 1.14 (0.78, 1.68), respectively.

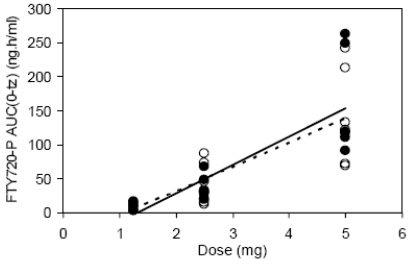
Dose proportionality:

Relationships of FTY720 dose levels versus FTY720-P Cmax and AUC are shown in the figure below:

Figure 7-5 FTY720-phosphate dose-exposure relationships



Relationship between FTY720 dose and FTY720-phosphate Cmax in white subjects (*open circles*) and Asian subjects (*filled circles*). Shown are the corresponding regression lines (*dashed* and *solid*, respectively).



As in left panel for FTY720-phosphate AUC(0-t).

Regression of FTY720 dose levels versus metabolite exposure parameters are shown in the table below:

Table 7-9 Dose-exposure regressions: metabolites

Analyte	Parameter	Ethnicity	Slope (90%CI) (ln transformed*
FTY720-phosphate	$C_{max,b}$	Asian	0.72 (0.57, 0.87)
		White	0.64 (0.47, 0.80)
	$AUC(0\text{-}t_z)_b$	Asian	2.24 (1.82, 2.66)
		White	1.52 (1.08, 1.96)
Hexanoic acid metabolite M2	Ae(0-48)	Asian	1.15 (0.86, 1.43)
		White	1.08 (0.79, 1.37)
Butanoic acide metabolite M3	Ae(0-48)	Asian	1.17 (0.87, 1.48)
		White	0.91 (0.60, 1.21)

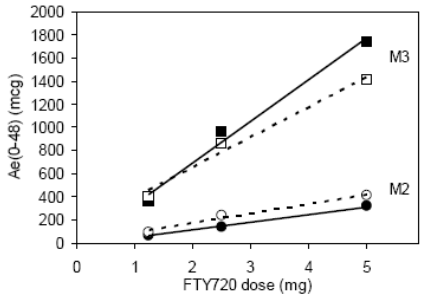
* Dose-proportionality over the full dose range 0.125 – 5 mg concluded if 90%CI for slope was contained in the interval (0.84, 1.16).

- There did not appear to be any differences or trends in peak or total exposure between the ethnic groups.
- FTY720-P Cmax,b rose under-proportionally with dose in both ethnic groups by −28% for Asians and −36% for whites.

- There were no interethnic difference in intercepts [−0.13 (-0.38, 0.12) ng/ml] or slopes [0.08 (−0.14, 0.31) ng/ml/mg].
- For both ethnicities, the dose-AUC(0-tz)b relationship was over-proportional by +124% for Asians and +52% for whites.
- There were significant interethnic differences for intercepts [−0.87 (-1.55, −0.19) ng.h/ml] and slopes [0.72 (0.11, 1.32) ng.h/ml/mg].

Relationships of FTY720 dose levels versus M2 and M3 excreted in the urine are shown in the figure below:

Figure 7-6 Metabolite dose-excretion relationships



Relationship between FTY720 dose and amounts of metabolites M2 and M3 excreted in 48 hours [Ae(0-48)] for white subjects (*open symbols*) and Asian subjects (*filled symbols*). Shown are the corresponding regression lines (*dashed* and *solid*, respectively).

Hexanoic (M2) and butanoic acid (M3) metabolites.

- The amounts excreted of both M2andM3 exhibited high interindividual variability of between 30 to 53 percent. When normalized for dose and pooled, the amount of M2 excreted was 31% lower in Asians compared with whites: ratio (90%CI) 0.68 (0.55, 0.85). Whereas, no difference in the amount of M3 was apparent: 1.04 (0.84, 1.29).
- The amounts of each metabolite excreted rose with increasing dose, but deviated slightly from strict dose-proportionality. There were no marked interethnic differences in intercepts or slopes for either metabolite: M2 intercept difference −0.44 (−0.87, −0.01) mcg, slope difference 0.07 (−0.34, 0.47) mcg/mg; M3 intercept difference −0.21 (−0.68, 0.25) mcg, slope difference 0.27 (−0.17, 0.71) mcg/mg.

Multiple-dose pharmacokinetics

<u>FTY720 and FTY720-P multiple dose pharmacokinetics in blood:</u>

Mean FTY720 and FTY720-P PK parameters after 5 mg FTY720 QD multiple doses administration are shown in the following table:

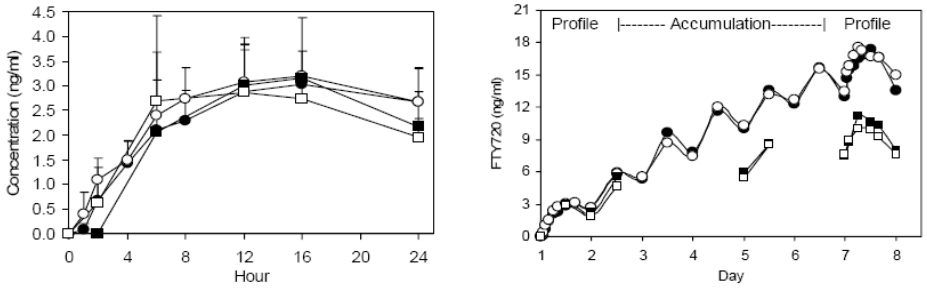
Table 7-10 Multiple-dose pharmacokinetics: FTY720 and FTY720-phosphate

Parameter	FTY720		FTY720-phosphate	
	Asian (n=6)	White (n=6)	Asian (n=6)	White (n=6)
Day 1:				
t_{max} (h)	14 (6-16)	14 (12-16)	14 (6-16)	12 (6-16)
$C_{max,b}$ (ng/ml)	3.1 ± 0.8	3.3 ± 0.5	3.7 ± 1.1	3.3 ± 0.9
$AUC_{\tau,b}$ (ng.h/ml)	54 ± 12	59 ± 11	52 ± 14	53 ± 14
Day 7:				
R	7.0 ± 0.7	6.6 ± 0.4	4.6 ± 1.1	4.2 ± 0.6
$C0_b$ (ng/ml)	13.0 ± 3.0	13.4 ± 3.5	7.4 ± 2.3	7.6 ± 2.0
t_{max} (h)	12 (6-16)	7 (6-16)	9 (6-16)	6 (0-12)
$C_{max,b}$ (ng/ml)	18.2 ± 4.8	17.9 ± 3.4	11.3 ± 3.5	10.9 ± 1.8
$AUC_{\tau,b}$ (ng.h/ml)	382 ± 106	390 ± 73	236 ± 76	219 ± 42
$C_{avg,b}$ (ng/ml)	15.9 ± 4.4	16.3 ± 3.0	9.8 ± 3.2	9.1 ± 1.7
PTF (%)	32 ± 8	28 ± 6	40 ± 10	34 ± 23
$t_{1/2}$ (days)	7.9 ± 2.0	7.4 ± 0.8	6.0 ± 2.4	7.1 ± 2.0

Values are median (range) for tmax and mean ± sd for all others.
Data source: Appendix 4 Table 2 and Table 4.

Mean FTY720 and FTY720-P blood concentration-time plots after 5 mg FTY720 QD multiple doses administration on day 1 and the accumulation phase are shown in the following figure:

Figure 7-7 FTY720 multiple-dose profiles and accumulation



Mean concentration profiles over the 24-hour dose interval on day 1 in Asian subjects (*filled symbols*) and white subjects (*open symbols*). Shown are FTY720 (*circles*) and FTY720-phosphate (*squares*). *Bars* represent 95% confidence intervals.

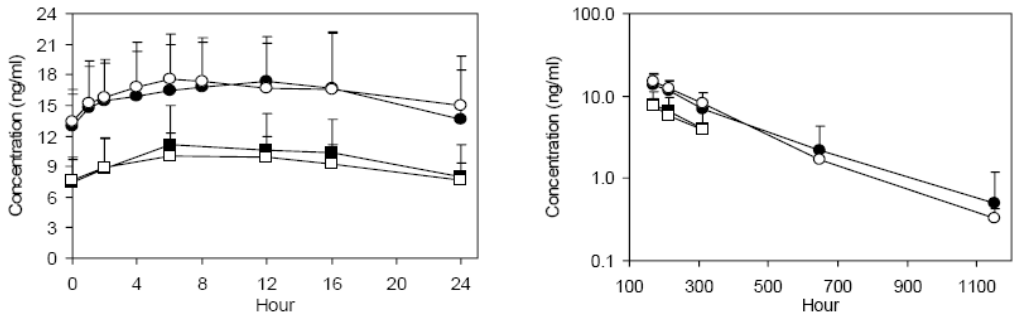
Mean concentration profiles on days 1 and 7 with the mean predose C0 and peak C12 concentrations on days 2 through 6. Symbols as defined in left panel,

Day 1 exposure.

- No differences in exposure to either analyte were apparent in the two ethnic groups with Asian/white parameter ratios as follows: 0.93 (0.81, 1.07) and 0.91 (0.78, 1.05) for FTY720 Cmax,b and AUCτ,b and 1.10 (0.86, 1.40) and 0.98 (0.78, 1.25) for FTY720-phosphate Cmax,b and AUCτ,b.

Mean FTY720 and FTY720-P blood concentration-time plots after 5 mg FTY720 QD multiple doses administration on day 7 and the elimination phase are shown in the following figure:

Figure 7-8 FTY720 and FTY720-phosphate multiple-dose profiles



Mean concentration profiles over the 24-hour dose interval on day 7 in Asian subjects (*filled symbols*) and white subjects (*open symbols*). Shown are FTY720 (*circles*) and FTY720-phosphate (*squares*). *Bars* represent 95% confidence intervals.

As in left panel during the washout after day 7. the assay quantification limit for FTY720 was 0.08 ng/ml and for FTY720-phosphate was 1 ng/ml.

Day 2 to 7 accumulation.

- FTY720 blood levels accumulated over the week with exposure on day 7 averaging 7-fold higher than on day 1.
- FTY720-P accumulated less, averaging 4-fold.
- The respective Asian/white ratios for accumulation were 1.06 (0.98, 1.14) for FTY720 and 1.06 (0.85, 1.33) for FTY720-P.
- On day 7, the FTY720 blood level profiles were notable for their flatness with a peak-trough fluctuation of around 30 percent consistent with the long half-life relative to the short dosing interval.
- With multiple dosing the concentration-time patterns for both analytes on day 7 were roughly similar but FTY720-P was shifted downward on the concentration scale relative to FTY720 compared with the patterns on day 1.
- The FTY720-P/FTY720 molar ratio based on AUCτ,b was 0.62 ± 0.07 in Asian subjects and 0.56 ± 0.09 in white subjects.
- Asian/white parameter ratios on day 7 were: 1.00 (0.85, 1.17) and 0.96 (0.83, 1.10) for FTY720 Cmax,b and AUCτ,b and 1.01 (0.78, 1.31) and 1.05 (0.87, 1.26) for FTY720-phosphate Cmax,b and AUCτ,b

Washout and elimination half-life.

- After the last dose, FTY720 and FTY720-P blood levels declined in a log-linear manner in parallel.
- The half-life of FTY720 averaged 8 days while that of FTY720-P was more difficult to estimate since blood levels fell below the assay quantification limit in most subjects by 648 hours postdose. Nonetheless, the half-life of FTY720-P appeared similar to that of FTY720.
- The respective Asian/white half-life ratios were 1.05 (0.86, 1.29) for FTY720 and 0.82 (0.52, 1.29) for FTY720-phosphate.

Urinary excretion of metabolites

Table 7-11 Multiple-dose pharmacokinetics: metabolites

Parameter	Metabolite M2		Metabolite M3	
	Asian	White	Asian	White
Day 1 Ae(0-24) (mg)	0.2 ± 0.1	0.3 ± 0.1	0.9 ± 0.5	1.5 ± 0.7
Day 7 Ae(0-24) (mg)	1.2 ± 1.0	2.1 ± 0.7	6.5 ± 4.9	12.4 ± 4.1

Note: these data are in mg; whereas the single-dose data in Table 7-8 are in mcg.
Data source: Appendix 4 Table 5.

- Excretion of M2 was quantitatively less than that of M3 on both days.
- From day 1 to day 7, excretion increased roughly 7-fold consistent with FTY720 accumulation in blood over this time period.
- Excretion of both metabolites was about 2-fold higher in whites compared with Asians on both days based on the Asian/white ratios but the 90% confidence intervals were wide: 0.60 (0.36, 1.01) for M2 and 0.52 (0.27, 1.01) for M3 on day 1 and 0.48 (0.24, 0.95) for M2 and 0.47 (0.25, 0.90) for M3 on day 7.

Pharmacodynamics results:

Lymphocyte responses: single-dose FTY720

Lymphocyte counts and derived response parameters are summarized in the table below:

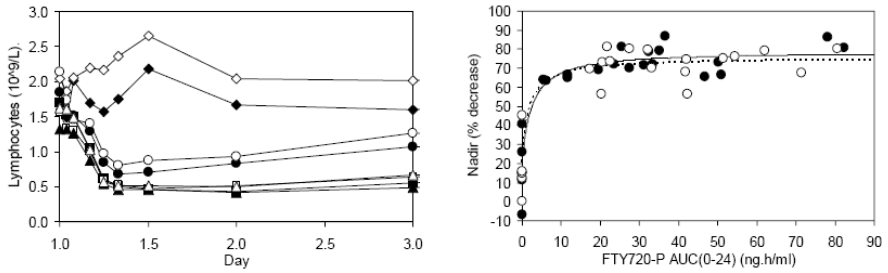
Table 7-12 Lymphocyte response parameters: single-dose FTY720

Parameter	1.25 mg		2.5 mg		5 mg	
	Asian	White	Asian	White	Asian	White
Acute response:						
Predose (10^9/L)	1.85 ± 0.57	2.14 ± 0.83	1.69 ± 0.36	1.56 ± 0.44	1.32 ± 0.38	1.62 ± 0.33
Time of nadir (h)	8 (6-48)	10 (8-12)	12 (8-24)	12 (6-24)	36 (24-96)	18 (8-24)
Nadir (10^9/L)	0.62 ± 0.20	0.72 ± 0.22	0.41 ± 0.07	0.43 ± 0.16	0.30 ± 0.12	0.41 ± 0.09
Nadir (% predose)	33 ± 3	36 ± 11	25 ± 4	28 ± 8	24 ± 9	26 ± 5
AUE(1) (10^9/L x day)	0.9 ± 0.3	1.0 ± 0.3	0.7 ± 0.1	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.1
Recovery:						
Day 14 count (10^9/L)	1.42 ± 0.39	1.57 ± 0.47	1.29 ± 0.25	1.43 ± 0.26	0.69 ± 0.33	1.25 ± 0.45
Day 21 count (10^9/L)	1.69 ± 0.47	1.93 ± 0.55	1.55 ± 0.44	1.39 ± 0.43	0.91 ± 0.43	1.51 ± 0.28
Day 28 count (10^9/L)	1.74 ± 0.51	1.93 ± 0.56	1.74 ± 0.33	1.56 ± 0.43	1.06 ± 0.36	1.69 ± 0.43
AUE(1-28) (10^9/L x day)	40 ± 11	45 ± 12	33 ± 6	34 ± 8	21 ± 7	33 ± 7

Values are mean ± sd except for time of nadir which is median (range).
Data source: Appendix 3 Table 3.3.8.1, Appendix 4 Table 6.

Lymphocyte counts trajectories are shown in the figure below:

Figure 7-9 Lymphocyte acute response plots: single-dose FTY720



Mean lymphocyte trajectories to day 3 after single-dose FTY720 in Asians (*filled symbols*) and whites (*open symbols*) receiving placebo (*diamonds*), 1.25mg (*circles*), 2.5mg (*squares*), and 5mg (*triangles*). Synoptic plots for each dose group are in Appendix 4 Figure 2.

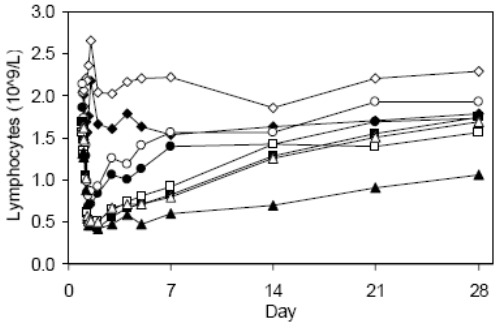
Scatterplot of FTY720-phosphate exposure on day 1 postdose versus percent decrease from baseline in lymphocytes at nadir and the fits of an inhibitory Emax model to the data in Asians (*filled circles, solid line*) and whites (*open circles, dashed line*).

Acute lymphocyte decrease.

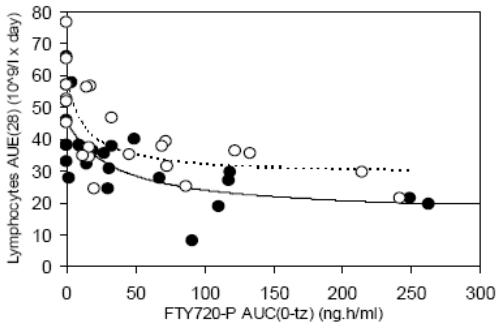
- Both the nadir lymphocyte count and the time at which it occurred showed an association with dose. With increasing dose, the lymphocyte nadir decreased as follows: 0.6 x 10^9/L at 1.25mg, 0.4 x 10^9/L at 2.5mg, and 0.3 x 10^9/L at 5mg. The time of nadir occurrence was later with increasing dose: 8-10 hours, 12 hours, and 18-36 hours, respectively.
- The corresponding percent decrease from predose averaged 66% at 1.25mg, 74% at 2.5mg, and 75% at 5mg. There were no notable interethnic differences in nadirs as percent decrease from predose.
- The decrease to nadir in placebo-treated subjects (E0) likely represented day-to-day variation in lymphocyte counts and was 21.1 ± 3.5 versus 20.7 ± 4.3 percent in Asians and whites.
- The FTY720-P AUC(0-24)b yielding a half-maximal decrease (EC50) was 2.5 ± 1.3 versus 1.7 ± 3.7 ng.h/ml in Asians and whites. The high standard error for whites was due to the lack of data at low exposures for this relationship.
- The maximal decrease from baseline in lymphocytes (Emax) was well estimated and amounted to 79.0 ± 3.4 versus 75.4 ± 6.3 percent for Asians and whites, respectively.

Recovery

Figure 7-10 Lymphocyte recovery plots: single-dose FTY720



Mean lymphocyte trajectories over the full study course after single-dose FTY720 in Asians (*filled symbols*) and whites (*open symbols*) receiving placebo (*diamonds*), 1.25mg (*circles*), 2.5mg (*squares*), and 5mg (*triangles*). Synoptic plots for each dose group are in Appendix 4 Figure 2.



Scatterplot of FTY720-phosphate overall exposure versus lymphocyte area under the effect curve over the full study course with the fits of an inhibitory effect Emax model to the data in Asians (*filled circles, solid line*) and whites (*open circles, dashed line*).

- Mean lymphocyte count recovery trajectories do not reveal any interethnic differences after single doses of 1.25mg or 2.5mg but recovery appeared slower in Asians than whites after single-dose 5mg.
- Using linear regression on lymphocyte count versus time from day 4 to 28 yielded recovery rates of 0.0242 ± 0.0099 versus 0.0441 ± 0.0149 cells x 10^9/L/day for Asians versus whites.
- The AUE(1-28) was 36% lower in Asians.
- AUE(1-28) after placebo in Asians was lower than in whites: E0 45.6 ± 3.5 versus 57.8 ± 4.0 10^9/L x days.
- The FTY720-P AUC(0-tz)b yielding a half-maximal decrease (EC50) was 35.2 ± 32.3 versus 14.2 ± 11.7 ng.h/ml in Asians and whites, both with high standard errors.
- The maximal decrease in AUE(1-28) or Emax was 16.5 ± 8.2 versus 28.5 ± 5.6 10^9/L x days for Asians and whites, respectively.

- By day 28, the mean lymphocyte counts had recovered back to the predose range for all groups with the exception of Asian subjects receiving 5mg.

Lymphocyte responses: multiple-dose FTY720

Lymphocyte counts and derived response parameters are summarized in the table below:

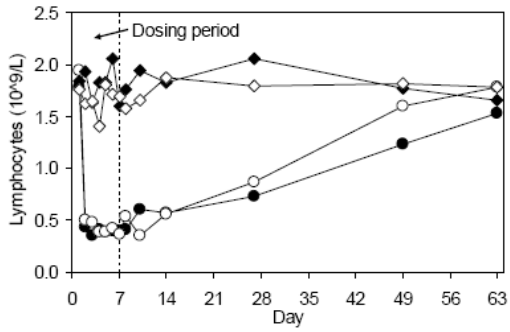
Table 7-13 Lymphocyte response parameters: multiple-dose FTY720

Parameter	Asian	White
Acute response:		
Predose (10^9/L)	1.78 ± 0.44	1.94 ± 0.43
Time of nadir (day)	7 (3-8)	7 (5-10)
Nadir (10^9/L)	0.27 ± 0.15	0.25 ± 0.11
Nadir (% predose)	15 ± 5	13 ± 4
AUE(1-8) (10^9/L x day)	3.5 ± 1.5	3.8 ± 1.2
Recovery:		
Day 27 count (10^9/L)	0.74 ± 0.41	0.87 ± 0.44
Day 49 count (10^9/L)	1.24 ± 0.52	1.60 ± 0.52
Day 63 count (10^9/L)	1.53 ± 0.70	1.78 ± 0.41
AUE(1-63) (10^9/L x day)	57 ± 18	67 ± 18

Values are mean ± sd except for time which is median (range).
Data source: Appendix 3 Table 3.3.8.1, Appendix 4 Table 6.

Lymphocyte counts trajectories are shown in the figure below:

Figure 7-11 Lymphocyte response plots: multiple-dose FTY720



Mean lymphocyte trajectories after multiple-dose FTY720 in Asians (*filled symbols*) and whites (*open symbols*) receiving placebo (*diamonds*) and 5mg/day (*circles*) from day 1 to 7. Synoptic plots in Appendix 4 Figure 2.

- The percent decrease from baseline to nadir was on average 85%.
- One week after the last dose, return of lymphocytes to the blood was evident and reached nearly half the predose value by 3 weeks after the last dose .
- Full recovery was apparent at the end-of-study visit (day 63).
- Lymphocyte responses were similar in both ethnic groups over the short-term with Asian/white response parameter ratios of 1.04 (0.85, 1.28) for nadir count and 0.90 (0.69, 1.18) for AUE(1-8).
- Over the long-term to day 63, the recovery in lymphocytes appeared slightly slower for Asians based on the Asian/white AUE(1-63) ratio of 0.85 (0.65, 1.10).
- Linear regression on the lymphocyte-time data from day 8 to 63 yielded a recovery rates of 0.0219 ± 0.0070 vs 0.0260 ± 0.0066 cells x 10^9/L/day for Asians and

whites, respectively. This indicates a mean 16% slower recovery rate for Asians compared with whites.

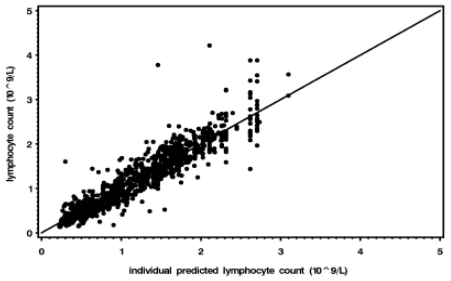
Lymphocyte response: mechanistic model

Table 7-14 Indirect-response model parameters

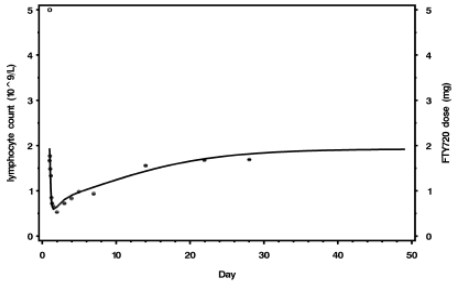
Parameter	Estimate	Standard error (CV %)	Inter-subject CV (%)
Imax	0.883	2.7%	-
IC50 (ng/ml)	0.537	9.9%	38.5%
Kout (1/h)	0.419	7.2%	-
Kin (10^9/L/h)	0.739	8.3%	22.7%

Data source: Appendix 9.

Figure 7-12 Indirect-response diagnostic plots for lymphocytes



Individual measured versus model-predicted lymphocyte counts from all subjects in the study. Shown is the identity line.



Representative lymphocyte-time plot (*filled circles*) for subject 5221 receiving 5mg FTY720 on day 1 (*open circle*). The curve is the model-predicted fit to the data.

- The population indirect-response model described the time course of lymphocyte counts as a function of FTY720- P blood concentration.
- The population means of the parameters could be precisely estimated, with small standard errors (coefficients of variation, 2-10%). The steady-state value of lymphocyte counts in the absence of FTY720-P was 1.76 x 10^9/L (=kin/kout).
- The residual standard deviation was 0.26 x 10^9/L, which corresponds to a coefficient of variation of 15% at the steady-state value of lymphocyte counts.
- There was no apparent difference in model parameters between Asian and white subjects. This is also supported by the statistical analysis based on the difference of the objective function between the basic model and the extended model with a covariate for ethnicity (p =0.14).

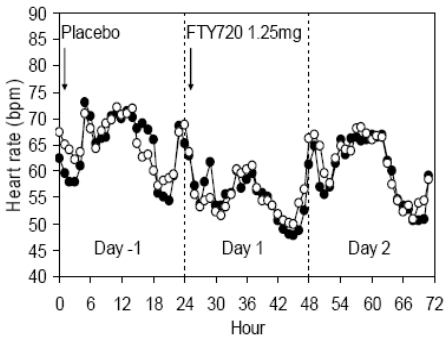
Lymphocyte responses: lymphocyte subsets (T cells and B cells)

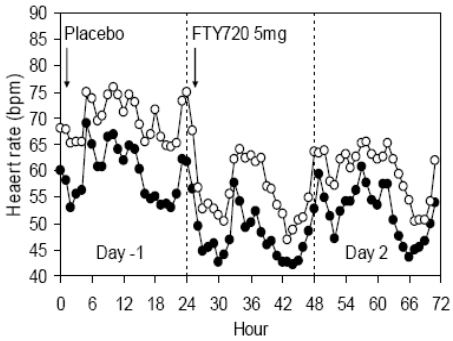
- Temporal patterns were similar to those for total lymphocytes with the exception of monocytes which did not appear to decrease postdose.
- Trajectories did not appear to be ethnic sensitive.

Heart rate responses: single-dose FTY720

The heart rate trajectories are shown in the figure below:

Figure 7-14 Heart rate response plots: single-dose FTY720





Mean heart rate trajectories after single-dose administration of placebo on day -1 (0-24 h) and 1.25mg FTY720 on day 1 (24-72 hours) in Asians (*filled circles*) and whites (*open circles*).

As in left panel for subjects receiving 5mg FTY720. Synoptic plots at each dose level are in Appendix 4 Figure 3.

Day 1: acute response to FTY720.

- There were no within-dose interethnic differences noted based on the Asian/white ratios for nadir heart rate of 1.06 (0.98, 1.16) at 1.25mg; 1.04 (0.96, 1.11) at 2.5mg; and 1.02 (0.91, 1.15) at 5mg.
- Similar ethnic ratios were noted for the parameter AUE(0-4).

Day 2 and end-of-study: recovery.

- A slight recovery toward the baseline values was evident on day 2.
- Supine pulse at the end-of-study visit on day 28 demonstrated full recovery of the morning heart rate: 63 ± 11 bpm at 1.25mg, 62 ± 8 bpm at 2.5mg, 60 ± 7 bpm at 5mg pooled across all subjects.

Heart rate responses: multiple-dose FTY720

Heart rate responses after multiple doses of FTY720 are shown in the table below:

Table 7-16 Heart rate response parameters: multiple-dose FTY720

Parameter	Day -1		Day 1	
	Asian	White	Asian	White
Morning rate (bpm)	71 ± 7	68 ± 9	73 ± 9	68 ± 9
Day nadir time (h)	4 (3-11)	4 (3-4)	5 (3-7)	3 (3-5)
Day nadir (bpm)	62 ± 7	59 ± 7	46 ± 7	43 ± 6
AUE(0-4) (bpm.h)	267 ± 30	261 ± 33	241 ± 37	223 ± 36
Night nadir time (h)	18 (17-20)	19 (16-23)	17 (15-20)	18 (15-18)
Night nadir (bpm)	55 ± 7	53 ± 7	45 ± 8	45 ± 8
AUE(0-24) (bpm.h)	1585 ± 189	1558 ± 239	1245 ± 201	1254 ± 198
	Day 2		Day 7	
	Asian	White	Asian	White
Morning rate (bpm)	61 ± 7	60 ± 10	69 ± 7	66 ± 9
Day nadir time (h)	4 (2-10)	4 (3-9)	4 (3-4)	4 (3-4)
Day nadir (bpm)	51 ± 9	47 ± 9	61 ± 7	59 ± 9
AUE(0-4) (bpm.h)	213 ± 54	220 ± 40	262 ± 28	258 ± 35
Night nadir time (h)	20 (18-20)	17 (16-21)	18 (18-20)	19 (18-22)
Night nadir (bpm)	47 ± 6	46 ± 7	54 ± 6	53 ± 9
AUE(0-24) (bpm.h)	1242 ± 196	1247 ± 186	1482 ± 169	1439 ± 205

Values are mean ± sd except for time of nadir which is median (range).
Data source: Appendix 4 Table 7.

- The heart rate trajectories were similar for Asians and whites in the FTY720-treated group on days −1, 1 and 2.
- On day 1, the daytime nadir rate was reduced from day −1 by an average −26% in Asians and −27% in whites.
- This interethnic similarity was also noted for the day 1 Asian/white ratio for daytime nadirs of 0.96 (0.86, 1.07) and for AUE(0-4) of 0.98 (0.88, 1.08). By day 7 with continued dosing, morning heart rate (07:00) and the daytime nadir (11:00) were nearly back to the day −1 baseline values.
- Nonetheless, the overall heart rate vs time AUE(0-24) on day 7 was not fully recovered to day −1 values averaging −6% in Asians and −8% in whites compared to day −1.

Conclusions:

- **Single-dose pharmacokinetics.** There were no clinically relevant differences in the dose-Cmax or dose-AUC relationships between Asian and white subjects for either FTY720 or FTY720-P. The amount of M2 excreted in urine was slightly lower in Asians than whites at 5mg but the amount excreted of M3 was similar between ethnicities.
- **Multiple-dose pharmacokinetics.** There were no clinically relevant interethnic differences in FTY720 or FTY720-P accumulation, exposure, or elimination. The amount of both M2 and M3 excreted in urine on day 1 and 7 was about two fold lower in Asians compared with whites but against a background of high variability.
- **Lymphocyte responses.** The acute effects of single and multiple FTY720 doses on lymphocytes were similar between ethnic groups. The lymphocyte recovery rate after 5mg single-dose and 5mg/day multiple-dose FTY720 was reduced slightly in Asian subjects.
- **Heart rate responses.** The transient, acute decrease in heart rate after the first dose of FTY720 and the subsequent recovery was similar in Asian and white subjects after both single-dose and multiple-dose FTY720.

Reviewer's Comment:

- *Although the finding is minor and doesn't seem to be of significance, the lower excretion of metabolite M2 in Asian at 5 mg after single dose and the two-fold lower excretion in M2 and M3 in Asian after multiple doses and in addition, the decreased recovery rate for lymphocyte counts in Asian at 5 mg single and multiple doses, all together, might suggest there might be ethic differences in metabolism or excretion which might lead to different pharmacological outcomes after long term use.*
- *It is known that CYP4F2 exist polymorphisms however relatively limited information is available up to date. Based on the finding in the present study, there might be CYP4F2 genetic variants between Caucasian and Asian however require further investigations.*

4.1-3. HUMAN PK STUDIES
4.1-3.4 Extrinsic factors

Study FTY720 A107: A two-period, randomized, crossover study to evaluate the effect of Neoral on the bioavailability of FTY720 in psoriatic patients

Study Rationale
Initially FTY720 (fingolimod) a sphingosine-1-phosphate receptor agonist was developed as an immunomodulator for prophylaxis of acute rejection after organ transplantation. It was intended to be used in multidrug immunosuppressive regimens with agents such as cyclosporine. In order to provide administration guidance, this study was conducted to assess whether coadministration of FTY720 with cyclosporine alters the disposition of either agent.

Objectives	The primary objective was to determine the effect of multiple doses of Neoral on the pharmacokinetics of a single dose of FTY720 in psoriatic patients. The secondary objectives were (1) to determine the effect of a single dose of FTY720 on the pharmacokinetics of Neoral and (2) to determine the safety and tolerability of FTY720 in combination with Neoral in psoriatic patients.
Study Design	Open-label, randomized, two-period, two-treatment, crossover study. Treatment A: single 1 mg dose of FTY720 on day 1. Treatment B: cyclosporine (Neoral) 200 mg twice-daily for 8 days and a single 1 mg dose of FTY720 on day 5.
Study Population	Male and female subjects aged 18 to 65 years with psoriasis.
Investigational Drug	FTY720 1 mg capsule (center 1: Lot X1680998, KN3754538.00.001; center 2: Lot H05624, KN3754538.00.003A). Neoral soft gelatin capsules containing 100 mg cyclosporine (commercially purchased by the centers). Treatment A: Study medication on day 1 and a total of 5 days of evaluations. Treatment B: Study medications days 1 to 8 and a total of 9 days of evaluations.
Blood Sampling:	Venous blood samples were obtained before FTY720 administration and then at 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96 hours postdose. Each blood sample was 3 ml in volume collected in an EDTA-containing vacuum tube. Venous blood samples were obtained on days 4 and 6 before the morning cyclosporine administration and then at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours postdose. Each blood sample was 3 ml in volume collected in an EDTA-containing vacuum tube.
Assay	Plasma concentrations of fingolimod, fingolimod-phosphate and cyclosporine were determined separately by validated liquid chromatography methods with tandem mass spectrometry (LC/MS/MS).
PK Assessment	Whole blood samples for FTY720 in treatments A and B were drawn over a 96-hour period after each FTY720 dose and analyzed using LC/MS/MS. Whole blood samples for cyclosporine in treatment B were drawn over a 12-hour steady-state morning dose interval on days 4 and 6 and analyzed by a radioimmunoassay method. Standard noncompartmental pharmacokinetic parameters were derived for both analytes.
Pharmacodynamic Evaluations	Peripheral blood lymphocyte counts and supine heart rate were obtained at screening and each morning of treatment A and B. Derived response parameters included the nadir value, time to reach the nadir, and area-under-the-effect curve.

Statistical methods	Pharmacokinetic and response parameters were compared between treatments in ANOVA. Lack of a drug interaction was based on conventional bioequivalence criteria on pharmacokinetic parameters.

Dose Selection:
Subjects were dosed with 1 mg fingolimod, which is the twice the dose sought for clinical use and 200 mg cyclosporine twice-daily is the commonly used therapeutic maintenance dosage regimen.

Reviewer's Comment
Blood sampling for PK analysis of fingolimod (t1/2= ~9 days) was not adequate to fully characterize PK profile of drug due its long half life.

Bioanalytical

Assay performance for Fingolimod and cyclosprine was acceptable during the study sample analysis.

Fingolimod Assay Performance during Study

Parameter	**Quality Control Samples (ng/mL)**	**Standard Curve Samples (ng/mL)**
Quality Control Samples or Standard Curve Samples (ng/mL)	0.129, 2.1 and 21	0.102, 0.512, 1.36, 2.727, 9.738 and 25.56
Between Batch Accuracy (% nominal)	94.1 to 123	95.5 to 105
Linearity (ng/mL)	Weighted Linear equation ($1/X^2$), mean r= 0.9996	
Linear Range (ng/mL)	0.1 to 25.5	
Sensitivity (Lower Limit of Quantitation, LLOQ) ng/mL	0.1	

Cyclosporine Assay Performance during Study

Parameter	**Quality Control Samples (ng/mL)**	**Standard Curve Samples (ng/mL)**
Quality Control Samples or Standard Curve Samples (ng/mL)	50, 200, 900 and 1800	15.63, 31.25, 62.5, 125, 250, 500, 1000, and 2000
Between Batch Precision (% CV)	3.2 to 9.4	0.9 to 10.7
Between Batch Accuracy (% nominal)	95.7 to 102	90 to 103
Linearity (ng/mL)	Weighted Linear equation ($1/X^2$), mean r= 0.9995	
Linear Range (ng/mL)	15 to 2000	
Sensitivity (Lower Limit of Quantitation, LLOQ) ng/mL	15	

Reviewer's Comments: Fingolimod-phosphate (active metabolite) concentrations were not determined in this study.

RESULTS

FTY720 pharmacokinetics Results:

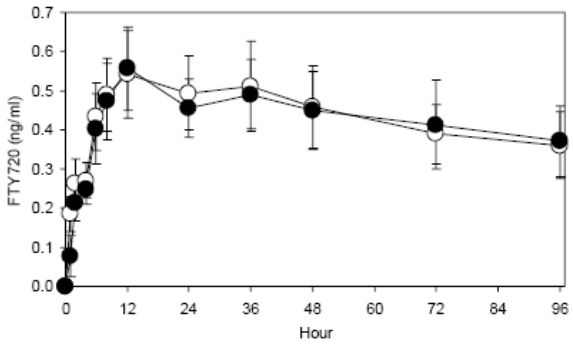
Lack of an effect of cyclosporine coadministration on FTY720 pharmacokinetics was specifically demonstrated for FTY720 Cmax and the truncated AUC(0-tz) but not for the extrapolated AUC0-inf since the sampling time points were limited to day 5 were inadequate to fully characterize fingolimod's prolonged elimination half-life for AUC extrapolation.

Due to inadequate sampling time points to fully characterize PK profile of fingolimod large portion of the AUC0-inf was extrapolated (61%). Overall fingolimod exposure (AUC0-inf) did not fulfill equivalence criteria: 1.16 (0.80 – 1.67).

Following table represents PK parameters for fingolimod obtained from the study.

Fingolimod pharmacokinetic parameters and PK profiles

Parameter	Alone	Coadministration
Cmax (ng/ml)	0.58 ± 0.19	0.57 ± 0.17
AUC(0-tz) (ng h/ml)	41± 13	41 ± 13
AUC(0-b) (ng h/ml)	110 ± 49	138 ± 75
t1/2 (days)	6.0 ± 4.8	7.0 ± 3.2



Mean fingolimod concentration profiles after administration alone (open circles) and coadministration with cyclosporine (filled circles). Bars represent 95% confidence intervals.

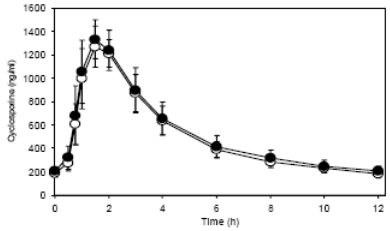
Cyclosporine pharmacokinetics: Steady-state cyclosporine pharmacokinetics were not influenced by single-dose coadministration of FTY720.

Following table and figure represents PK parameters and PK profile for cyclosporine obtained from the study.

Cyclosporine pharmacokinetic parameters and PK profile

Parameter	Alone	Coadministration
$C_{min,b}^{ss}$ (ng/ml)	186 ± 50	207 ± 49
t_{max} (h)	1.5 (1 – 3)	1.5 (1 – 2)
$C_{max,b}^{ss}$ (ng/ml)	1376 ± 149	1452 ± 308
$C(2)_b$ (ng/ml)	1212 ± 183	1239 ± 273
$AUC_{t,,b}^{ss}$ (ng.h/ml)	6031 ± 1051	6385 ± 1578
$C_{avg,b}^{ss}$ (ng/ml)	503 ± 88	532 ± 131
PTF (%)	241 ± 43	238 ± 43

Data are mean ± sd except for time parameters which are median (range).



Mean cyclosporine concentration profiles after administration alone (*open circles*) and coadministration with FTY720 (*filled circles*). *Bars* represent 95% confidence intervals. Individual subject

Cyclosporine concentration profiles

Following table represents PK parameters of fingolimod and cyclosporine when administered alone and coadministered along with the point estimates and 90% confidence intervals.

Pharmacokinetic parameters and point estimates

Drug and parameter	Administration alone	Coadministration	Point estimate (90%CI)
FTY720:			
t_{max} (h)	18 (8 - 48)	12 (12 – 72)	--
$C_{max,b}$ (ng/ml)	0.58 ± 0.19	0.57 ± 0.17	0.99 (0.92 – 1.07)
AUC(0-t_z)$_b$ (ng·h/ml)	41 ± 13	41 ± 13	1.00 (0.93 – 1.08)
AUC_b (ng·h/ml)	110 ± 49	138 ± 75	1.16 (0.80 – 1.67)
Cyclosporine:			
$C_{min,b}^{ss}$ (ng/ml)	186 ± 50	207 ± 49	1.13 (0.99 – 1.29)
t_{max} (h)	1.5 (1 – 3)	1.5 (1 – 2)	--
$C_{max,b}^{ss}$ (ng/ml)	1376 ± 149	1452 ± 308	1.04 (0.97 – 1.12)
$AUC_{t,b}^{ss}$ (ng·h/ml)	6031 ± 1051	6385 ± 1578	1.05 (0.99 – 1.11)

Values are mean ± standard deviation except for tmax which is median (range).
The subscript *b* designates measurement in whole blood.

Reviewer's Comment:
Fingolimod-phosphate concentration was not determined in this study. Since there was no drug-drug interaction seen on the fingolimod, fingolimod phosphate pharmacokinetics are not likely to be effected.

Pharmacodynamics: Influence of FTY720 on lymphocyte counts and influence of FTY720 on heart rate were not changed when cyclosporine was coadministered with fingolimod.

CONCLUSIONS:

- Cyclosporine did not modify fingolimods overall exposure AUC(0-tz) or peak concentration (Cmax).
- The pharmacokinetics of steady-state cyclosporine were not altered during coadministration with single-dose fingolimod.
- Mean lymphocyte counts decreased from baseline by approximately 40 percent over the first two days after FTY720 administration and thereafter increased back to prestudy values by day 5 postdose. This temporal pattern was similar in the presence and absence of cyclosporine.
- The morning mean supine heart rate decreased approximately 10 percent after FTY720 administration alone and with cyclosporine. Mean heart rate returned to prestudy values by day 5 postdose. Heart rate changes were asymptomatic in all study participants. One subject experienced second degree type 1 atrioventricular (Wenckebach) block.

Study FTY720 A2311: **An open-label, two-period, single-sequence, crossover study to evaluate the influence of ketoconazole on the pharmacokinetics of FTY720 in healthy volunteers.**

Study Rationale

Fingolimod is reversibly phosphorylated to the active moiety fingolimod-phosphate and irreversibly oxidized via cytochrome CYP4F2 to a series of carboxylic acid metabolites. There is currently limited knowledge about this isozyme. The CYP4F family primarily metabolizes endogenous substances such as fatty acids, ecosinoids, and leukotrienes. Ketoconazole is an inhibitor of CYP4F with an inhibitory constant Ki of approximately 1 µmol. The ketoconazole plasma C_{max} in patients receiving ketoconazole treatment is around 10 µmol. This yields an anticipated C_{max}/Ki ratio of 10 and suggests that a drug interaction of ketoconazole on fingolimod is likely in clinical setting. Therefore this study was conducted to evaluate the influence of ketoconazole on the pharmacokinetics of fingolimod.

Objectives	The primary objective was to evaluate the influence of ketoconazole on the pharmacokinetics of fingolimod in healthy volunteers. The secondary objectives were to assess tolerability and safety when FTY720 is administered alone and with multiple-dose ketoconazole and to perform exploratory pharmacogenetic assessments to examine whether individual genetic variation in genes relating to drug metabolism and the drug target pathway confer differential response to fingolimod.
Study Design	This was an open-label, two-period, single-sequence, crossover study intended for 20 healthy subjects. In period 1 (days 1-35) subjects received a single 5 mg dose of FTY720 on day 1 with pharmacokinetic blood sampling and clinical assessments up to day 35. In period 2 (days 36-73) subjects received ketoconazole 200 mg twice-daily for 9 days (days 36-44) and a single 5 mg dose of FTY720 coadministered on the fourth day of ketoconazole treatment (day 39). Pharmacokinetic blood sampling and clinical assessments were performed up to day 73.
Study Population	Male and female subjects, aged 18 to 50 years with psoriasis. A total of 28 subjects were randomized and 22 completed the study.
Investigational and coadministerd Drug	FTY720 2.5 mg final market image capsules (Lot US03082, KN3752938.006) Ketoconazole 200 mg immediate release tablet (b) (4))
Duration of Treatment	1 day single-dose FTY720 in period 1; 9 days of multiple-dose ketoconazole with a single dose of FTY720 on day 4 in period 2
Blood Sampling:	For fingolimod 2 ml venous blood samples were collected in EDTA vacuum tubes and for fingolimod-phosphate 2 ml venous blood samples were collected in sodium citrate vacuum tubes predose and then at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 312, 480, 648, and 816 hour postdose. For ketoconazole 5 ml venous blood samples were collected in heparin vacuum tubes before the morning dose on days 38, 39, 40, 42, and 44.
Assay	Plasma concentrations of fingolimod, fingolimod-phosphate and ketoconazole were determined separately by validated liquid chromatography methods with tandem mass spectrometry (LC/MS/MS).
PK	Single-dose pharmacokinetics of fingolimod and fingolimod-phosphate; trough levels

Assessment	of ketoconazole. Whole blood concentrations of fingolimod and fingolimod-phosphate were determined by LC-MS/MS methods with lower quantification limits of 0.08 ng/ml and 1 ng/ml. Ketoconazole plasma concentrations were measured by UV-HPLC with a lower quantification limit of 0.01 ug/ml.
Statistical methods	Fingolimod and fingolimod-phosphate pharmacokinetic parameters were log-transformed and compared between treatments in a linear mixed-effects model from which point estimates and 90% confidence intervals for the treatment ratios were generated. Ketoconazole trough concentrations in the presence and absence of fingolimod were compared in a mixed-effects model.

Dose Selection:
Subjects were dosed with 5 mg fingolimod, which is the highest clinically-used dose in development trials and 200 mg ketoconazole twice-daily is the highest commonly used therapeutic regimen. However, final dose of fingolimod sought for labeling was 0.5 mg.

Reviewer's Comment
Dosing regimen for ketoconazole and finfolimod are appropriate. Blood sampling for PK analysis for fingolimod (t1/2= 9 days), ketoconazole (t1/2= 8 hrs) is adequate.

Bioanalytical

Assay performance for fingolimod, fingolimod-phosphate and ketoconazole was acceptable during the study sample analysis.

Fingolimod Assay Performance during Study

Parameter	**Quality Control Samples (ng/mL)**	**Standard Curve Samples (ng/mL)**
Quality Control Samples or Standard Curve Samples (ng/mL)	0.24, 2, 5 and 25	0.08, 0.2, 1, 3, 10, 20 and 30
Between Batch Precision (% CV)	6.1 to 7.8	2.3 to 7.4
Between Batch Accuracy (% nominal)	96.7 to 103	96.3 to 106
Linearity (ng/mL)	Weighted Linear equation ($1/X^2$), mean r= 0.9956	
Linear Range (ng/mL)	0.08 to 30	
Sensitivity (Lower Limit of Quantitation, LLOQ) ng/mL	0.08	

Fingolimod- Phosphate Assay Performance during Study

Parameter	**Quality Control Samples (ng/mL)**	**Standard Curve Samples (ng/mL)**
Quality Control Samples or Standard Curve Samples (ng/mL)	2, 7.5 and 40	1, 2.5, 5, 10, 20, 30, and 50
Between Batch Precision (% CV)	3.6 to 5.4	2.2 to 3.8
Between Batch Accuracy (% nominal)	96.0 to 104	94.1 to 104
Linearity (ng/mL)	Weighted Linear equation ($1/X^2$), mean r= 0.997	

Linear Range (ng/mL)	1 to 50
Sensitivity (Lower Limit of Quantitation, LLOQ) ng/mL	1

Ketoconazole Assay Performance during Study

Parameter	**Quality Control Samples (ng/mL)**	**Standard Curve Samples (ng/mL)**
Quality Control Samples or Standard Curve Samples (ng/mL)	20, 2500 and 4000	10, 50, 250, 500, 1000, 2500 and 5000
Between Batch Precision (% CV)	3.2 to 9.6	1.6 to 5.3
Between Batch Accuracy (% nominal)	96.7 to 108	92.8 to 113
Linearity (ng/mL)	Weighted Linear equation ($1/X^2$), mean r= 0.996	
Linear Range (ng/mL)	10 to 5000	
Sensitivity (Lower Limit of Quantitation, LLOQ) ng/mL	10	

RESULTS

Pharmacokinetic Results:

Fingolimod pharmacokinetics.

Ketoconazole did not affect fingolimod tmax while there was an increase in average Cmax of 1.22-fold. Nearly all subjects had in increase in total exposure (AUCb) in the presence of ketoconazole. Over the full study population, AUC was increased 1.71-fold. Fingolimod half-life was not changed by ketoconazole coadministration.

Fingolimod-phosphate Cmax was unaffected by ketoconazole; whereas, AUC(0-tz) was increased to a similar extent (1.67-fold) as fingolimod AUC.

Following table indicates pharmacokinetic parameters of fingolimod and fingolimod-phosphate when fingolimod was administered alone and in the presence of ketoconazole.

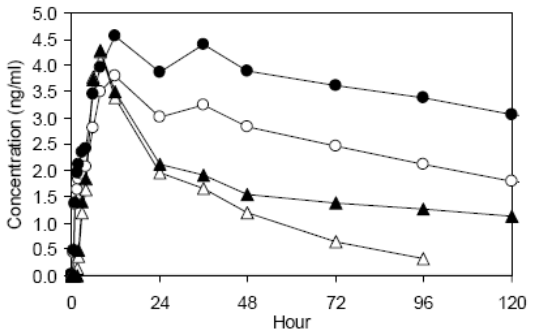
Fingolimod and fingolimod-phosphate pharmacokinetics

Parameter	Fingolimod alone	Fingolimod with ketoconazole	Ratio of geometric means (90%CI)
Fingolimod:			
t_{max} (h)	12 (8-36)	12 (3-48)	--
$C_{max,b}$ (ng/ml)	3.9 ± 0.7	4.8 ± 1.1	1.22 (1.15 – 1.30)
AUC_b (ng.h/ml)	665 ± 202	1124 ± 293	1.71 (1.53 – 1.91)
$t_{1/2}$ (days)	5.1 ± 1.6	5.8 ± 1.6	1.15 (1.06 – 1.26)
Fingolimod-phosphate:			
t_{max} (h)	8 (6-12)	8 (6-12)	--
$C_{max,b}$ (ng/ml)	4.5 ± 1.3	4.4 ± 1.1	0.99 (0.92 – 1.06)
$AUC(0\text{-}t_z)_b$ (ng.h/ml)	128 ± 49	217 ± 99	1.67 (1.50 – 1.85)

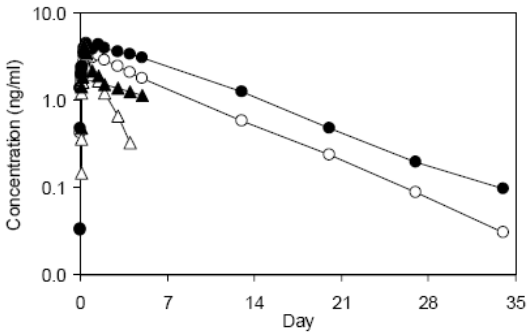
Values are mean ± sd except for temporal parameters which are median (range).
b denotes parameter from blood concentrations.

Following figure represents pharmacokinetic profiles of fingolimod and fingolimod-phosphate when fingolimod was administered alone and in the presence of ketoconazole.

Fingolimod and fingolimod-phosphate profiles



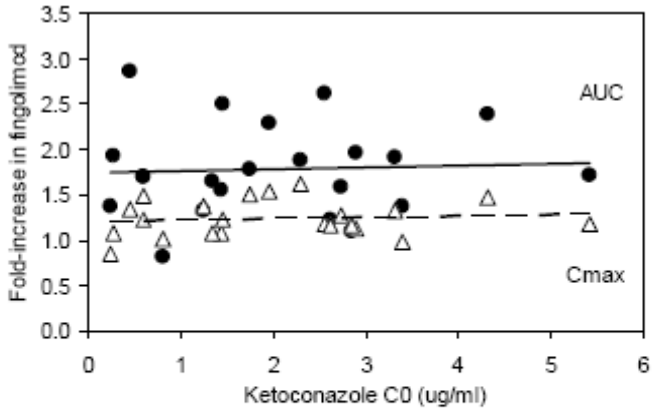
Mean concentration-time profiles to day 5 postdose for fingolimod alone (*open circles*) and with ketoconazole (*filled circles*) and for fingolimod-phosphate alone (*open triangles*) and with ketoconazole (*filled triangles*).



As in left panel with concentrations on a logarithmic scale plotted over the full 35-day assessment periods.
Individual plots in Appendix 4, Figure 1.

Following scatter plot represents (fold) increase AUC and Cmax fingolimod when fingolimod was administered in the presence of ketoconazole.

Scatter plot for fingolimod exposure



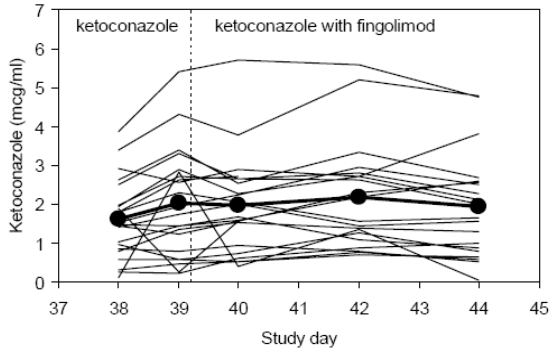
Scatterplot of ketoconazole predose concentration (C0) before FTY720 administration and the fold-increase in fingolimod Cmax (*open triangles; dashed regression line*) or AUC (*filled circles, solid regression line*). Additional correlations are in Appendix 6.

Ketoconazole pharmacokinetics. Ketoconazole trough plasma concentrations over the study course are shown in the figure below.

Mean predose ketoconazole concentrations reached steady-state by the fourth day of twice daily administration: 1.6 ± 1.0 µg/ml on day 38 and 2.0 ± 1.4 µg/ml on day 39 just before the dose of fingolimod. Coadministration of fingolimod did not alter ketoconazole trough levels: 2.0 ± 1.2 µg/ml (day 40), 2.2 ± 1.4 µg/ml (day 42), and 2.0 ± 1.3 µg/ml (day 44).

Statistical comparison of the values on day 39 to those on days 40 to 44 show that through concentrations of ketoconazole were not effected in the presence of fingolimod.

Ketoconazole individual trough concentrations



Synoptic view of ketoconazole trough concentrations (*lines*) and mean trajectory (*filled circles*) in the absence and presence of fingolimod.

Discussion

Ketoconazole is an inhibitor of CYP4F with an inhibitory constant Ki of approximately 1 µmol. The ketoconazole plasma C_{max} in patients receiving ketoconazole treatment is

approximately 10 μmol. This yields an anticipated [I]/Ki ratio of 10 and suggesting that a drug interaction of ketoconazole on fingolimod is likely in man.

Since fingolimod has a potent immunomodulatory activity. Therefore fingolimod was not dosed to steady state in healthy subjects. It was administered FTY720 as a single dose with steady-state ketoconazole.

Ketoconazole administration was not continued until the end of fingolimod sampling given the prolonged half-life of fingolimod requiring blood sampling for 1 month postdose. Instead ketoconazole was coadministered for 5 days to cover fully fingolimod's absorption phase. Continuous coadministration of ketoconazole throughout the period of fingolimod characterization would have shown a stronger interaction effect on fingolimod. Therefore, the changes in fingolimod PK parameters seen in this study may be lower than actual values.

CONCLUSIONS:

- Coadministration of a single 5 mg dose of FTY720 with steady-state ketoconazole 200 mg twice-daily increased both fingolimod peak exposure, Cmax by 1.2-fold and overall exposure AUC by 1.7-fold.
- Fingolimod-phosphate AUC(0- tz) was increased to a similar extent (1.7-fold) as the parent fingolimod but Cmax was unaffected by ketoconazole.
- Trough concentrations of ketoconazole were not effected by coadministration of fingolimod.

Labeling Recommendation: The AUC_{0-t} and C_{max} of fingolimod increased by approximately 1.7 and 1.2 fold respectively upon coadministration of ketoconazole with fingolimod. Fingolimod should be administered with caution in subjects taking ketoconazole.

4.1-4. HUMAN PD STUDIES
4.1-4.1 Healthy subject PD

Study FTY720A 0114*:* **A partially-blinded, partially-randomized, three period, cross-over study to evaluate the effect of single-dose FTY720 with steady-state concentrations of beta-blocker, Tenormin® LA (atenolol), or calcium channel blocker, Cardizem® LA (extended-release diltiazem), on cardiac rate in healthy volunteers**

PD, DDI study: SD FTY720 coadministered with steady state Atenolol and Diltiazem, two types of antihypertensive medications known to decrease heart rate

A brief overview of some essential components of the study design is given below:

Study Design	partially-blinded, partially-randomized, three period, cross-over study
Study Population	N=36 enrolled, 33 completed Age: 18-49 years (mean 29.5 years) Gender: 25 males, 11 females Weight: 50.6-94 kg (mean 70.8 kg) Race: 12Whites, 20 Blacks, 3Asians and 1 other ethnicity
Dosage and Administration	**Part 1: test administration of low-dose FTY720 with atenolol and diltiazem.** Group A (n = 4) received 50 mg atenolol once-daily for 5 days and group B (n = 4) received 240 mg diltiazem once-daily for 5 days. Both groups received a single 0.5 mg dose of FTY720 on day 5. **Part 2: coadministration of FTY720 with atenolol(n=12 completed).** Groups C (n=7) and D (n=7). In **period 1:** all subjects received 50 mg atenolol once-daily for 5 days followed by a 7-day washout In **period 2:** subjects were randomized to receive either atenolol 50 mg/day or placebo for 5 days in groups C and D. Both groups received 5 mg FTY720 on day 5. In **period 3:** the groups received the alternate treatment. Periods 2 and 3 were separated by a 33-day washout **Part 3:coadministration of FTY720 with diltiazem(n=13 completed)** This assessment was identical to part 2 with the exception that atenolol was replaced with extended-release diltiazem 240 mg once daily and the groups were designated E (n=7) and F (n=7).

Table 3-1 Study treatment scheme

Part	Group	Period 1	Period 2	Period 3
1	A	Atenolol with low-dose FTY720	--	--
	B	Diltiazem with low-dose FTY720	--	--
2	C	Atenolol alone	Placebo with FTY720	Atenolol with FTY720
	D	Atenolol alone	Atenolol with FTY720	Placebo with FTY720
3	E	Diltiazem alone	Placebo with FTY720	Diltiazem with FTY720
	F	Diltiazem alone	Diltiazem with FTY720	Placebo with FTY720

Atenolol = 50 mg/day on days 1-5; *diltiazem* = 240 mg/day on days 1-5.
Low-dose FTY720 = 0.5 mg on day 5; all other *FTY720* doses were 5 mg on day 5.

Study medication was administered with 240 ml of water after a light breakfast.

Lot No.

FTY720 0.5 mg capsules (FMI):

	batch H-05930, control number 04-0470US FTY720 2.5 mg capsules (FMI): batch US03082, control number 04-0978CH Atenolol 50 mg tablets: Tenormin, batch 101132, Astra Zeneca Dilitiazem 240 mg ER tablets: Cardizem LA, batch 04G011P, Biovail Pharmaceuticals FTY720 placebo capsules: batch US03096, control number 04-0978CH Generic placebo capsules: batch 50243, (b) (4) <u>Diet:</u> No fluids were allowed until 2 hours after dosing. Otherwise, subjects had a fluid intake of at least 200 ml every 4 hours during waking hours in addition to fluid taken with meals. Xanthine-containing beverages were allowed but was to be discontinued 48 hours before dosing of study drug(s) Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood/plasma	For FTY720 and FTY720-P (Blood): At predose (0 hour), and 2, 4, 6, 8, 10, 12 and 24 hours. For atenolol and diltiazem (Plasma): At predose (0 hour), and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours.
Analysis (Blood)	<u>Method</u> LC/MS/MS <u>Lower Limits of Quantitation</u> <u>Blood</u> FTY720 0.08 ng/mL FTY720-P 1 ng/mL <u>FTY720:</u> Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 16.6% Inter-day accuracy: < 11.7 % <u>FTY720-P:</u> Linear range : 1.5-500 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 5.2% Inter-day accuracy: < 6.4 %
Analysis (Plasma)	<u>Method</u> HPLC with fluorescence detection <u>Lower Limits of Quantitation</u> <u>Urine</u> Atentolol 25 ng/mL <u>Atenolol:</u> Linear range : 25-1000 ng/mL in plasma Inter-day Precision (%CV for Quality Controls) : < 4.4% Inter-day accuracy: < 7.4 % Short term Stability: ~10 % degradation at 5± 5°C for 7 days
Analysis (Plasma)	<u>Method</u> HPLC with ultraviolet detection <u>Lower Limits of Quantitation</u>

	Urine Diltiazem 2.5 ng/mL Diltiazem: Linear range : 2-250 ng/mL in urine Inter-day Precision (%CV for Quality Controls) : < 9.3% Inter-day accuracy: < 2.4 % Short term Stability: No degradation at 5± 5°C for 7 days
PK Assessment	FTY720 and FTY720-P in blood: Cmax, Tmax, T ½, AUC0-t Atenolol and diltiazem in plasma: Cmin, Cmax, Tmax, T ½, AUC0-t, Cave, FTF
PD Assessment	**Cardiovascular responses:** The nadir response and area under the effect-time curve over 12 hours postdose were derived from the heart rate and mean arterial pressure data on day 5 in study parts 2 and 3. **Table 3-2 Study assessment schedule** **Assessment** / **Timing** Physical exam: Screening, baseline before each period, end-of-study Blood pressure + pulse rate: Screening, baseline before each period, day 1 (0, 1, 2, 3, 4 hours), day 5 (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24 hours), end-of-study ECG: Screening, baseline before each period, day 5 (0, 4, 12 hours), end-of-study Telemetry: Continuous on day 5 from the time of dosing to 24 hours postdose Lab parameters: Screening, baseline before each period, end-of-study
Safety Assessment	Assessment of physical examination findings, vital signs, telemetry, electrocardiogram (ECG), adverse events (AEs), laboratory parameters.

Results:

Study Part 1--Pharmacokinetics Results:

The pharmacokinetic parameters of atenolol, diltiazem, and FTY720 are summarized in the following table:

Table 7-2 Pharmacokinetics (study part 1)

Parameter	Atenolol + low-dose FTY720		Diltiazem + low-dose FTY720	
	Atenolol	Fingolimod	Diltiazem	Fingolimod
Cmin (ng/ml)	29 ± 11	0	65 ± 44	0
tmax (h)	3.5 (2 – 4)	18 (12 – 24)	10 (10 – 12)	18 (12 – 24)
Cmax (ng/ml)	346 ± 95	0.5 ± 0.1	107 ± 30	0.4 ± 0.1
AUC(0-24) (ng.h/ml)	2999 ± 493	9.3 ± 1.1	1947 ± 725	7.9 ± 1.9
Cavg (ng/ml)	125 ± 21	--	81 ± 30	--
PTF (%)	253 ± 63	--	63 ± 37	--
t1/2 (h)	6.3 ± 1.4	--	--	--

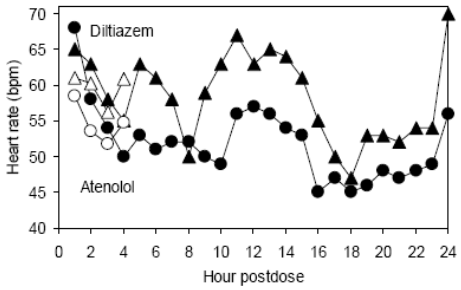
Values are mean ± sd except for tmax which is median (range).
Fingolimod-phosphate could not be quantified after low-dose FTY720.
Source data: Appendix 4 Tables 1-8.

- The steady-state pharmacokinetics of atenolol and diltiazem were consistent with reviously reported data based on the sponsor.
- Fingolimod exposure was similar for both treatment groups in this study part.
- Fingolimod-P could not be quantified after single-dose of 0.5 mg FTY720.

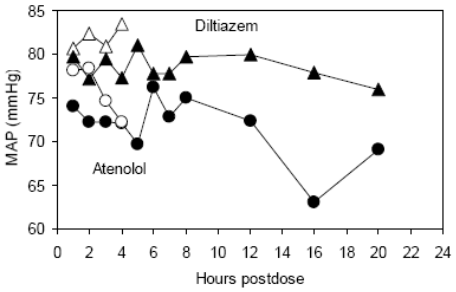
Pharmacodynamics results:

The mean heart rate and mean arterial pressure trajectories are shown in the figures below:

Figure 7-1 Heart rate and blood pressure trajectories (study part 1)



Mean heart rate trajectories after atenolol alone and with low-dose FTY720 (*open and filled circles*) and after diltiazem alone and with low-dose FTY720 (*open and filled triangles*).



As in left panel for mean arterial pressure (MAP).

- Mean heart rate to 4 hours postdose exhibited a conventional circadian decline in the morning when both antihypertensives were given alone or with low-dose FTY720.
- Atenolol alone elicited a mean 6 mmHg decreased in mean arterial pressure (MAP) over the first 4 hours postdose (78 to 72 mmHg); whereas, MAP after diltiazem alone exhibited a relatively flat pattern over 4 hours postdose.
- The addition of low-dose FTY720 on day 5 did not appear to change the MAP trajectories for either antihypertensive.
- These heart rate and MAP data allowed the study to progress to parts 2 and 3 with higher-dose FTY720.

Study Part 2: Coadministration of FTY720 and atenolol

Pharmacokinetics Results:

The pharmacokinetic parameters of atenolol and FTY720 are summarized in the following table:

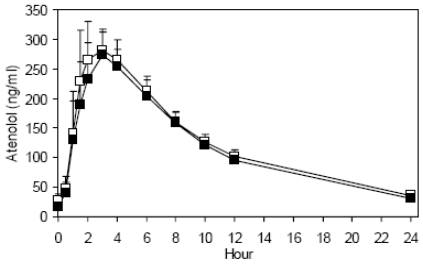
Table 7-3 Atenolol and fingolimod pharmacokinetics (study part 2)

Parameter	Atenolol		Fingolimod		Fingolimod-phosphate	
	Alone	Combined	Alone	Combined	Alone	Combined
Cmin (ng/ml)	35 ± 6	34 ± 10	--	--	--	--
tmax (h)	2 (1.5 – 4)	3 (1.5 – 6)	12 (8 – 24)	12 (8 – 24)	6 (6 – 12)	6 (6 – 10)
Cmax (ng/ml)	315 ± 70	291 ± 65	4.7 ± 1.0	4.8 ± 1.1	5.4 ± 1.1	5.3 ± 1.4
AUC(0-24) (ng.h/ml)	3023 ± 485	2834 ± 554	90 ± 17	92 ± 21	72 ± 14	75 ± 16
Cavg (ng/ml)	126 ± 20	118 ± 23	--	--	--	--
PTF (%)	221 ± 32	217 ± 20	--	--	--	--
t1/2 (h)	7.2 ± 0.5	7.2 ± 1.2	--	--	--	--

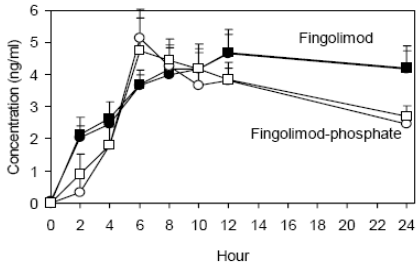
Values are mean ± sd except for tmax which is median (range).
Source data: parameters in Appendix 4 Tables 1-8 and statistical comparisons in Appendix 6 Table 6.1.

Mean concentration-time profiles of atenolol and FTY720 are shown in the following figure:

Figure 7-2 Atenolol and fingolimod concentration profiles (study part 2)



Mean concentration profiles of atenolol at steady state when administered alone (*open squares*) and with single-dose fingolimod (*filled squares*). *Bars* represent 95% confidence intervals. Individual and synoptic plots in Appendix 4 Figure 3.



Mean concentration profiles of fingolimod (*filled symbols*) and fingolimod-phosphate (*open symbols*) administered alone (*circles*) and with atenolol (*squares*). Bars represent 95% confidence intervals. Individual and synoptic plots in Appendix 4 Figure 1.

- Atenolo PK was not altered. Addition of single-dose FTY720 to steady-state atenolol did not change atenolol Cmaxss with a geometric mean ratio (90%CI) of 0.92 (0.81-1.05) or AUC(0-24)ss with a ratio of 0.93 (0.85-1.02).
- FTY720 PK was not changed. Addition of single-dose FTY720 to steady-state atenolol did not change fingolimod Cmax with a geometric mean ratio of 1.02 (0.96-1.08) or AUC(0-24) with a ratio of 1.00 (0.96-1.05).
- A statistical sequence effect was noted for fingolimod Cmax ($p < 0.10$) the relevance of which is unknown based on the sponsor.
- FTY720-P PK was not changd. Addition of single-dose FTY720 to steady-state atenolol did not change fingolimod-phosphate Cmax with a geometric mean ratio of 0.98 (0.87-1.10) or AUC(0-24) with a ratio of 1.05 (0.99-1.11).

Reviewer's note:
A sequence effect was noted; however the relevance is not clear.

Pharmacodynamics Results:

Cardiovascular responses from FTY720 and atenolol:

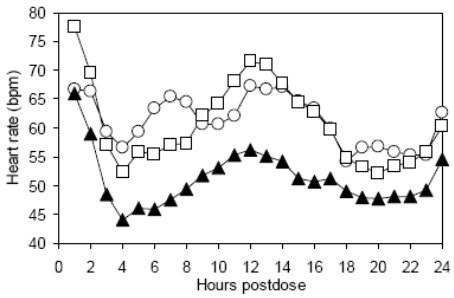
Table 7-4 Cardiovascular responses: atenolol and FTY720 (study part 2)

Response	Atenolol	FTY720	FTY720 + atenolol
Heart rate:			
Predose (bpm)	60 ± 8	73 ± 9	60 ± 7
Nadir (bpm)	55 ± 7	51 ± 9	42 ± 7
Time of nadir (h)	4 (3 -11)	4.5 (3 -8)	4 (3 – 8)
AUEC(0-12) (bpm.h)	687 ± 81	674 ± 120	561 ± 82
Mean arterial pressure:			
Nadir (mmHg)	77 ± 5	79 ± 6	74 ± 4
AUEC(0-12) (mmHg.h)	934 ± 51	953 ± 68	881 ± 57

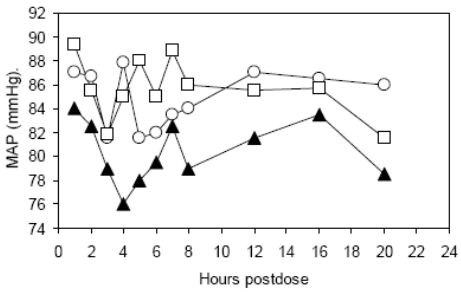
Values are mean ± sd except for nadir time which is median (range).
Heart rate data are from telemetry with the exception of predose rate from vital signs.
Statistical comparisons are in Appendix 6 Table 6-1.

Mean trajectories are shown in the figure below:

Figure 7-3 Heart rate and blood pressure trajectories (study part 2)



Mean heart rate trajectories from telemetry after atenolol alone (*open circles*), FTY720 alone (*open squares*), and FTY720 + atenolol (*filled triangles*).



As in left panel for mean arterial pressure from vital signs recordings.

- FTY720 alone elicited an 8% lower heart rate nadir (51 bpm) compared with atenolol alone (55 bpm) (p= 0.03), but the FTY720/atenolol ratio and 90% confidence interval remained in the equivalence bounds: 0.92 (0.86-0.98).
- The combination FTY720+atenolol elicited a 15% lower morning heart rate nadir (42 bpm) compared with FTY720 alone (51 bpm). The treatment response ratios and 90% confidence intervals were not within the standard equivalence bounds.
- MAP responses were similar when the two drugs were given alone, whereas the effect-time curve (AUEC) was 8% lower for FTY720 + atenolol versus FTY720 alone, but the treatment response ratios and 90% confidence intervals were within the standard equivalence bounds.
- Although the heart rate and MAP trajectories were shifted downward on the measurement scale after FTY720+atenolol, they retained their normal circadian patterns.

Reviewer's note:

Similarly, the evaluation of heart rate nadir and mean arterial blood pressure AUEC(0-12) in periods 2 and 3 showed significant period effects. Also sequence effects were also noted. The sponsor stated this is likely a true sequence effects however again the relevance is not known.

Study Part 3: Coadministration of FTY720 and diltiazem

Pharmacokinetics Results:

The pharmacokinetic parameters of diltiazem and FTY720 are summarized in the following table:

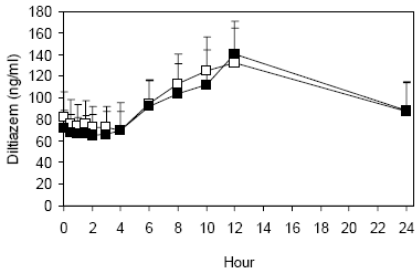
Table 7-5 Diltiazem and fingolimod pharmacokinetics (study part 3)

Parameter	Diltiazem		Fingolimod		Fingolimod-phosphate	
	Alone	Combined	Alone	Combined	Alone	Combined
Cmin (ng/ml)	87 ± 45	88 ± 43	--	--	--	--
tmax (h)	12 (8 – 24)	12 (8 – 24)	12 (8 -24)	12 (10 – 24)	6 (6 – 8)	6 (6 – 10)
Cmax (ng/ml)	144 ± 51	142 ± 51	4.6 ± 0.9	4.8 ± 1.0	5.3 ± 1.2	5.7 ± 1.8
AUC(0-24) (ng.h/ml)	2491 ± 944	2465 ± 972	91 ± 15	93 ± 16	77 ± 14	80 ± 21
Cavg (ng/ml)	104 ± 39	103 ± 40	--	--	--	--
PTF (%)	63 ± 33	70 ± 24	--	--	--	--

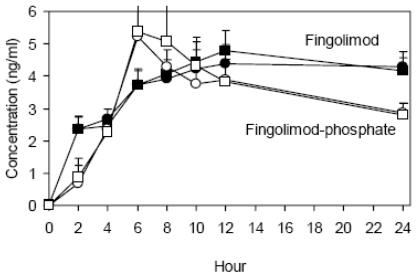
Values are mean ± sd except for tmax which is median (range)

The mean concentration-time profiles of diltiazem and FTY720 are shown in the following figure:

Figure 7-5 Diltiazem and fingolimod concentration profiles (study part 3)



Mean concentration profiles of diltiazem at steady state when administered alone (*open squares*) and with single-dose fingolimod (*filled squares*). *Bars* represent 95% confidence intervals. Individual and synoptic plots in Appendix 4 Figure 3.



Mean concentration profiles of fingolimod (*filled symbols*) and fingolimod-phosphate (*open symbols*) administered alone (*circles*) and with diltiazem (*squares*). Bars represent 95% confidence intervals. Individual and synoptic plots in Appendix 4 Figure 1.

- Diltiazem PK was not altered. Addition of single-dose FTY720 to steady-state diltiazem did not change diltiazem Cmaxss with a geometric mean ratio (90%CI) of 0.99 (0.83-1.19) or AUC(0-24)ss with a ratio of 0.99 (0.87-1.13).
- FTY720 PK was not changed. Addition of single-dose FTY720 to steady-state diltiazem did not change fingolimod Cmax with a geometric mean ratio of 1.05 (0.97-1.13) or AUC(0-24) with a ratio of 1.02 (0.95-1.10).
- FTY720-P PK was not chnged. Addition of single-dose FTY720 to steady-state diltiazem did not change fingolimod-phosphate Cmax with a geometric mean ratio of 1.05 (0.96-1.15) or AUC(0-24) with a ratio of 1.02 (0.94-1.09).

Pharmacodynamics Results:

Cardiovascular responses from FTY720 and diltiazem:

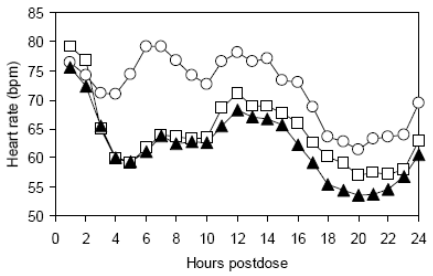
Table 7-6 Cardiovascular responses: diltiazem and FTY720 (study part 3)

Response	Diltiazem	FTY720	FTY720 + diltiazem
Heart rate:			
Predose (bpm)	70 ± 9	72 ± 10	69 ± 9
Nadir (bpm)	67 ± 7	55 ± 5	56 ± 8
Time of nadir (h)	5 (2 – 11)	5 (3 – 10)	5 (3 – 7)
AUEC(0-12) (bpm.h)	828 ± 86	724 ± 77	710 ± 84
Mean arterial pressure:			
Nadir (mmHg)	78 ± 7	79 ± 9	78 ± 7
AUEC(0-12) (mmHg.h)	949 ± 80	935 ± 95	918 ± 80

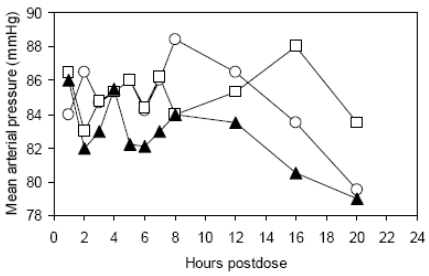
Values are mean ± sd except for nadir time which is median (range).
Heart rate data are from telemetry with the exception of predose rate from vital signs.
Statistical comparisons are in Appendix 6 Table 6-1.

Mean trajectories are shown in the figure below:

Figure 7-6 Heart rate and blood pressure trajectories (study part 3)



Mean heart rate trajectories from telemetry after diltiazem alone (*open circles*), FTY720 alone (*open squares*), and FTY720 + diltiazem (*filled triangles*).



As in left panel for mean arterial pressure from vital signs recordings.

- The mean heart rate nadir and the AUEC(0-12) were significantly lower by 18% and 13% for FTY720 compared with diltiazem ($p < 0.001$). The corresponding FTY720/diltiazem ratios the 90% confidence intervals were: 0.82 (0.78-0.85) and 0.87 (0.84-0.90). MAP nadir and AUEC(0-12) were similar between treatments: 1.00 (0.97-1.04) and 0.98 (0.96-1.01).
- Heart rate and MAP responses were not different from FTY720 alone compared with FTY720+diltiazem.
- The heart rate trajectory after FTY720+diltiazem retained a normal circadian pattern.

Reviewer's note:

There were statistically significant sequence effects for heart rate nadir and AUEC(0-12) in periods 2 and 3. The sponsor stated these are likely to be true sequence effects rather than statistical carryover effects given similar finding previously but not knowing the relevance.

AEs

Based on the sponsor, all ECG recordings were reported as either normal or having clinically insignificant abnormality. Five subjects (*5109, 5115, 5118, 5119, 5120*) had prolonged *uncorrected* QT intervals on day 5 of period 2 at 4 hours postdose (and at 24 hours in subject 5118) ranging from 504 to 564 msec (normal <500 msec). These values were recorded in the presence of the heart rate lowering effect of FTY720 and are not corrected for heart rate.

Reviewer's note:

QTc prolongation potential will be reviewed by relevant division. The significance/importance of the potential QT prolongation by FTY720 should be addressed in the QTc review.

Conclusions:

- Single-dose FTY720 administered alone and with steady-state atenolol or diltiazem was well tolerated by healthy subjects based on the sponsor.
- Single-dose FTY720 administered with steady-state atenolol or diltiazem did not alter the pharmacokinetics of fingolimod, fingolimod-phosphate, atenolol, or diltiazem.
- The mean nadir heart rate was 15% lower when FTY720 was combined with atenolol compared with FTY720 alone: 55 bpm for atenolol alone, 51 bpm for FTY720 alone, and 42 bpm for the combination.
- The mean nadir heart rate from FTY720 was not altered when combined with diltiazem.
- There was no clinically relevant change in daytime MAP responses when FTY720 was administered with atenolol or diltiazem compared with administration of the drugs alone in normotensive subjects.

Study FTY720A 0118*:* **A single-blind, randomized, cross-over study to evaluate the temporal effect of concurrent and delayed intravenous atropine administration on the negative chronotropic effect of 5 mg oral FTY720 in healthy volunteers**

PD, DDI study: SD FTY720 dosed with atropine i.v. infusion concurrently or 4 hours post FTY720 dose.

A brief overview of some essential components of the study design is given below:

Study Design	single-blind, randomized, cross-over study
Study Population	N=30 enrolled, 24 completed Age: 20-48 years (mean 35.8 years) Gender: 11 males, 19 females Weight: 50.8-91 kg (mean 71 kg) Race: 16 Whites, 1 Blacks, and 13 other ethnicity
Dosage and Administration	**Part 1: concurrent administration of atropine and FTY720 (n=12).** Group A: Period 1 D1 placebo p.o.+ atropine i.v. titration D2 5 mg FTY720 + atropine i.v. titration Period 2 D1 placebo + saline placebo titration D2 5 mg FTY720 + saline placebo titration Group B: Period 1 D1 placebo p.o.+ saline placebo titration D2 5 mg FTY720 + saline placebo titration Period 2 D1 placebo + atropine i.v. titration D2 5 mg FTY720 + atropine i.v. titration **Part 2: delayed administration of atropine and FTY720(n=12).** Groups C and D identical to part 1 with the exceptions that the atropine/placebo titrations were begun 4 hours after the oral placebo/FTY720 dose Periods 2 and 3 were separated by a 33-day washout. **Table 3-1 Study treatment scheme** Study Part / Group / Period 1: Day 1, placebo po / Day 2, FTY720 po / Period 2: Day 36, placebo po / Day 37, FTY720 po 1 / A / concurrent atropine iv / concurrent atropine iv / concurrent placebo iv / concurrent placebo iv 1 / B / concurrent placebo iv / concurrent placebo iv / concurrent atropine iv / concurrent atropine iv 2 / C / delayed atropine iv / delayed atropine iv / delayed placebo iv / delayed placebo iv 2 / D / delayed placebo iv / delayed placebo iv / delayed atropine iv / delayed atropine iv Study medication was administered with 240 ml of water after a light breakfast. Lot No. FTY720 2.5 mg capsules (FMI): batch US02030, KN375938.005 FTY720 placebo capsules: batch AEUS/2002-0137, KN3752961.003 Atropine sulfate 1 mg/ml: lots 322284 and 332856; (b) (4) Diet: No fluids were allowed until 2 hours after dosing. Otherwise, subjects had a fluid intake of at least 200 ml every 4 hours during waking hours in addition to fluid taken with meals. Xanthine-containing beverages were allowed but was to be discontinued 48 hours before dosing of study drug(s)

<table>
<tr><td></td><td>Alcohol was prohibited for 72 hours prior to dosing until study completion.</td></tr>
<tr><td>Sampling:
Blood/plasma</td><td>For FTY720 and FTY720-P (Blood):
At predose (0 hour), and 2, 4, 6, 12 and 24 hours.</td></tr>
<tr><td>Analysis (Blood)</td><td><u>Method</u>
LC/MS/MS
<u>Lower Limits of Quantitation</u>
Blood
FTY720 0.08 ng/mL
FTY720-P 1.5 ng/mL

<u>FTY720:</u>
Linear range : 0.08-30 ng/mL in blood
Inter-day Precision (%CV for Quality Controls) : < 16.1%
Inter-day accuracy: < 12.9 %

<u>FTY720-P:</u>
Linear range : 1.5-500 ng/mL in blood
Inter-day Precision (%CV for Quality Controls) : < 6.3%
Inter-day accuracy: < 5.9 %</td></tr>
<tr><td>PK Assessment</td><td>FTY720 and FTY720-P in blood: Cmax, Tmax, AUC0-t</td></tr>
<tr><td>PD Assessment</td><td>Cardiovascular responses:
The nadir response and area under the effect-time curve over 12 hours postdose were derived for heart rate from telemetry data
Table 3-2 Study assessment schedule
<table>
<tr><th>Assessment</th><th>Timing</th></tr>
<tr><td>Physical exam</td><td>Screening, baseline-1, day 3, baseline-2, end-of-study</td></tr>
<tr><td>Blood pressure + pulse rate</td><td>Screening, baseline-1, baseline-2, day 1-2 (0, 1, 2, 3, 4, 5, 6, 8, 12 hours) in both periods, day 3, end-of-study</td></tr>
<tr><td>ECG</td><td>Screening, baseline-1, baseline-2, days 1-2 (4 hours postdose) in both periods, day 3, end-of-study</td></tr>
<tr><td>Telemetry</td><td>Continuous on days 1-2 in both periods for 48 hours from the time of placebo/FTY720 dosing</td></tr>
<tr><td>Lab parameters</td><td>Screening, baseline-1, baseline-2, day 3, end-of-study</td></tr>
</table></td></tr>
<tr><td>Safety Assessment</td><td>Assessment of physical examination findings, vital signs, telemetry, electrocardiogram (ECG), adverse events (AEs), laboratory parameters.</td></tr>
</table>

Results:

Study Part 1: Concurrent administration of FTY720 and atropine

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 and FTY720-P are summarized in the following table:

Table 7-2 Pharmacokinetics: concurrent administration

Parameter	Fingolimod			Fingolimod-phosphate		
	Placebo	Atropine	Ratio (90%CI)	Placebo	Atropine	Ratio (90%CI)
t_{max} (h)	12 (12-12)	12 (12-12)	--	6 (6-12)	12 (6-12)	--
$C_{max,b}$ (ng/ml)	3.9 ± 0.6	3.8 ± 0.5	0.97 (0.90, 1.05)	5.2 ± 1.4	4.5 ± 1.1	0.89 (0.77, 1.01)
$AUC(0\text{-}24)_b$ (ng.h/ml)	74 ± 11	65 ± 9	0.90 (0.83, 0.97)	77 ± 21	69 ± 15	0.93 (0.83, 1.05)

Values are arithmetic mean ± sd except for tmax which is median (range).
Ratio (90%CI) is the ratio of geometric means and 90% confidence interval.
Source data: Appendix 4 Table 1 and 2; Appendix 6 Tables 6.1-3 and 6.1-5.

- When FTY720 was administered concurrently with atropine, fingolimod Cmax,b and AUC(0-24)b were not affected.

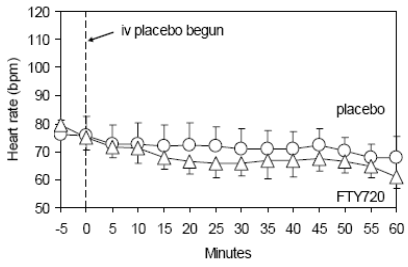
- Fingolimod-phosphate Cmax,b was 11% lower with concurrent atropine; however, the AUC(0-24)b was not affected.

Pharmacodynamics Results:

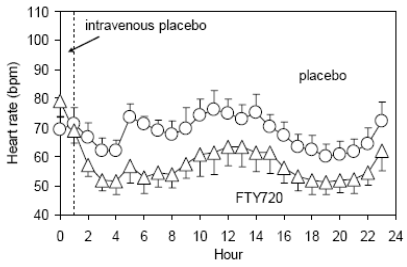
Heart rate response to oral placebo and FTY720 in the absence of atropine

Mean trajectories are shown in the figure below:

Figure 7-1 Heart rate trajectories for placebo or FTY720 and concurrent intravenous placebo



Mean heart rate trajectories over 60 minutes after oral administration of placebo (*circles*) or FTY720 (*triangles*) with concurrent intravenous titration of placebo. *Data points* are means over 5-minute intervals plotted at the start of each interval and *bars* are from the 95% confidence intervals.



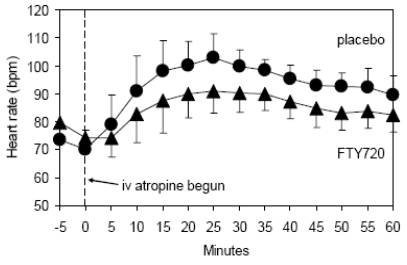
As in left panel for mean heart rate trajectories over 24 hours. *Data points* are means over hourly intervals plotted at the start of each hour and *bars* are from the 95% confidence intervals.

- The heart rate trajectory in FTY720 group shows a clear decline from the placebo group after the first hour but maintains the same circadian pattern.
- FTY720 elicited a mean daytime nadir heart rate 18% lower than oral placebo (49 vs 60 bpm) and this occurred at a median 4 hours postdose. This percent difference between placebo and FTY720 was also present for AUEC(0-12) and AUEC(0-24).

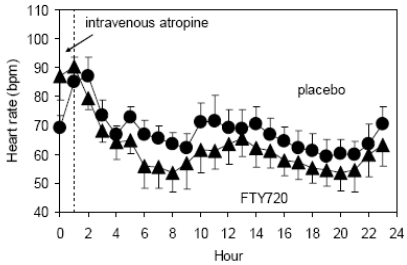
Heart rate response to oral placebo and FTY720 with atropine

Mean trajectories are shown in the figure below:

Figure 7-3 Heart rate trajectories for placebo or FTY720 and concurrent atropine



Mean heart rate trajectories over 60 minutes after oral administration of placebo (*circles*) or FTY720 (*triangles*) with concurrent intravenous titration of atropine. *Data points* are means over 5-minute intervals plotted at the start of each interval and *bars* are from the 95% confidence intervals.



As in left panel for mean heart rate trajectories over 24 hours. *Data points* are means over hourly intervals plotted at the start of each hour and *bars* are from the 95% confidence intervals.

- The average maximal heart rate was 95 bpm (12% lower than atropine + oral placebo) occurring at a median 25 minutes and was 82 bpmby the end of the first hour postdose.
- By 5 hours postdose the heart rates recovered and resumed their normal circadian pattern. The heart rate trajectory in FTY720 group showed similarly elevated rates for the first 5 hours postdose albeit shifted slightly lower compared to placebo-treated subjects.

- Thereafter, the morning nadir heart rate (51 bpm) was similar to that when FTY720 was given in the absence of atropine (49 bpm) but shifted to a median 8 hours postdose compared with 4 hours without atropine.

Study Part 2: Delayed administration of FTY720 and atropine

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 and FTY720-P are summarized in the following table:

Table 7-4 Pharmacokinetics: delayed administration

Parameter	Fingolimod			Fingolimod-phosphate		
	Placebo	Atropine	Ratio (90%CI)	Placebo	Atropine	Ratio (90%CI)
t_{max} (h)	12 (12-12)	12 (12-24)	--	12 (12-12)	12 (12-12)	--
$C_{max,b}$ (ng/ml)	4.2 ± 0.7	4.0 ± 0.8	0.95 (0.86, 1.05)	5.3 ± 0.9	5.3 ± 1.1	0.99 (0.88, 1.11)
$AUC(0\text{-}24)_b$ (ng.h/ml)	76 ± 12	73 ± 10	0.96 (0.89, 1.03)	81 ± 12	81 ± 13	1.00 (0.93, 1.07)

Values are arithmetic mean ± sd except for tmax which is median (range).
Ratio (90%CI) is the ratio of geometric means and 90% confidence interval.
Source data: Appendix 4 Table 3 and 4; Appendix 6 Tables 6.1-4 and 6.1-6.

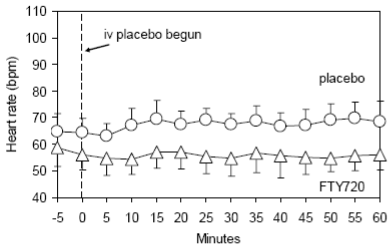
- When FTY720 was administered with delayed atropine, fingolimod and fingolimod-phosphate Cmax,b and AUC(0-24)b were not affected.

Pharmacodynamics Results:

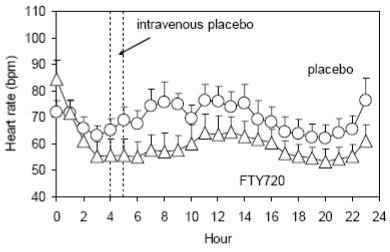
Heart rate response to oral placebo and FTY720 in the absence of atropine:

Mean trajectories are shown in the figure below:

Figure 7-5 Heart rate trajectories for placebo or FTY720 and delayed intravenous placebo



Mean heart rate trajectories over 60 minutes after oral administration of placebo (*circles*) or FTY720 (*triangles*) with delayed intravenous titration of placebo. *Data points* are means over 5-minute intervals plotted at the start of each interval and *bars* are from the 95% confidence intervals.

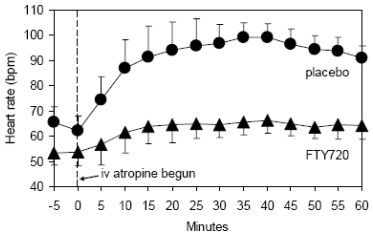


As in left panel for mean heart rate trajectories over 24 hours. *Data points* are means over hourly intervals plotted at the start of each hour and *bars* are from the 95% confidence intervals.

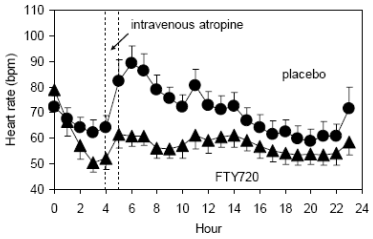
- The heart rate trajectory in FTY720 group shows a downward divergence from the placebo group about 2 hours postdose but maintains the same circadian pattern.
- FTY720 elicited a mean morning nadir heart rate 14% lower than oral placebo (54 vs 63 bpm) occurring a median 5 hours postdose.

Heart rate response to oral placebo and FTY720 with atropine

Figure 7-7 Heart rate trajectories for placebo or FTY720 and delayed atropine



Mean heart rate trajectories over 60 minutes after oral administration of placebo (*circles*) or FTY720 (*triangles*) with delayed intravenous titration of atropine. *Data points* are means over 5-minute intervals plotted at the start of each 5-minute period and *bars* are from the 95% confidence intervals.



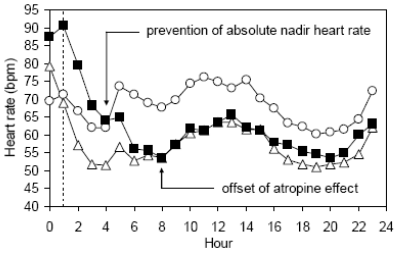
As in left panel for mean heart rate trajectories over 24 hours. *Data points* are means over hourly intervals plotted at the start of each hour and *bars* are from the 95% confidence intervals.

- By the end of the hour observation period, average heart rate was 64 bpm.
- Delaying the atropine titration allows the typical morning heart rate nadir to occur around 4 hours after FTY720 administration (50 bpm) which was 19% lower than for subjects receiving oral placebo.
- Since the atropine intervention was made after the nadir heart rate was reached, the mean morning nadir was the same regardless if atropine (50 bpm) or placebo (54 bpm) was subsequently given.
- The elevation in heart rate during and after the atropine titration is clear.
- Normal heart rates and circadian patterns were resumed 6 hours after the atropine titration (10 hours after the FTY720 dose).

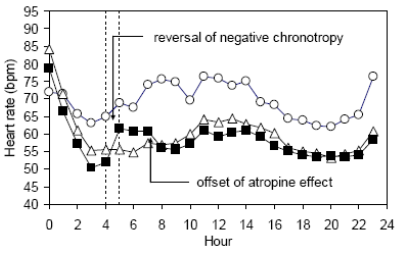
Comparison of concurrent and delayed atropine

Mean trajectories are shown in the figure below:

Figure 7-9 Comparison of heart rate responses to FTY720 with concurrent and delayed atropine



Mean heart rate trajectories for normal heart rate (*open circles*), FTY720 alone (*open triangles*), and FTY720 with concurrent atropine (*filled squares*). Concurrent atropine is able to prevent the daytime nadir heart rate observed for FTY720 alone.



Mean heart rate trajectories for normal heart rate (*open circles*), FTY720 alone (*open triangles*), and FTY720 with delayed atropine (*filled squares*). Delayed atropine is able to reverse the daytime nadir heart rate observed for FTY720 alone.

Mean heart rate trajectory for concurrent atropine.

- FTY720 with a concurrent atropine titration raised mean heart rate to normal or above-normal values for a median 5.5 hours after FTY720. The typical heart rate nadir that occurs 3-4 hours after the first dose of FTY720 was prevented.
- By 6 hours and thereafter, the effect of an early atropine titration was no longer present and mean heart rate conformed to the typical FTY720-associated negative chronotropic response pattern fluctuating between 54 and 66 bpm.

Mean heart rate trajectory for delayed atropine.

- When the atropine titration was delayed to 4 hours after FTY720, the typical first-dose nadir had already occurred between 3-4 hours postdose. Titrating atropine at this time was able to reverse the maximal negative chronotropic effect of FTY720.
- During the observation hour of the atropine titration, all subjects either reached or exceeded their corresponding normal heart rate at that time of the day.
- Heart rates remained elevated relative to those after FTY720 alone for a median duration of 3.5 hours.
- By 8 hours and thereafter, mean heart rate resumed the typical pattern due to the negative chronotropic effect of FTY720 fluctuating between 53 and 61 bpm.

Maximal dynamic responses (MDRs): concurrent atropine.

- FTY720 alone decreased the mean heart rate nadir 18% relative to oral placebo.
- When atropine was titrated concurrently with FTY720, the nadir decreased by 15% relative to atropine alone. The difference in MDRs over the 12-hour observation period (ratio = 0.85) was minimal because, although concurrent atropine prevented the early, absolute nadir (3-4 hours after FTY720), nonetheless, the typical negative chronotropic response to FTY720 later in the day occurred between 6 to 12 hours—a time interval when typical heart rates are still near the absolute nadir.

Maximal dynamic responses (MDRs): delayed atropine.

- FTY720 alone decreased the mean heart rate nadir 16% relative to oral placebo.
- When atropine was titrated at 4 hours after FTY720, the nadir decreased by 17% relative to atropine alone. There was no difference in MDRs over the 12-hour observation period (ratio = 0.98) because the absolute nadir (3-4 hours after FTY720) occurred for both treatments (FTY720 alone and FTY720 with atropine). Only after the nadir occurred was atropine administered in this scenario.

Conclusions:

- Titrating intravenous atropine concurrently with oral administration of FTY720 prevented the early absolute heart rate nadir that typically occurs 4 hours postdose. The prophylactic effect of atropine lasted 5.5 hours.
- Delaying the intravenous atropine titration to 4 hours after oral administration of FTY720 was able to reverse the negative chronotropic effect of FTY720. The reversal effect on heart rate lasted 3.5 hours.
- Single-dose FTY720 administered with atropine did not alter the pharmacokinetics of fingolimod or fingolimod-P to a clinically relevant extent.

Study FTY720A 0119*:* **A single-blinded, randomized, placebo-controlled, crossover study to evaluate the influence of intravenous isoproterenol administration on the negative chronotropic effect of 5 mg oral FTY720 in healthy volunteers**

A brief overview of some essential components of the study design is given below:

Study Design	single-blind, randomized, placebo-controlled, two-treatment, crossover study
Study Population	N=16 enrolled, 14 completed Age: 18-74 years (mean 29.3 years) Gender: 7 males, 9 females Weight: 50.8-78.7 kg (mean 66.3 kg) Race: 6 Whites, and 10 other ethnicity
Dosage and Administration	**Treatment A: Isoproterenol+FTY720** **Treatment B: Placebo+FTY720** Dosing regimen is listed below Periods 1 and 2 were separated by a 33-day washout **Table 3-1 Overview of study interventions** Hour | Isoproterenol/placebo | FTY720 | Heart rate, blood pressure measurements | PK -2 | Infusion 1 (30-min) | -- | Every 2 min during infusion then hourly | -- 0 | -- | 5 mg | Hourly | Predose 3 | Infusion 2 (30-min) | -- | Every 2 min during infusion then every 10 min | 3h 4 | Infusion 3 (30-min) | -- | Every 2 min during infusion then every 10 min | -- 5 | Infusion 4 (30-min) | -- | Every 2 min during infusion then every 10 min | 5h 6 | Infusion 5 (30-min) | -- | Every 2 min during infusion then every 10 min | -- 7-24 | -- | -- | Hourly | 7h, 12h, 24 h *PK* = pharmacokinetics; *h* = hours postdose. Study medication was administered with 240 ml of water after a light breakfast. Lot No. FTY720 2.5 mg capsules (FMI): batch US03082, package number 04-1433CH Isoproterenol: Isuprel (Abbott), ampules of 1mg/5ml, lot number 116253A Placebo: 5% dextrose solution (D5W) for intravenous infusion (b) (4), 1000ml per bag, lot numbers C622068 and C624163. Diet: No fluids were allowed until 2 hours after dosing. Otherwise, subjects had a fluid intake of at least 200 ml every 4 hours during waking hours in addition to fluid taken with meals. Xanthine-containing beverages were allowed but was to be discontinued 48 hours before dosing of study drug(s) Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood/plasma	For FTY720 and FTY720-P (Blood): At predose (0 hour), and 3, 5, 7, 12 and 24 hours.

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 1.5 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 8.6% Inter-day accuracy: < 11.7 % FTY720-P: Linear range : 1.5-500 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 4.0% Inter-day accuracy: < 4.8 %
PK Assessment	FTY720 and FTY720-P in blood: Cmax, Tmax, AUC0-t
PD Assessment	**Cardiovascular responses:** Heart rate measurements from telemetry and systolic/diastolic blood pressure (SBP/DBP) measurements from vital signs were used as the pharmacodynamic raw data. Mean arterial pressure (MAP) was calculated as: MAP = 1/3 SBP + 2/3 DBP.
Safety Assessment	Assessment of Physical examinations, vital signs, ECGs, clinical laboratory parameters (hematology, biochemistry, urinalysis), and adverse event.

Results:

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 and FTY720-P are summarized in the following table:

Table 7-1 Pharmacokinetic parameters

Parameter	Placebo	Isoproterenol	Ratio of geometric means (90%CI)
Fingolimod:			
t_{max} (h)	12 (12-24)	12 (7-24)	--
$C_{max,b}$ (ng/ml)	4.2 ± 1.0	3.9 ± 0.9	0.93 (0.88, 0.99)
$AUC(0\text{-}7)_b$ (ng.h/ml)	12 ± 2	13 ± 4	1.04 (0.94, 1.15)
$AUC(3\text{-}7)_b$ (ng.h/ml)	9 ± 2	10 ± 3	1.05 (0.96, 1.15)
$AUC(0\text{-}24)_b$ (ng.h/ml)	75 ± 14	73 ± 15	0.97 (0.92, 1.03)
Fingolimod-phosphate:			
t_{max} (h)	12 (7-12)	12 (5-12)	
$C_{max,b}$ (ng/ml)	4.5 ± 1.0	4.4 ± 1.0	0.98 (0.93, 1.03)
$AUC(0\text{-}7)_b$ (ng.h/ml)	15 ± 2	13 ± 5	0.84 (0.70, 1.01)
$AUC(3\text{-}7)_b$ (ng.h/ml)	12 ± 2	11 ± 4	0.91 (0.80, 1.03)
$AUC(0\text{-}24)_b$ (ng.h/ml)	77 ± 14	73 ± 16	0.94 (0.89, 0.99)

Data are arithmetic mean ± sd except for tmax which is median (range).

Source data: Appendix 4 Tables 1, 2, 3 and Appendix 6 Tables 6.1-1 and Appendix 6 Tables 6.1-2.

- Concomitant administration of isoproterenol did not alter the pharmacokinetics of FTY720 or FTY720-P.

Pharmacodynamics Results:

Descriptive overview of heart rate responses

Heart rate response to isoproterenol alone: infusion 1 and recovery

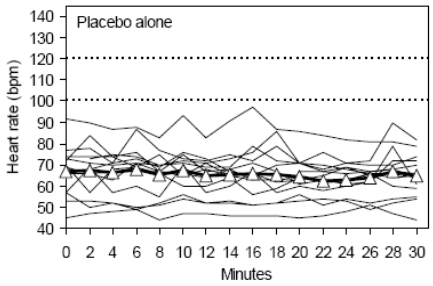
Table 7-2 Heart rate responses to isoproterenol and placebo: infusions 1 and 2

Response	Infusion 1 alone		Infusion 2 with fingolimod	
	Placebo	Isoproterenol	Placebo	Isoproterenol
Preinfusion (bpm)	67 ± 12	68 ± 9	53 ± 7	57 ± 8
Isoproterenol dose (mcg)	0	69 ± 27	0	97 ± 6
Subjects reaching max infusion rate*	--	1	--	10
Subjects reaching target heart rate*	0	14	--	9
E_{max} (bpm)	74 ± 14	122 ± 15	57 ± 7	105 ± 21
E_{max} (% of preinfusion)	9 ± 11	80 ± 21	7 ± 5	85 ± 29
E_{avg} (bpm)	66 ± 9	101 ± 13	54 ± 7	86 ± 17
AUE (bpm x h)	33 ± 5	51 ± 7	27 ± 3	43 ± 8

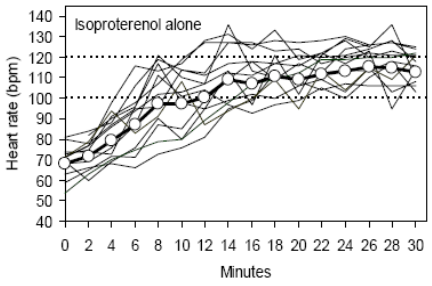
Values are mean ± sd. Source data: Appendix 6, Table 5.2-1.
* *Maximum infusion rate:* 5 mcg/min; *target heart rate:* 100-120 bpm.

Mean trajectories are shown in the figure below:

Figure 7-2 Heart rate responses: infusion 1



Individual heart rate trajectories during the 30-minute placebo infusion 1. Shown is the mean trajectory (*open symbols*) and the target heart rate range of 100-120 bpm (*dashed lines*).



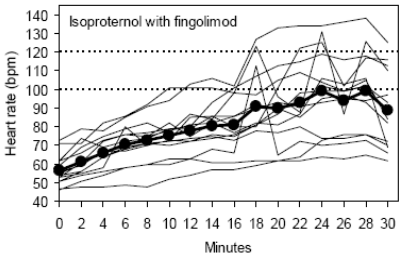
As in the left panel for isoproterenol infusion 1.

- Baseline morning heart rate averaged 67-68 bpm before placebo and isoproterenol infusion.
- On average the target heart rate was reached by 12 minutes into the 30-minute infusion.
- The maximum heart rate attained during the infusion (Emax) represented an 80% increase from the preinfusion heart rate.
- After the isoproterenol infusion the average heart rate recovered from 113 ± 14 bpm at end-of-infusion; to 74±9 bpm at -1 hour; to 73±11 bpm at 0 hour before FTY720.

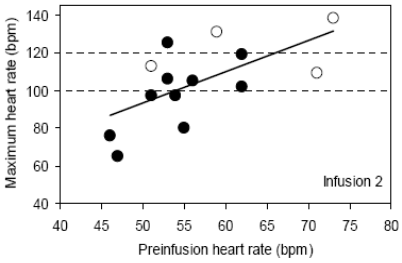
Heart rate response to FTY720 and isoproterenol: infusion 2

Mean trajectories are shown in the figure below:

Figure 7-3 Heart rate responses: infusion 2



Individual heart rate trajectories during the 30-minute isoproterenol infusion 2 in the presence of fingolimod. Shown is the mean trajectory (*circles*) and the target heart rate range of 100-120 bpm (*dashed lines*).



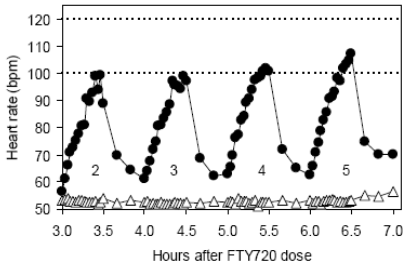
Relationship between preinfusion heart rate and maximum heart rate during infusion 2 (isoproterenol with fingolimod). Indicated are subjects reaching the maximum allowed infusion rate of 5 mcg/min (*filled circles*) and the regression line: r = 0.64, p=0.01.

- On average the full titration time specified in the protocol (20 minutes) was needed to reach the target or the maximum heart rate.
- The maximum heart rate attained during the infusion (Emax) represented an 85% increase from the preinfusion heart rate.
- The average total isoproterenol dose was 97 mcg. This dose was 41% higher than during infusion 1 in the absence of FTY720 to elicit a similar percent increase from baseline in Emax compared to infusion 1 (85% versus 80%).

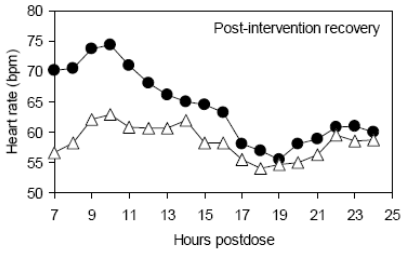
Heart rate response to FTY720 and isoproterenol: infusions 3-5 and recovery

Mean trajectories are shown in the figure below:

Figure 7-4 Heart rate responses: infusions 2 to 5 and recovery



Mean heart rate trajectories during infusions 2-5 of isoproterenol (*filled circles*) and placebo (*open triangles*). Shown is the target heart rate range of 100-120 bpm (*dashed lines*).



Mean heart rate trajectories after the interventional infusions of placebo (*open triangles*) and isoproterenol (*filled circles*).

Placebo infusions

- Placebo infusions had no effect on average heart rate which remained at the nadir of 52 ± 7 bpm.
- After infusion 5 (between 6.5 and 7 hours after the FTY720 dose) there appeared to be a slight recovery in heart rate (57 ± 8 bpm) despite the fact that FTY720-P blood levels were still increasing.
- Between 7 and 24 hours postdose, there was a clear release from the nadir heart rate (52 bpm) with a return to a circadian rhythm in the average heart rate trajectory.
- By 24 hours postdose on day 2, heart rate was 59 ± 8 bpm and still depressed compared to the pre-FTY720 heart rate at the same clocktime on day 1 of 72 ± 11 bpm.

Isoproterenol infusions

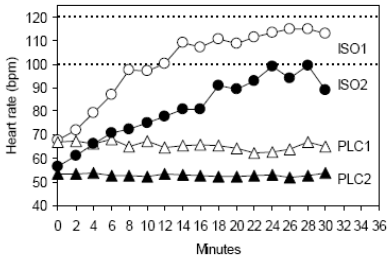
- In the half-hour interval after each isoproterenol infusion, heart rate recovered back to a relatively stable preinfusion value before infusions 3 to 5.

- Heart rate responses to infusions 3 and 4 were similar to those of infusion 2.
- Infusion 5 appeared to elicit a slightly greater response compared with infusions 2 through 4.
- Although the AUE of infusion 5 was unchanged, more subjects reached the target heart rate yielding a marginally higher Emax response during the final infusion.
- Heart rate recovered back to the value in the placebo treatment by 17 hours after the FTY720 dose (10.5 hours after the last isoproterenol infusion).

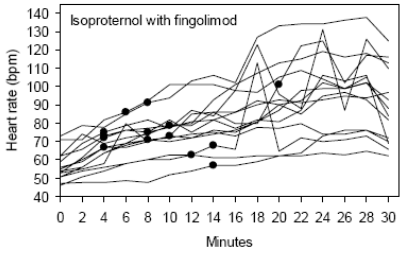
Effect of isoproterenol on heart rate and blood pressure responses to FTY720

Mean trajectories are shown in the figure below:

Figure 7-5 Heart rate trajectories: infusion 1 vs infusion 2



Mean heart rate trajectories during placebo (PLC) and isoproterenol (ISO) infusion 1 (alone) and infusion 2 (after FTY720 administration). Shown is the target heart rate range of 100-120 bpm (*dashed lines*).



Individual heart rate trajectories during infusion 2 of isoproterenol after FTY720 administration. Shown are the time points when each subject's heart rate returned to the pre-FTY720 baseline value (*filled circles*).

Heart rate without isoproterenol

- A 5 mg single dose of FTY720 elicited a 28% decrease in heart rate from a predose value of 72 ± 11 bpm to a nadir of 52 ± 7 bpm at 3.3 hours postdose.
- This nadir heart rate was maintained thereafter essentially for the full time period when the interventional infusions were givenfrom 3 to 7 hours postdose.

Ability of isoproterenol to return heart rate to pre-FTY720 values

- During each of the isoproterenol infusions 2 through 5, heart rate in all subjects reached and surpassed their heart rate before administration of FTY720.
- The average time to exceed the predose heart rate was 9 minutes into the infusion at an average infusion rate of 2.3 ± 1.2 mcg/min.
- Hence, isoproterenol was able to counteract the negative chronotropic effect of FTY720 when FTY720 was exerting its strongest influence on heart rate (3-7 hours postdose).

Ability of isoproterenol to increase heart rate relative to placebo in presence of FTY720

Table 7-4 Isoproterenol effect on fingolimod responses

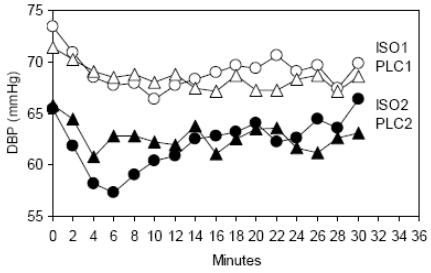
Parameter	Response	Placebo/FTY720	Isoproterenol/FTY720
Time-averaged response E_{avg}(3-7 hr)	Heart rate (bpm)	53 ± 6	80 ± 13
	SBP (mmHg)	104 ± 7	116 ± 8
	DBP (mmHg)	65 ± 4	67 ± 5
	MAP (mmHg)	78 ± 5	83 ± 5
Total response: AUE(3-7 hr)	Heart rate (bpm.h)	212 ± 26	319 ± 50
	SBP (mmHg.h)	417 ± 27	464 ± 31
	DBP (mmHg.h)	260 ± 17	268 ± 19
	MAP (mmHg.h)	313 ± 19	334 ± 21
Mean maximal response E_{max}(3-7 hr)	Heart rate (bpm)	56 ± 7	107 ± 18
	SBP (mmHg)	109 ± 7	132 ± 11
	DBP (mmHg)	70 ± 6	73 ± 5
	MAP (mmHg)	82 ± 6	90 ± 6

Values are mean ± sd. Source data: Appendix 6, Table 5.2-2.

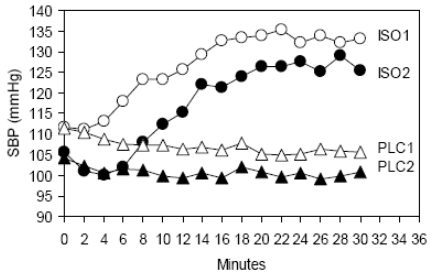
- As administered in this study to a maximum dose of 100 mcg over 30 minutes, isoproterenol significantly increased both the time-averaged and total heart rate response by 50% compared with placebo ($p<0.001$).
- Isoproterenol also significantly increased the mean maximal response by 90% compared to placebo ($p<0.001$).

Blood pressure responses to isoproterenol relative to placebo in presence of FTY720

Figure 7-6 Blood pressure trajectories: infusion 1 vs infusion 2



Mean diastolic blood pressure trajectories during placebo (PLC) and isoproterenol (ISO) infusion 1 (alone) and infusion 2 (after FTY720 administration).



As in left panel for systolic blood pressure.

- As FTY720 was exerting its negative chronotropic effect postdose, SBP decreased from 109 ± 8 to 104 ± 10 mmHg and DBP decreased from 70 ± 5 to 66 ± 6 mmHg by 3 hours postdose before the interventional infusions.
- The downward shift in both plots between infusion 1 and 2 was due to the response to FTY720 administration.
- DBP patterns over the 30-min infusions were similar between placebo and isoproterenol.
- SBP remained relatively stable during placebo infusions and increased during isoproterenol infusions in parallel with heart rate.
- DBP, SBP, and MAP response parameters (Eavg, mean Emax, AUE) summed over the interval 3-7 hours postdose were not significantly different between isoproterenol and placebo as all isoproterenol/placebo response ratios had 90% confidence intervals.

Influence of FTY720 on heart rate and blood pressure responses to isoproterenol

Table 7-5 Fingolimod effect on isoproterenol responses

Parameter	Response	Isoproterenol	Isoproterenol/FTY720
Time-averaged response: E_{avg}	Heart rate (bpm)	101 ± 13	86 ± 17
	SBP (mmHg)	127 ± 11	122 ± 12
	DBP (mmHg)	69 ± 5	64 ± 6
	MAP (mmHg)	88 ± 6	84 ± 7
Total response: AUE	Heart rate (bpm.h)	51 ± 7	43 ± 8
	SBP (mmHg.h)	63 ± 6	61 ± 6
	DBP (mmHg.h)	34 ± 2	32 ± 3
	MAP (mmHg.h)	44 ± 3	42 ± 4
Maximal response: E_{max}	Heart rate (bpm)	122 ± 15	105 ± 21
	SBP (mmHg)	140 ± 13	135 ± 12
	DBP (mmHg)	78 ± 5	71 ± 7
	MAP (mmHg)	97 ±6	91 ± 7

Isoproterenol = infusion 1; *isoproterenol/FTY720* = infusion 2. Source data: Appendix 6, Table 5.2-6.

- Isoproterenol administered alone yielded a time-averaged heart rate of 101 bpm.
- Isoproterenol given in the presence of FTY720 yielded a time-averaged heart rate of 86 bpm.
- The percent increase from pre-infusion to maximal response was similar for infusion 1 (80%) and infusion 2 (85%).
- The isoproterenol dose required to achieve this percentage increase in heart rate was 41% higher for isoproterenol in the presence of FTY720 (97 ± 6 mcg) versus isoproterenol alone (69 ± 27 mcg).
- FTY720 had no influence on blood pressure responses to isoproterenol as all infusion 2 / infusion 1 response ratios were within 90% confidence intervals.

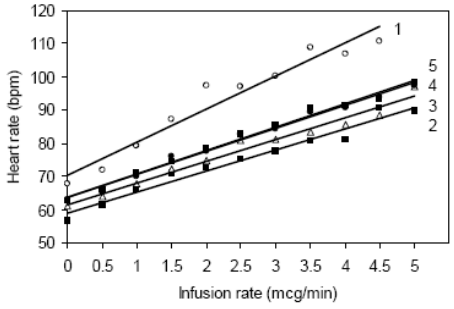
Influence of FTY720 on heart rate sensitivity to isoproterenol

Table 7-6 Heart rate sensitivity parameters

Treatment	Alone	With FTY720			
	Infusion 1	Infusion 2	Infusion 3	Infusion 4	Infusion 5
Isoproterenol	16.6 ± 10.7	6.4 ± 2.3	6.8 ± 3.2	7.0 ± 3.5	7.9 ± 3.1
Placebo	-0.5 ± 1.2	-0.2 ± 0.4	-0.1 ± 0.5	-0.1 ± 0.5	0.2 ± 0.5

Units are bpm per mcg/min for isoproterenol and bpm per ml/min for placebo.
Values are mean ± sd. Source data: Appendix 6, Table 5.2-3 and 5.2-5.

Figure 7-7 Plots of heart rate sensitivity to isoproterenol



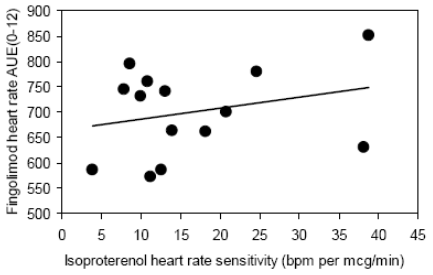
Regression lines through mean heart rate vs isoproterenol infusion rate during infusion 1 (alone) and infusions 2 through 5 (with FTY720).

- Coadministration of FTY720 reduced the heart rate sensitivity to isoproterenol by 2.6-fold (slopes 16.6 versus 6.4 bpm per mcg/min, p = 0.002).
- With each subsequent infusion, the mean sensitivity remained significantly lower than infusion 1 (pvalues 0.002 – 0.009).
- Mean slopes increased sequentially for each isoproterenol infusion from 2 through 5 suggesting a recovery in sensitivity with time; however, the data were highly variable as indicated by the large standard deviations.

- Heart rate was not sensitive to placebo infusion with essentially flat relationships (slopes near zero).

Correlation between heart rate sensitivity to isoproterenol and heart rate response to FTY720

Figure 7-8 Heart rate sensitivity to isoproterenol and FTY720



Regression of FTY720 heart rate AUE(0-12) and heart rate sensitivity to isoproterenol.

- There was no significant correlation between heart rate sensitivity to isoproterenol and heart rate response to FTY720 regardless whether the latter was parameterized as heart rate AUE(0-12) (p = 0.349) or mean Emax(0-12) (p = 0.150).

Conclusions:

- Coadministration of isoproterenol and FTY720 did not alter the pharmacokinetics of FTY720 or FTY720-P.
- Isoproterenol was able to counteract fully the negative chronotropic effect of FTY720 returning heart rate to the pre-FTY720 baseline value.
- Blood pressure was not significantly affected.
- In order to increase heart rate by a given percentage, a higher dose of isoproterenol was needed in the presence of FTY720 than when isoproterenol was used alone.
- As dosed in this study, an 85% increase in heart rate required a 41% higher isoproterenol dose relative to the dose of isoproterenol when used alone.
- Heart rate sensitivity to isoproterenol (the slope of the increase in heart rate versus the increase in isoproterenol infusion rate) was 2.6-fold lower in the presence of FTY720 than when isoproterenol was used alone. Heart rate sensitivity to isoproterenol was not correlated with the heart rate response to FTY720.

Study FTY720A 2213*:* **A double blind, parallel group, placebo controlled, multiple dose study to evaluate the effects of FTY720 (1.25 and 5 mg) on cardiac rate and rhythm**

A brief overview of some essential components of the study design is given below:

Study Design	double blind, parallel group, placebo-controlled, multiple-dose study
Study Population	N=66 enrolled, 60 completed **Table 7.2-1 Demographics** Treatment | Sex (M/F) | Age (yrs) | Weight (kg) | Height (cm) | Ethnicity (%) White, Black, Other Placebo | 14/9 | 31 (19-44) | 80 (50-111) | 175 (160-188) | 52, 4, 44 FTY720 1.25 mg | 11/12 | 27 (18-42) | 71 (52-93) | 170 (159-186) | 52, 4, 44 FTY720 5.0 mg | 7/13 | 29 (19-44) | 71 (54-88) | 172 (160-187) | 50, 5, 45 Values are mean (range).
Dosage and Administration	Subjects were randomization to one of three treatments: FTY720 1.25 mg (n=20), 5 mg (n=20), or placebo (n=20). Each subject participated in a 21-day screening period, a 2-day placebo run-in phase, and an 8-day treatment period (7 days of study drug administration). Study medication was administered with 180 ml of water after at least a 10 h fast and continue to fast for at least 4 hours thereafter. Drug Name | Expiry dates | Batch number / KN # | Form | Quantity FTY720 1.25 mg | 07/2002 | H-05673 3761319.00.001 | Capsules | 12 bottles of 35 capsules/bottle FTY720 2.5 mg | 01/2003 | H-05663 3752938.00.002 | Capsules | 25 bottles of 35 capsules/bottle Matching Placebo | 01/2006 | H-05666 3755030.00.16 | Capsules | 4 bottles of 300 capsules/bottle Diet: Subjects had a fluid intake of at least 200 ml every 4 hours during waking hours in addition to fluid taken with meals. Xanthine-containing beverages were to be discontinued 48 hours before dosing of study drug(s) Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood/plasma	For FTY720 and FTY720-P (Blood): Day 1: predose, 1, 2, 4, 6, 8, 12 and 16 h post daily dose. Day 7: predose, 1, 2, 4, 6, 8, 12 16 and 24 h post daily dose. Days 2-6: predose and window of 6-8 h post daily dose Study completion: Day 3, 7, 14 21 and 28 following last FTY720/Placebo dose
PD measurements	12 lead ECG monitoring Tracings were recorded every 2 hours for 24 h on Days –1, 1 and 7. Mortorola Holter Monitor Continuous 24 hr recording on Days –1, 1 and 7.

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720: Linear range : 0.08-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 9.6% Inter-day accuracy: < 8.7 %
PK Assessment	FTY720 in blood: Cmax, Tmax, AUC0-24, Cx, Cmin, Cave, PTF, R, t1/2
PD Assessment	12 lead ECG, Holter monitoring, telemetry, lymphocyte count and sphingosine-1-phosphate measurements
Safety Assessment	Assessment of Vital Signs, adverse events, and standard clinical laboratory variables (biochemistry, hematology, urinalysis).

Results:

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 on Day 1 are summarized below:

Table 7.4-1 Pharmacokinetic parameters: Day 1

Parameter	1.25 mg/day	5 mg/day
t_{max} (h)	12 (12 - 16)	12 (8 - 16)
$C_{max,b}$ (ng/ml)	1.1 ± 0.2	4.2 ± 0.8
$C(24)_b$ (ng/ml)	0.9 ± 0.2	3.3 ± 0.5
$AUC(0\text{-}24)_b$ (ng·h/ml)	20 ± 4	79 ± 13

Values are mean ± standard deviation except for tmax which is median (range).

- Cmax,b was consistent with dose-proportionality based on the geometric mean ratio (95% confidence interval) for dose-normalized values of 1.04 (0.92 - 1.17).
- AUC(0-24)b was also consistent with dose-proportionality after the first dose with a ratio of 1.00 (0.90 - 1.12).
- Both Cmax,b and AUC(0-24)b exhibited moderate interindividual variability with coefficients of variation between 16 and 20 percent.

Drug accumulation

Trough (C0) and peak (C7) concentrations of FTY720 are summarized below:

Table 7.4-2 Daily peak/trough concentrations

Day	$C(0)_b$ (ng/ml)		$C(7)_b$ (ng/ml)	
	1.25 mg/day	5 mg/day	1.25 mg/day	5 mg/day
2	0.9 ± 0.2	3.3 ± 0.5	1.7 ± 0.3	6.3 ± 1.1
3	1.6 ± 0.3	6.0 ± 1.1	2.4 ± 0.5	9.3 ± 1.5
4	2.2 ± 0.5	8.1 ± 1.5	2.9 ± 0.7	10.9 ± 2.4
5	2.9 ± 0.7	10.5 ± 2.0	3.8 ± 0.9	14.6 ± 2.9
6	3.4 ± 0.8	12.6 ± 2.3	4.2 ± 0.9	15.4 ± 3.2
7	3.7 ± 0.8	14.0 ± 2.7	--*	--*

$C(x)_b$ = concentration in blood obtained x hours postdose.
Values are mean ± standard deviation.
* $C(7)_b$ not sampled on day 7.

- From day to day the ratio of C(0)b's between dose levels remained relatively constant and averaged 3.8 reflecting the 4-fold difference in doses.
- A similar pattern occurred for C(7)b.
- The overall accumulation in blood over 7 days of daily dosing was 5-fold regardless of dose level.

Reviewer's note:
Subject 5108, received 1.25 mg/day, has a FTY720 concentration of 15.1 ng/ml on day 6, 7h postdose and at no other timepoint in the entire study course did his concentration exceed 3.9 ng/ml. The sponsor stated that the concentration of 15.1 ng/ml on day 6 was considered likely not from this subject and may reflect contamination or mislabeling. This unexplainable result along with the sponsor's statement raised the concern of the quality of the study conduct. This might question the reliability of the study results.

Day 7 profiles and elimination phase

The pharmacokinetic parameters of FTY720 on Day 7 are summarized below:

Table 7.4-3 Pharmacokinetic parameters: day 7

Parameter	1.25 mg/day	5 mg/day
$C_{min,b}$ (ng/ml)	3.7 ± 0.8	14.0 ± 2.7
t_{max} (h)	12 (6 - 16)	12 (6 - 16)
$C_{max,b}$ (ng/ml)	5.0 ± 1.0	18.2 ± 4.1
$AUC(0\text{-}24)_b$ (ng·h/ml)	109 ± 24	399 ± 85
$C_{avg,b}$ (ng/ml)	4.5 ± 1.0	16.6 ± 3.5
$C(24)_b$ (ng/ml)	4.3 ± 1.1	15.7 ± 3.5
PTF (%)	27 ± 8	25 ± 6
$t_{1/2}$ (days)	7.9 ± 2.2	8.1 ± 2.0

Values are mean ± standard deviation except for tmax which is median (range).

- The difference in exposure between dose levels was consistent with the four-fold difference of doses based on the dose-normalized parameter ratio (95% CI) for Cmin,b of 1.06 (0.94 - 1.21), Cmax,b of 1.09 (0.96 - 1.25), and AUC(0-24)b of 1.09 (0.95 - 1.25).
- The fact that C(24)b was higher than the predose concentration (Cmin,b) indicated that steady state had not be reached in 7 days, as anticipated.
- The decline in concentrations after the last dose was gradual and parallel at the two dose levels.

Pharmacodynamics Results:

Blood pressure

Table 7.3-3 Blood pressure: area under the effect curve, day 1

	Supine		Standing	
	SBP (mmHg)	DBP (mmHg)	SBP (mmHg)	DBP (mmHg)
Placebo	1517 ± 115	941 ± 65	1633 ± 108	1091 ± 72
FTY720 1.25 mg (vs. Placebo)	1540 ± 104 (0.538)	921 ± 61 (0.336)	1576 ± 119 (0.157)	1041 ± 75 (0.075)
FTY720 5 mg (vs. Placebo)	1459 ± 131 (0.027)	886 ± 64 (0.008)	1536 ± 149 (0.015)	1005 ± 103 (0.002)
P value (1.25 mg vs. 5 mg)	0.129	0.087	0.319	0.189

Values are mean ± sd.

Table 7.3-4 Blood pressure: Emax, day 1

	Supine		Standing	
	SBP (mmHg)	DBP (mmHg)	SBP (mmHg)	DBP (mmHg)
Placebo	103 ± 9	62 ± 5	109 ± 8	71 ± 8
FTY720 1.25 mg (vs. Placebo)	100 ± 9 (0.371)	59 ± 7 (0.141)	103 ± 10 (0.061)	67 ± 7 (0.234)
FTY720 5 mg (vs. Placebo)	95 ± 8 (0.004)	56 ± 5 (0.001)	100 ± 12 (0.007)	63 ± 9 (0.006)
P value (1.25 mg vs. 5 mg)	0.048	0.098	0.439	0.114

Emax is defined as the lowest observed blood pressure from 0-14 h post dose
Values are mean ± sd.

- FTY720 treatment was associated with a mild decrease in mean blood pressure, systolic blood pressure and diastolic pressure decreasing by approximately 5-12 mm Hg and 3-8 mm Hg, respectively . Statistical significance was only observed 5 mg dose. This decrease was only evident on Day 1 as the three treatment groups present comparable pre-dose supine diastolic blood pressure on Days 1-8.
- There was no difference between the log- transformed area under the effect curve. However, a dose dependent difference between the maximal reduction (Emax) in supine systolic blood pressure was significant (p=0.048).
- The time of maximal effect of FTY720 on blood pressure appeared to be approximately 8 hours post dose. After this time, blood pressure remained constant or began to increase.

ECG derived heart rate

Table 7.5-1 Mean ECG derived PD parameters following Placebo or FTY720 administration

Parameter	Treatment	Day –1	Day 1	Day 7
AUE (0-4)	Placebo	247	246	263
	FTY720 1.25 mg	247	224	242
	FTY720 5 mg	249	210	241
AUE (0-16)	Placebo	1046	1052	1094
	FTY720 1.25 mg	1034	930	994
	FTY720 5 mg	1043	899	996
AUE (0-24)	Placebo	1553	1578	1625
	FTY720 1.25 mg	1526	1380	1477
	FTY720 5 mg	1540	1335	1477

Mean trajectories are shown in the figures below:

Figure 7.5-2 Mean ECG derived heart rate following a single placebo/FTY720 administration (Day 1)

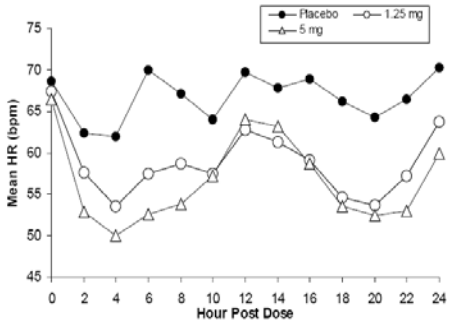
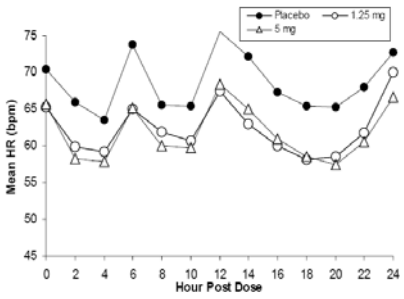


Figure 7.5-3 Mean ECG derived heart rate following multiple placebo/FTY720 administration (Day 7)



- The administration of the first, single dose of FTY720 (1.25 and 5.0 mg) resulted in a acute, dose dependent decrease in mean heart rate, with the nadir mean heart rate observed at 4 hours post dose.
- By 12 hours after the first FTY720 dose, the reduction in mean heart rate no longer appeared to be dose-dependent. Both FTY720 treatment groups manifested a similar 10 BPM decrease in mean heart rate compared to placebo.
- AUE in the FTY720 treatment groups decreased in a significant ($p<0.001$), dose dependent manner following the first dose on Day 1. This dose dependent decrease was not observed on Day 7.
- By day 7 of FTY720 treatment, it was no longer possible to detect an acute effect of FTY720 treatment on heart rate. The increase and decrease in mean heart rate over the day and night matched the diurnal, baseline rhythm, changing to a similar degree in both FTY720 and placebo treatment groups.

Assessment of QRS, PR, RR and QT interval

- A significant increase in the PR interval on Day 1 of 1.25 mg and 5 mg FTY720 treatment,101 ms and 140 ms, respectively) is shown. Although the magnitude of change decreased (25 ms and 29 ms) on Day 7, both increases were significant compared to Placebo.
- The increased RR interval was reflected in a significant reduction in HR on Day 1 of FTY720 treatment (1.25 mg resulted in a 6.1 bpm reduction and 5 mg in a 8.4 bpm reduction). Although the magnitude of this reduction was diminished (1.9 and 2.4 bpm) by Day 7, both treatment groups significantly differed from Placebo throughout the dosing interval.
- The mean QRS change from baseline was comparable for all treatment groups on Days 1 and 7.

QT change from baseline

Table 1 QT mean ms (+/- SD) change from baseline (Day –1)

Interval (ms)	Placebo		FTY720 1.25 mg		FTY720 5 mg	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
QT	-4.3 (5.9)	-4.0 (11.9)	16.9 (9.0)*	11.4 (15.2)*	17.2 (9.8)*	2.0 (12.8)
QTcF	-1.5 (4.0)	2.1 (8.6)	4.5 (5.2)*	7.7 (13)	-0.6 (6.1)	-2.5 (10.3)
QTcB	- 0.1 (5.6)	5.3 (9.7)	-1.8 (5.8)	5.7 (13.3)	-9.6 (6.2)*	-4.9 (11.4)*

* $p<0.05$ compared to placebo

- The uncorrected QT duration significantly increased following the single administration of FTY720 (~ 18 ms for both 1.25 and 5 mg) on day 1.

- In the case of the Frederica correction, (QTcF), a significant increase in QTcF was noted with the 1.25 mg dose on day 1 (4.5 ms) and this increase was still detected on Day 7 (7.7 ms).
- In the case of Bazett's correction (QTcB), the opposite phenomenon was seen, with FTY720 treatment resulting a decrease in QTcB for both the 1.25 and 5 mg treatment groups (-1.8 and -9.6, respectively) with statistical significance achieved at the 5 mg dose. This significant reduction of QTcB was also noted for the 5 mg group on Day 7 (-4.9 ms). The QTcB for the 1.25 mg dose group was also decreased by 5.7 ms.

Table 7.5-3 Frequency distribution of maximum change from baseline for QTcB over <30, 30-60 and >60 ms

Day	Treatment	< 30 ms	30-60 ms	>60 ms
Day 1	Placebo	13	7	0
	FTY720 1.25 mg	17	3	0
	FTY720 5.0 mg	20	0	0
Day 7	Placebo	11	9	0
	FTY720 1.25 mg	10	10	0
	FTY720 5.0 mg	17	3	0

Table 7.5-4 Frequency distribution of maximum change from baseline for QTcF over <30, 30-60 and >60 ms

Day	Treatment	< 30 ms	30-60 ms	>60 ms
Day 1	Placebo	17	3	0
	FTY720 1.25 mg	16	4	0
	FTY720 5.0 mg	19	1	0
Day 7	Placebo	14	6	0
	FTY720 1.25 mg	9	11	0
	FTY720 5.0 mg	18	2	0

- These data suggested there being no clear FTY720 dose or concentration effect on QT interval.

Holter Monitor Event Data

A summary of twenty-four hour Holter data for day –1, day 1 and day 7 are shown for all treatment groups in the tables below:

Table 7.5-5 Holter data following a single dose of Placebo (Day -1)

Day –1	Total number			
Treatment	Heart beats (24 h) Mean (+/-SD)	Atrial Fibrillations	Supraventricular Ectopy* Total beats (# of subjects)	Ventricular Ectopy ** Total beats (# of subjects)
Placebo	88385 (24307)	0	52 (10)	47 (8)
FTY720 1.25 mg	92840 (9515)	0	1599*** (11)	65 (5)
FTY720 5 mg	91088 (11546)	0	28 (12)	8 (5)

*SupraVentricular Ectopy includes supraventricular premature beats, single beats, pairs and runs

** Ventricular Ectopy includes ventricular premature beats, single beats, pairs, runs and R on T.

*** Subject 6137 presented 1310 of these beats. Total beats reduced to 289 when this subject is excluded.

Table 7.5-6 Holter data following a single dose of Placebo or FTY720 (Day 1)

Day 1	Total number			
Treatment	Heart beats (24 h) Mean (+/-SD)	Atrial Fibrillations	Supraventricular Ectopy* Total beats (# of subjects)	Ventricular Ectopy ** Total beats (# of subjects)
Placebo	101513 (+/-10386)	0	91 (11)	169 (10)
FTY720 1.25 mg	81111 (+/-15641)	0	485 (15)	46 (8)
FTY720 5 mg	76353 (+/- 19372)	0	445 (17)	15 (7)

*SupraVentricular Ectopy includes supraventricular premature beats, single beats, pairs and runs

** Ventricular Ectopy includes ventricular premature beats, single beats, pairs, runs and R on T.

Table 7.5-7 Holter data following multiple daily doses of Placebo or FTY720 (Day 7)

Day 7	Total number			
Treatment	Heart beats (24 h) Mean (+/-SD)	Atrial Fibrillations	Supraventricular Ectopy* Total beats (# of subjects)	Ventricular Ectopy ** Total beats (# of subjects)
Placebo	95593 (+/-26415)	0	53 (8)	101 (8)
FTY720 1.25 mg	90487 (+/- 7743)	0	182 (14)	35 (12)
FTY720 5 mg	88729 (+/-9613)	0	86 (16)	1 (1)

*SupraVentricular Ectopy includes supraventricular premature beats, single beats, pairs and runs

** Ventricular Ectopy includes supraventricular premature beats, single beats, pairs, runs and R on T.

- Although slight, a significant difference in the magnitude of heartbeat reduction as measured by AUE (0-4) was noted on day 1. Both FTY720 dose groups were comparable on both days 7.
- FTY720 did not induce atrial fibrillation.
- Compared to the placebo group, FTY720 did not increase the rate of ventricular ectopy.
- 5 mg FTY720 appeared to increase the rate of supraventricular ectopy (premature atrial contractions) on day 1, however this effect became attenuated by day 7.

Summary of Sinus Pauses

The frequency of sinus pauses as well as the duration of these events were recorded by Holter monitor.

- In two or three subjects in each treatment group there was an increased incidence of low grade sinus pauses lasting 2-3 seconds on day 1, however the frequency of this event was higher in FTY720 treatment groups and was dose dependent.
- No high grade pauses, >3 seconds were detected in the FTY720 treatment groups on either day 1 or day 7. By day 7 of the study, no sinus pauses were detected in any treatment group.

Summary of Atrio-Ventricular Blocks

The Holter monitor was used to detect possible conduction blocks on days –1, 1 and 7 for the three study groups. The summary is shown in the following table:

Table 7.5-9 Summary of 2nd degree atrio-ventricular blocks, Mobitz type 1. (Wenckebach Block)

Subject #	Onset (Day/Treatment)	Cessation (Day/Treatment)
5105	Day –1/Placebo	Day 1/ FTY720 5 mg
5108	Day –1/Placebo	Day –1/Placebo
5125	Day –1/Placebo	Day 3*/FTY720 5 mg
5111	Day 1/FTY720 1.25 mg	Day 1/ FTY720 1.25 mg
5124	Day 1/FTY720 5 mg	Day 1/FTY720 5 mg
5126	Day 1/FTY720 1.25 mg	Day 3*/FTY720 1.25 mg

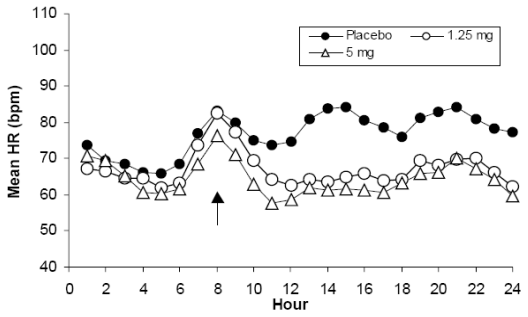
* per investigator

- No third degree or second degree, type 2 atrio-ventricular blocks were detected.
- Six subjects manifested transient second degree, type 1 (Wenckebach) atrio-ventricular blocks which were asymptomatic and did not require medical intervention while three of them (5105, 5108, 5125) were on placebo.
- Three subjects (5111, 5124, 5126) were on FTY720 treatment, when the AV block occurred. The onset of the block occurred on day 1 at the following times post dose: hour 15, hour 1, hour 5, respectively. In two of the subjects (5111 and 5126) the block was detected for at most one hour duration on day 1. One subject (5126) had two episodes of block lasting at most one hour on day 1. The investigator noted resolution of this AE on day 3.

FTY720 Effect on Heart Rate: Telemetry

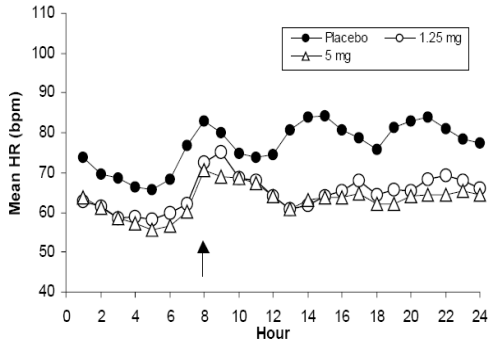
The continuous heart rate data derived from telemetry was summarized into hourly mean heart rate and this data for each day of study, 1 through 7, is presented in the following figures:

Figure 7.5-5 Day 1: Mean hourly heart rate



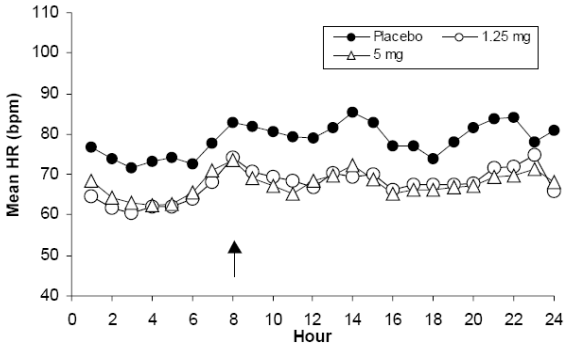
Arrow indicates time of dose administration.

Figure 7.5-6 Day 2: Mean hourly heart rate



Arrow indicates time of dose administration.

Figure 7.5-11 Day 7: Mean hourly heart rate



Arrow indicates time of dose administration.

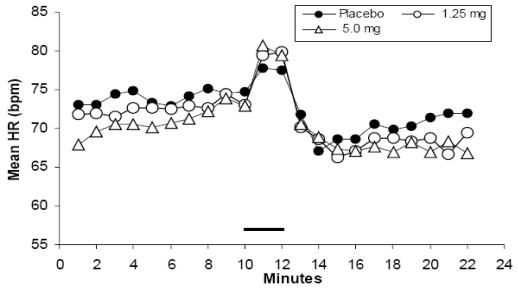
- On day –1, all treatment groups manifested an identical and consistent diurnal pattern (figure not shown).

- On day 1, FTY720 results in a dose dependent decrease in mean heart rate. This decrease in heart rate was first apparent approximately 2 hours after the FTY720 dose and heart rate reached its nadir at approximately three to four hours post dose. At nadir, the mean heart rate decreased by 10 and 18 beats per minute for the FTY720 1.25 and 5 mg treatment groups, respectively.
- The acute effect of FTY720 on heart rate was no longer observed on days 2 through 7 while FTY720 concentration was increasing by approximately 80% per day.

Cold Pressor Test

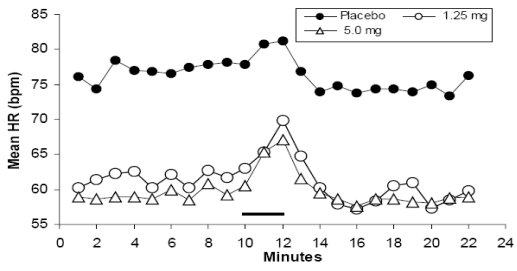
The Cold Pressor Test is a commonly used tool to evaluate para-sympathetic vagal function. This test has three segments. Resting heart rate is collected for a 10 minute interval. Subjects then immerse their hand in an ice bath for 2 minutes. Following immersion subjects remained in a resting state while heart rate is collected over the next 10 minutes. Three features of this test are shown in the following figures:

Figure 7.5-13 Day -2: Mean heart rate during Cold Pressor Test



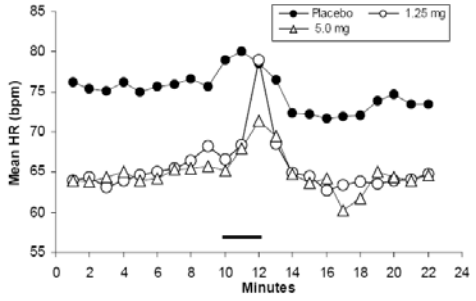
Note: Hand is immersed in ice bath during minutes 10-12.

Figure 7.5-14 Day 2: Mean heart rate during Cold Pressor Test



Note: Hand is immersed in ice bath during minutes 10-12.

Figure 7.5-16 Day 6: Mean heart rate during Cold Pressor Test



Note: Hand is immersed in ice bath during minutes 10-12

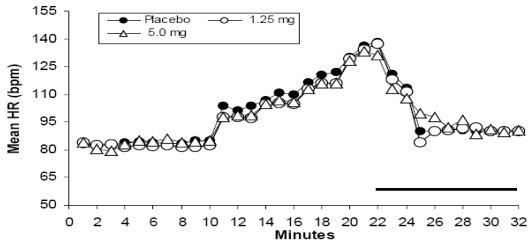
- All subjects demonstrated a rapid increase (3-8 beats/minute) in resting heart rate upon cold water bath hand immersion (Day –2). Once the external stimuli was removed, a sudden decrease in heart rate (~11 beats/min) was observed within 2 minutes and this was consistent with a vagal response. Heart rate gradually increased over the last 8 minutes of this test, however the final measurement was slightly lower than the resting rate (~2 beats/min)
- FTY720 dependent reduction in resting heart rate was not additive or synergistic with vagal stimulation. Although resting heart rate was reduced following FTY720 treatment, the same pattern was observed in all three treatment groups.

- There appeared to be a learning phenomenon during the repeated tests. The greatest increase of heart rate upon ice bath immersion was observed on Day –2, when the test was administered for the first time. This effect was blunted on Days 2-6 , regardless of treatment.

Exercise Stress Test

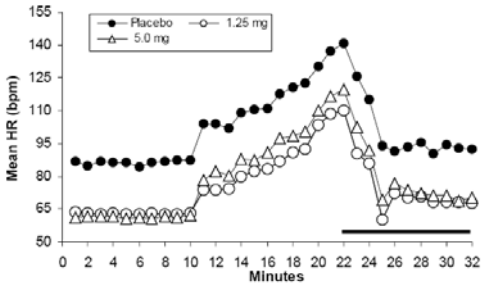
The Modified Bruce Protocol was used to evaluate cardiac response to sympathetic stimuli. Resting heart rate was collected for 10 minutes prior to exercise induction. Exercise intensity was increased every 3 minutes over the following 12 minute. Subjects were allowed to cool down over the next 2 minutes and heart rate continued to be monitored during the last 8 minutes. Two features were shown in the following figures:

Figure 7.5-17 Day -2: Mean heart rate during Exercise Stress Test



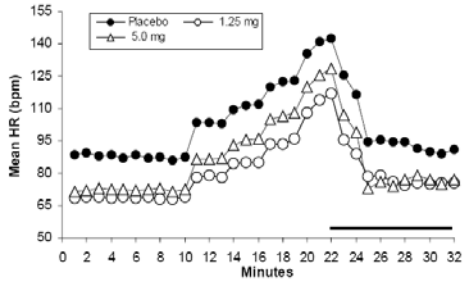
Recovery period indicated by horizontal line

Figure 7.5-18 Day 2: Mean heart rate during Exercise Stress Test



Recovery period indicated by horizontal line

Figure 7.5-20 Day 6: Mean heart rate during Exercise Stress Test



Recovery period indicated by horizontal line

- All subjects had a gradual increase in heart rate during exercise. The greatest increase was observed during minutes 20-22 and was ~ 50 bpm.
- FTY720 did not impair the cardiac response to external sympathetic stimuli. As observed previously, the resting heart rate was reduced in both FTY720 groups. This reduction in heart rate did not translate to a reduction of sympathetic response. In fact, both the 1.25 and 5 mg FTY720 treatment groups displayed a significantly greater percent change (77% and 94%) in HR (pre-test HR vs max HR) when compared to baseline (62%). This significant increase in HR is also observed on Days 4 and 6 of 5 mg FTY720 administration. All treatment groups generally had a reproducible 45-60 bpm mean increase on each test day.

FTY720 Effect on Lymphocyte Count

- In FTY720 groups, mean lymphocyte counts were decreased at the first post dose sampling on day 2 to 0.8 and 0.4 x 10^9/L in the low and high dose groups, respectively.

- The The model estimated a E0 of 1.76 x 10^9/L (CV, 4.0%) with a Emax by 1.61 x 10^9/L (CV, 93.4%) to an estimated minimal count on day 2 of 0.15 x 10^9/L. The AUC(0-24) yielding a half-maximal inhibitory effect was 13.5 ng·h/ml (CV, 35.0%).
- The absolute lymphocyte nadir occurred between days 3 to 7 . In 1.25 mg and 5mg gruops, the nadir correspondedto an 80 and 88 percent decrease from baseline, respectively.
- A gradual lymphocyte recovery was evident during the follow-up period in days 9 to 35. The recovery was faster at the lower compared with the higher dose group.
- At the end-of-study visit, mean absolute lymphocyte counts were 74 percent of baseline in the low-dose group and 47 percent of baseline in the high-dose group.

Conclusions:

- FTY720 resulted in an average decrease in heart rate of approximately 10 bpm. This decrease occurred within several hours of the first dose of FTY720 and then persisted for the remainder of the 7 day dosing interval. After day 1, additional daily doses of FTY720 did not result in an incremental decrease in heart rate.
- The mean ECG QRS interval did not increase in subjects on FTY720 treatment compared to placebo. Consistent with a reduction in heart rate there was a significant increase in ECG RR interval. ECG PR interval was increased on Day 1 of FTY720 treatment.
- There was no consistent effect of FTY720 on QT interval. QTcF was significantly increased, however QTcB was significantly decreased. No subject presented an interval greater than 450 ms (male) or 470 ms (female) or a QTc change from baseline > 60 ms.
- FTY720 treatment was not associated with an increase rate of atrial fibrillation or ventricular ectopy. A higher rate of atrial ectopy was detected in FTY treatment groups on day 1 of FTY720 dosing, however this treatment effect appeared to attenuate by day 7 of treatment.
- The rate of low grade sinus pauses (2-3 sec) increased on day 1 of FTY720 dosing. No sinus pauses were detectable in FTY720 treatment groups on day 7.
- Asymptomatic and transient second degree type 1 atrio-ventricular block (Wenckebach) was detected in three FTY720 treated subjects and three placebo treated subjects.
- FTY720 treatment was associated with 5-12 mm Hgdecrease in blood pressure on day 1. Over the remaining six day course of the study this difference in blood pressure between placebo and FTY720 treated groups diminished to approximately 2-3 mm Hg.
- Systemic exposure to FTY720 was consistent with dose-proportionality, exhibiting a four-fold difference between dose groups and a five-fold accumulation over the 7-day treatment period.
- Nadir lymphocyte counts were decreased by 80% and 88% from baseline in the low-dose and highdose groups, respectively.

Study FTY720A 2213-01*:* **A double blind, parallel group, placebo controlled, multiple dose study to evaluate the effects of FTY720 (1.25 and 5 mg) on cardiac rate and rhythm**

Evaluation of sphingosine-1-phosphate plasma levels

In study 2213, the sponsor analyzed the natural ligand of the receptor, S1P, after single and multiple dosing of FTY720. This supplement to the clinical study report presents the S1P data, its evaluation, and the interpretation.

A brief overview of some essential components of the study design is given below:

Sampling: Plasma	For sphingosine-1- phosphate: Day –2: pre-placebo dose Day 1: 6 hr post FTY720/Placebo dose Days 2 and 7: pre dose.
Analysis (Plasma)	Method HPLC Lower Limits of Quantitation Plasma Sphingosine-1-phosphate 40 ng/mL

Results:

Sphingosine-1-phosphate exposure:

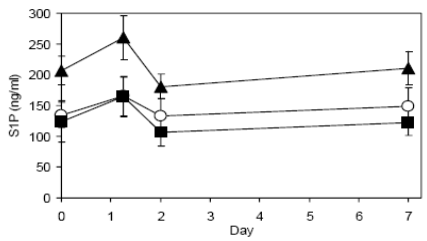
Table 3-1 Sphingosine-1-phosphate concentrations

Treatment	Baseline	Dose 1 6h postdose	Dose 1 24h postdose	Dose 7 predose
Placebo (*n* = 20)	135 ± 44	165 ± 68	133 ± 62	148 ± 66
FTY 1.25mg/day (*n* = 20)	124 ± 72	164 ± 69	107 ± 48	122 ± 45
FTY 5 mg/day (*n* = 20)	207 ± 49	260 ± 77	181 ± 44	211 ± 58

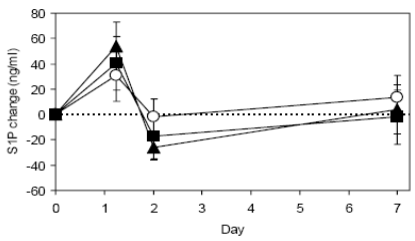
Values are mean ± standard deviation in ng/ml.
Source data: Post-text Table 1.

Sphingosine-1-phosphate (S1P) data are plotted in the following figure:

Figure 3-1 Sphingosine-1-phosphate concentration profiles



Mean sphingosine-1-phosphate (S1P) concentration profiles in subjects receiving placebo (*open circles*), FTY720 1.25 mg/day (*squares*), and FTY720 5 mg/day (*triangles*). *Bars* represent the 95% confidence intervals.



As in the left panel for change from baseline in S1P. Synoptic views of individual profiles are in Post-text Figure 1.

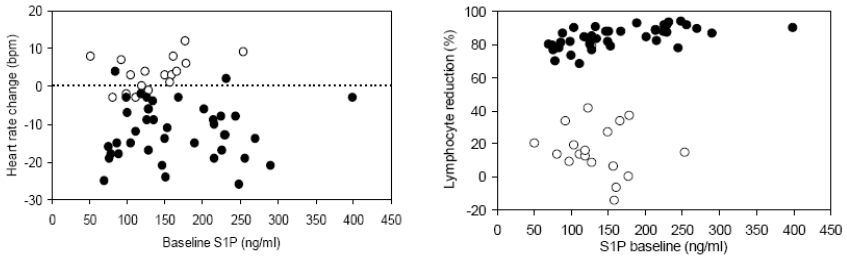
- Based on the placebo, there appeared to be a circadian pattern whereby concentrations rose about 20% in the afternoon (6 h postdose or about 13:00) compared with the morning value (predose or about 07:00).
- The morning concentration appeared consistent across days when comparing the baseline, day 2, and day 7 values in placebo-treated subjects.

- After subtracting the baseline value from post-baseline concentrations, sphingosine-1-phosphate concentrations and their temporal patterns were similar across treatments.
- The increases from baseline were 31 ± 44 ng/ml (placebo), 40 ± 45 ng/ml (FTY720 1.25 mg/day), and 54 ± 41 ng/ml (FTY720 5 mg/daywhere the change from baseline increased numerically with increasing dose (0, 1.25, 5 mg/day); however, the intersubject variability was very high and the values were not significantly different among treatments (p = 0.254).

Sphingosine-1-phosphate relationships to other parameters:

The relationship between heart rate change and lymphocyte reduction versus S1P baseline are plotted in the figures below:

Figure 3-2 Sphingosine-1-phosphate relationship to heart rate and lymphocyte reduction



Relationship between baseline S1P concentrations and change in heart rate on day 1 after the first dose of placebo (*open circles*) or FTY720 (*filled circles*).

As in left panel for lymphocyte reduction from baseline.

- The baseline sphingosine-1-phosphate concentration did not appear predictive of the change in heart rate from baseline to the 4-hour nadir on day 1 after the first dose of medication in either the placebo or FTY720 treatment groups.
- Likewise, there was no apparent relationship between these concentrations and the percent reduction in lymphocytes from baseline to nadir.

Conclusions:

- S1P was quantifiable in plasma in subjects receiving placebo and FTY720 and appeared to rise during the daytime perhaps due to a circadian rhythm in its plasma profile.
- The presence of FTY720 in vivo did not appear to influence S1Pplasma levels.
- There was no apparent relationship between baseline S1P plasma concentrations and the effect of FTY720 on heart rate or lymphocytes.

Study FTY720A 2213E1*:* **A single center, single blinded, multiple dose study to evaluate the effects of a desensitizing dosing regimen of FTY720 on cardiac heart rate in healthy volunteers**

PD, whether a low dose of FTY720 preceding a challenge dose could desensitize the subject to the decrease in heart rate: MD FTY720

A brief overview of some essential components of the study design is given below:

Study Design	randomized, single-blind, placebo-controlled, adaptive-design study
Study Population	N=19 enrolled, 17 completed Age: 19-45 years (mean 30.1 years) Gender: 12 males, 7 females Weight: 58.1-92.5 kg (mean 70.8 kg) Race: 12 Whites, 1 black, 3 Asians and 3 other ethnicity
Dosage and Administration	Day -1 was a run-in day in which subjects received placebo with 24-hour cardiac monitoring via telemetry. On day 1, subjects received a 0.5-mg FTY720 (n = 12) or placebo (n = 6) desensitizing dose at 0 hours, placebo (n = 18) at 3 hours, and a 5-mg FTY720 challenge dose (n = 18) at 6 hours. Study medication was administered with 240 ml of water. A light breakfast was provided 1 hour after the morning dose. Lot No. FTY720 0.5 mg capsules: Batch H-05930 FTY720 2.5 mg capsules: Batch 01-137US Placebo capsules: Batch H-05666 Diet: Subjects had a fluid intake of at least 200 ml every 4 hours during waking hours in addition to fluid taken with meals. Xanthine-containing beverages or foods was permitted but had to be noted on the CRF. Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood	For FTY720 (Blood): Day 1: predose (desensitization dose), 1, 2, 4, 6, 7, 8, 10, 12, 24, 72, and 144 h postdose.
PD measurements	Telemetry Before the first placebo dose on day −1 through 30 hours after the desensitization dose on day 1 for a total of 54 hours Lymphocyte Baseline on day –2; On day −1: at 0, 6 and 12 hours postdose; On day 1: at 0, 6, and 12 hours postdose; and On days 4 and 7.

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720: Linear range : 0.08-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 3.3% Inter-day accuracy: < 5.3 %
PK Assessment	FTY720 in blood: Cmax, Tmax, AUC0-t, Ct
PD Assessment	Heart rate by telemetry, total lymphocyte counts in blood. Response parameters were predose value, nadir value, and AUE(0-t).
Safety Assessment	Assessment of Physical examinations, ECGs, vital signs, standard clinical laboratory evaluations (biochemistry, hematology, urinalysis) and adverse event monitoring

Results:

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 after challenge dose are summarized below:

Table 7-2 FTY720 pharmacokinetic parameters after challenge dose

Parameter	Placebo desensitization	Active desensitization
$C(6)_b$ (ng/ml)	0	0.3 ± 0.1
tmax (h)	12	12
$C_{max,b}$ (ng/ml)	2.4 ± 1.0	2.3 ± 1.1
$AUC(6\text{-}12)_b$ (ng.h/ml)	5.7 ± 3.2	6.1 ± 3.0

Data are mean ± sd except for tmax which is median.
Time refers to hours after administration of the desensitizing dose (0h).
Source data: Appendix 4, Table 1 and Table 2.

- Regardless of pretreatment, both groups of study subjects had similar FTY720 exposure after the 5-mg challenge dose.

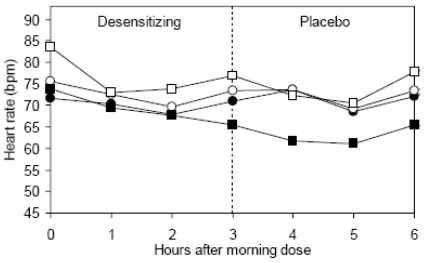
Pharmacodynamics Results:

Heart rate circadian rhythm in the absence of drug

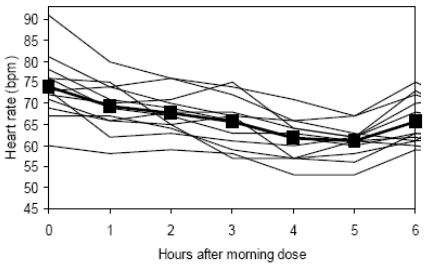
- The normal circadian rhythm in heart rate over 24 hours in the absence of drug is apparent and highly consistent among subjects.
- Mean heart rate undergoes four dips with nadirs at 2h, 5h, 11h, and 20h.

Heart rate response to the desensitizing dose (0-6 hours)

Figure 7-3 Desensitizing dose: heart rate response patterns



Mean heart rate trajectories from 07:00 to 13:00 on day -1 (*circles*) and day 1 (*squares*). On day 1 subjects received either a placebo dose (*open symbols*) or and active desensitizing dose (*filled symbols*) at 0h (07:00) and a placebo at 3h (10:00).

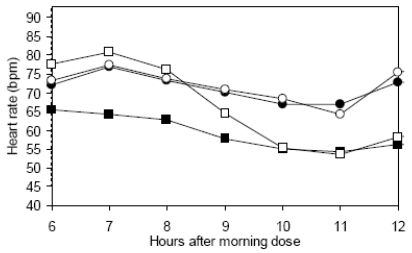


Synoptic view of individual trajectories in subjects receiving an active desensitizing dose on day 1. The mean trajectory (*filled squares*) is identical to that in the left panel.

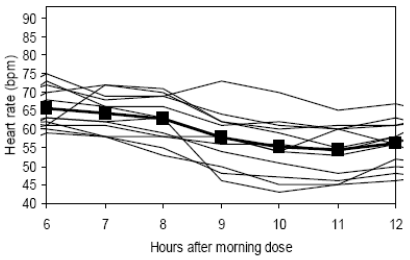
- The circadian rhythm in heart rate in the morning between 0 and 6 hours was consistent on days -1 and 1 in placebo desensitizing dose.
- In the active desensitizing dose group, the mean morning nadir was 8% lower than the nadir on day -1 ($p = 0.027$). The overall heart rate response over the 6-hour period as captured in the AUE(0-6) was reduced by 7% from that on day -1 ($p = 0.006$).

Heart rate response to the challenge dose (6-12 hours)

Figure 7-4 Challenge dose: heart rate response patterns



Mean heart rate trajectories from 6-12h (13:00-19:00) on day -1 (*circles*) and day 1 (*squares*). On day 1 subjects received at 0-3-6h either a placebo-placebo-active dose regimen (*open symbols*) or an active-placebo-active dose regimen (*filled symbols*).

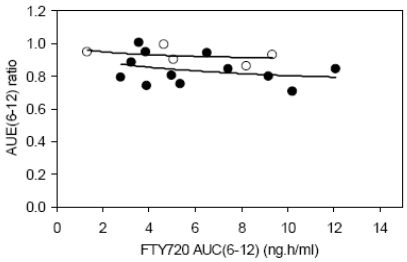


Synoptic view of individual trajectories in subjects receiving an active-placebo-active dose regimen on day 1. The mean trajectory (*filled squares*) is identical to that in the left panel.

- Both groups reached a similar mean heart rate of 53-54 bpm at 11h.
- In both groups, the nadir was 17% lower than the nadir over this period on day -1 ($p < 0.001$ for both).
- The corresponding AUE(6-12) was 17% reduced in the active desensitizing dose group ($p < 0.001$) and 7% reduced for the placebo desensitizing dose group ($p = 0.025$) compared with day -1.

Challenge dose: exposure-response

Figure 7-5 Challenge dose: exposure-response patterns

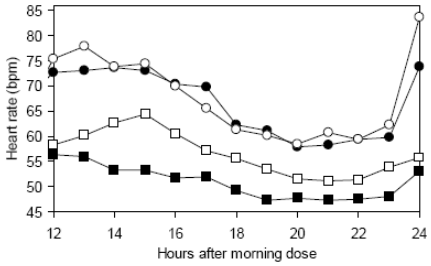


Scatterplot of FTY720 AUC(6-12) versus the day1/day−1 ratio of the area under the heart rate effect curve AUE(6-12) after the challenge dose. The lines are the best fit of a logarithmic function through the active desensitizing data (*filled circles*) and the placebo desensitizing data (*open circles*).

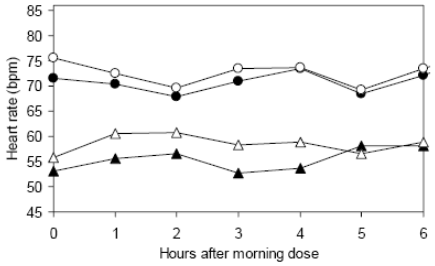
- The time-integrated heart rate response, AUE(6-12), was independent of concurrent systemic exposure to FTY720 over the range achieved in this study and was similar regardless if a subject received a desensitizing dose or not. Hence, the desensitizing dose did not attenuate the heart rate response to the challenge dose.

Postchallenge heart rate response (12-30 hours)

Figure 7-6 Night and day 2: heart rate response patterns



Mean heart rate trajectories from 12-24h (19:00-07:00) on day -1 (*circles*) and day 1 (*squares*). On day 1 subjects received at 0-3-6h either a placebo-placebo-active dose regimen (*open symbols*) or an active-placebo-active dose regimen (*filled symbols*).

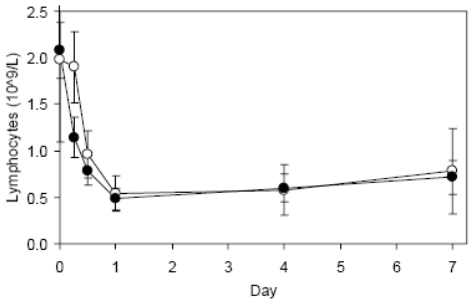


Mean heart rate trajectories from 0-6h (07:00-13:00) on day -1 (*circles*) and day 2 (*triangles*). On day 1 subjects received at 0-3-6h either a placebo-placebo-active dose regimen (*open symbols*) or an active-placebo-active dose regimen (*filled symbols*).

- The mean nadir on day 1 compared with day -1 was 14% and 21% lower in the placebo and active desensitizing groups, respectively.
- Reductions in the day 1 to day -1 nighttime AUE(12-24) were 18% and 23%, respectively.
- In the morning of day 2, heart rate patterns were nearly superimposible in the two treatment groups. Nadirs were 56 ± 7 versus 51 ± 7 bpm and AUE(24-30) were 352 ± 39 versus 332 ± 37 bpm.h for placebo versus active desensitizing dose groups, respectively.
- At the end-of-study visit, the morning supine heart rate was still depressed from baseline but recovering: 63 ± 5 and 61 ± 6 bpm in the placebo and active desensitizing dose groups, respectively

Lymphocyte responses

Figure 7-7 Lymphocyte response trajectories



Mean lymphocyte trajectories from day 1 to 7 in subjects receiving a placebo (*open circles*) or active (*filled circles*) desensitizing dose followed by an active challenge dose 6 hours later. *Bars* represent 95% confidence intervals. Synoptic views of individual trajectories are in Appendix 4, Figure 3.

- At 6 hours after the dose, counts remained stable at 1.9 x 10^9/L in placebo group and decreased to 1.1 x 109/L in 0.5 mg FTY720.
- The nadir count occurred in nearly all subjects at 24 hours reaching 0.5 ± 0.1 x 10^9/L in both groups.
- The corresponding AUE(0-7) was 4.9 ± 1.4 and 4.6 ± 1.5 cells x 10^9/L per day in the placebo and active desensitizing groups, respectively.
- At the end-of-study evaluation on day 7 mean lymphocyte counts had recovered slightly from nadir and were about 0.7 x 10^9/L in both groups.

Conclusions:

- Administering a 0.5-mg FTY720 dose six hours before a 5-mg challenge dose did not desensitize healthy subjects to the decrease in heart rate which normally occurs after starting treatment with FTY720.

Study FTY720A 2305*:* **Study to explore the effect of various intertreatment intervals on the heart rate response to FTY720**

PD. To address the concern regarding the potential effect of FTY720 on heart rate if patients discontinued use of FTY720 for a certain period of time and restarted FTY720. This study investigated the time when patients would again become vulnerable to the first-dose decrease in heart rate after restarting FTY720.

A brief overview of some essential components of the study design is given below:

Study Design	2 treatment groups: Group 1 (FTY720 5 mg) and Group 2 (FTY720 2.5 mg) contained 2 separate cohorts and used a 2-phase approach
Study Population	N=26 enrolled, 24 completed Age: 18-45 years (mean 28.5 years) Gender: 17 males, 9 females Weight: 54.2-88.4 kg (mean 69.9 kg) Race: 3 Caucasians, 18 blacks, 5 other ethnicity
Dosage and Administration	Group 1/Cohort 1=6; Group 1/Cohort 2=7; Group 2/Cohort 1=6; Group 2/Cohort 2=7 **Table 2 Study treatment assignment for PK/PD analysis** Clinical group | Clinical cohort | Bioanalysis assignment | Challenge dose (mg) | Loading regimen (mg/day) D1 D2 D3 D4 D5 D6 D7 | Intertreatment interval | Rechallenge dose (mg) 1 1 A 5 10 15 20 5 5 5 1 month 5 1 2 B 5 10 15 20 5 5 5 2 months 5 2 1 D 2.5 10 12.5 2.5 2.5 2.5 2.5 1 week 2.5 2 2 E 2.5 10 12.5 2.5 2.5 2.5 2.5 2 weeks 2.5 Loading regimen: D=day Study medication was administered with 240 ml of water. A light breakfast was provided 1 hour after the morning dose. Lot No. FTY720 2.5 mg capsules: batch US03082, control number 04-0475CH Placebo capsules: batch US03096, control number 04-0475CH Diet: Subjects had a fluid intake of at least 200 ml every 4 hours during waking hours in addition to fluid taken with meals. Xanthine-containing beverages or foods was permitted but had to be noted on the CRF. Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood/plasma	For FTY720 and FTY720-P (Blood): Day 1: predose (desensitization dose), 1, 2, 4, 6, 8, 12, 16 and 24 h postdose. Day 2-7: predose and 12h postdose Day 7: 24h postdose on Day 7 (Day 8 overall)
PD measurements	Telemetry In Phase 1 only, 6-hour continuous telemetry monitoring of heart rate was performed from 0 to 6 hours post-FTY720 dose on Day 2, Day 3, and Day 4. Holter monitoring On the placebo run-in day and the first FTY720 dosing day of each phase, 24-hour continuous ECG data was captured via a digital Holter monitor. Lymphocyte

	At screening, the initial baseline (Day -2), Day 1 (6 hours postdose), Days 3, 5, and 7 (all predose).
Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 8.0% Inter-day accuracy: < 8.8 % FTY720-P: Linear range : 1-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 5.7% Inter-day accuracy: < 5.0 %
PK Assessment	FTY720 in blood: For Period 1 and 2 on Day 1: Tmax, Cmax, AUC0-24hr, AUC0-12hr. For Period 1 only: Accumulation Ratio as determined by C(0hr) on Day 7 divided by C(24hr) on Day 1.
PD Assessment	Emax(0-12), Tmin, E4, AUCHR0-24hr, AUCHR0-12hr
Safety Assessment	Assessment of physical examinations, laboratory parameters, vital signs, telemetry, Holter monitoring, electrocardiograms, information on adverse events

Results:

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 and FTY720-P are summarized in the following table:

Table 7-6 Mean (SD) (except Tmax: median [minimum-maximum]) pharmacokinetic parameters of FTY720 and FTY720-P

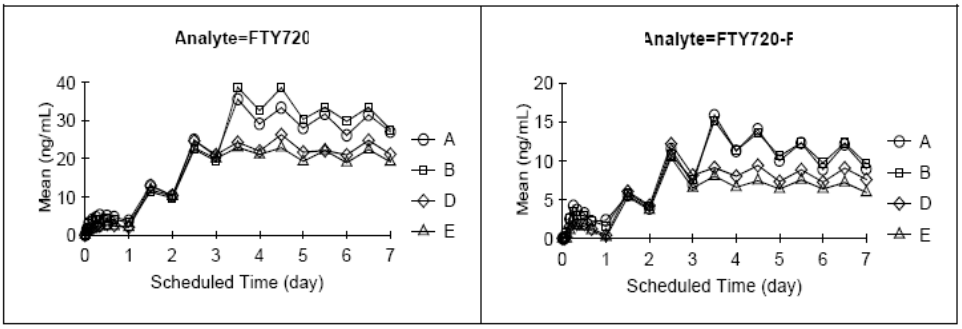
	Cohort 1				Cohort 2			
	Group A		Group B		Group D		Group E	
	FTY720	FTY720-P	FTY720	FTY720-P	FTY720	FTY720-P	FTY720	FTY720-P
Phase 1								
T_{max}	8 (4-12)	6 (6-24)	10 (4-12)	6 (6-12)	12 (12-12)	6 (6-6)	12 (8-12)	6 (6-12)
C_{max}	5.34±0.67	4.65±0.99	4.52±0.21	3.58±0.73	2.82±0.40	2.44±0.49	2.54±0.39	1.80±0.18
AUC0-24hr	102±13.1	60.0±9.0	87.8±8.0	53.1±11.0	53.2±8.6	30.3±9.3	48.4±8.5	23.4±6.2
AUC0-12hr	49.4±6.0	31.6±4.2	42.3±4.3	27.9±7.4	24.2±3.7	17.6±3.5	21.6±3.5	13.5±2.3
C24	3.75±0.58	2.32±1.97	3.41±0.27	1.60±0.25	2.10±0.42	0.42±0.65	2.06±0.53	0.183±0.449

Steady State								
C0	26.1±3.3	8.91±0.87	29.8±8.2	9.74±1.95	21.0±5.7	7.26±2.40	19.0±4.3	6.38±1.24
C24	27.0±4.0	8.96±0.42	27.6±5.8	9.61±1.58	21.2±6.0	7.60±2.96	19.2±4.4	5.93±1.42
Accum. Ratio	7.24±0.77	5.22±1.96	8.10±1.41	6.03±0.79	9.96±1.60	8.21±1.26	9.39±0.58	6.16 (N=1)
Phase 2								
C0	1.40±0.93	0.000	0.421±0.37	0.000	9.20±3.33	3.35±1.26	5.03±2.85	1.37±0.83
T_{max}	12 (4-16)	6 (6-6)	10 (6-16)	6 (6-12)	10 (6-12)	6 (4-6)	10 (4-24)	6 (6-6)
C_{max}	6.26±1.10	4.37±0.83	4.80±0.53	3.93±1.15	13.6±4.6	6.10±1.76	7.88±3.35	3.59±0.88
AUC0-24hr	126±22	66.8±10.3	96.0±14.9	56.0±17.6	293±97	120±39	164±70	62.8±22.5
AUC0-12hr	60.1±10.2	35.4±6.0	43.9±8.2	27.9±10.1	147±49	63.0±19.6	81.2±36.4	33.3±10.6
C24	5.07±0.90	2.05±0.27	4.00±0.56	1.74±0.53	11.1±4.0	3.99±1.51	6.42±2.42	1.91±1.07

Units: Tmax in hr, Concentrations in ng/mL, AUC in h*ng/mL, Accumulation Ratio = C0,day 7/C24,day 1 of Period 1, Steady State: Day 7

- In Phase 2, the predose levels of FTY720 in Groups A, B, D and E averaged 1.4 ng/mL, 0.4 ng/mL, 9.2 ng/mL, and 5.0 ng/mL, respectively.
- For FTY720-P, they were below the quantification limit in Groups A and B, and averaged 3.3 ng/mL in Group D and 1.4 ng/mL in Group E.
- Because of these residual predose concentrations, the mean total exposure to both analytes over the dose interval (AUC0-24hr) in Groups A, D, and E was about 1.2-fold, 5.4-fold, and 3.3-fold higher than after the first dose in Phase 1, respectively.
- For Group B, due to the longer washout period, exposure to both analytes was similar in the 2 study phases.

Figure 7-3 Mean concentrations of FTY720 and FTY720-P during Phase 1, Days 1-7



- In both cohorts, the loading regimen tended to overshoot steady-state exposure: In Cohort 1, FTY720 mean C0 and C12 concentrations declined during the maintenance dosing from Day 5 to 7. On day 7 mean C0 and C24 were similar and represented an 8-fold accumulation in blood levels from Day 1.
- FTY720-P concentrations followed a similar temporal pattern with a 6-fold accumulation. Day 7 C0 for FTY720 was about 27 ng/ml and for FTY720-P it was about 9 ng/ml.
- In Cohort 2, FTY720 mean C0 and C12 concentrations declined during the maintenance dosing from Day 4 to 7. On Day 7 mean C0 and C24 were similar and represented a 10-fold accumulation in blood levels from Day 1.
- FTY720-P followed a similar temporal pattern with a 6- to 8-fold accumulation. Day 7 C0 for FTY720 was about 20 ng/ml and for FTY720-P it was about 7 ng/ml.

Pharmacodynamics Results:

Heart rate circadian rhythm in the absence of drug

The mean heart rates collected in hourly intervals over 24 hours on the days prior to dosing and after dosing in Phase (period) 1 and 2 of the study are shown below:

Figure 7-4 Mean hourly heart rates during 24 hours pre and post first dose in Phase 1 and 2

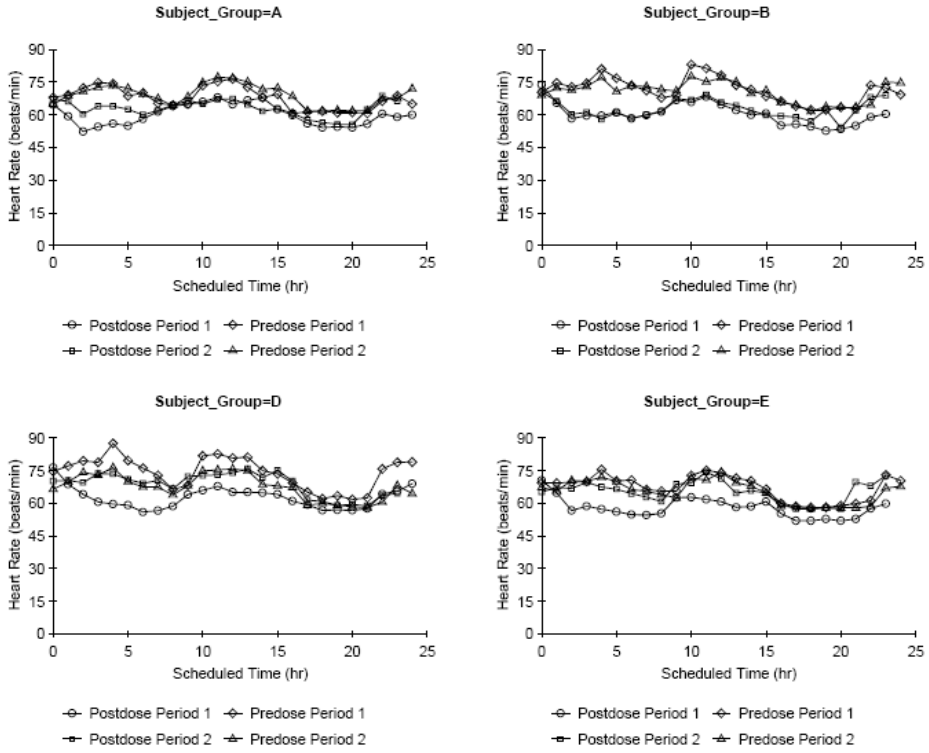
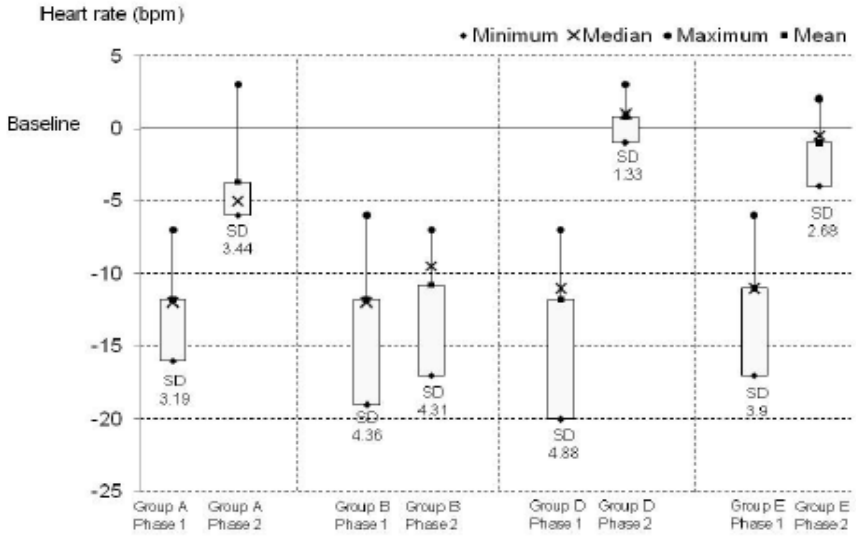


Figure 7-5 Change from Baseline in Emax(0-12)(bpm)



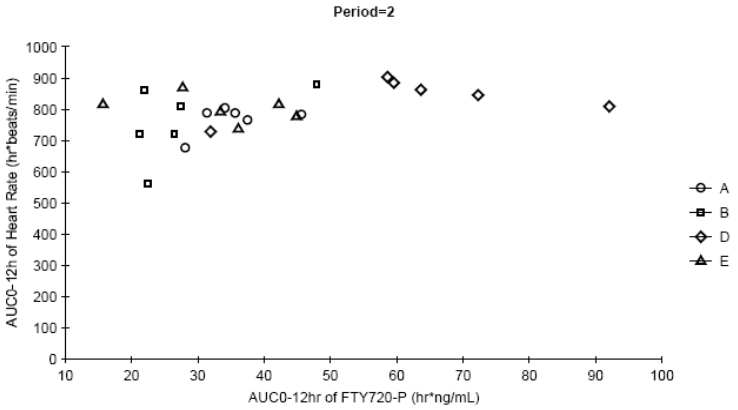
- The longer the washout period between the study phases the greater is the effect of a rechallenge with FTY720 on negative chronotropy. Group B had the longest washout period of 2 months and their heart rate in Phase 2 dropped to a similar level as after the first dose in Phase 1, whilst Groups D and E with a 1- or 2-week interval showed

almost similar heart rates upon rechallenge in Phase 2 compared to Phase 1. Group A with a 1-month washout is somewhat intermediate between the other groups.

- These data suggest that a period of 2 months is sufficient to completely wash out the attenuation of the effect of FTY720/FTY720-P on negative chronotropy upon multiple dosing (i.e., the subjects returned to a FTY720 "naïve" status in this respect). However, after a washout of 1 to 2 weeks the effect is still attenuated and a rechallenge does not lead to a pronounced negative chronotropy.

Pharmacokinetic-pharmacodynamic results

Figure 7-7 **AUC of FTY720-P versus AUC of heart rate from 0 to 12 hours in Phase 2**



- The sponsor stated that subjects with higher exposure to FTY720-P tended to have a higher AUC for heart rate.

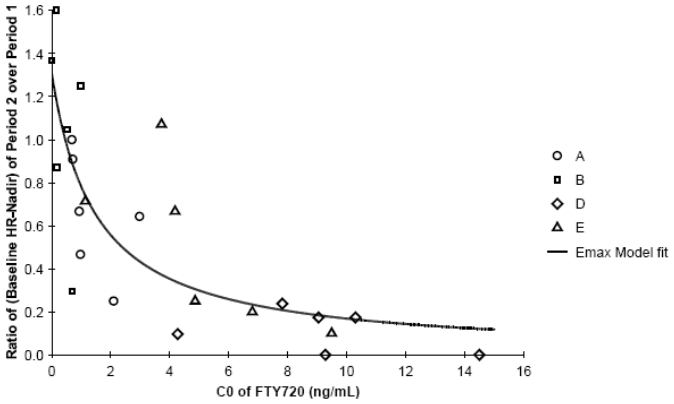
Reviewer's note:
The sponsor's statement is not a clear finding. Although there is a minor trend, the data is not sufficient to make the conclusion.

An inhibitory Emax model was fitted and the estimated parameters are shown below:

Table 7-7 **Parameters of Emax model fitted to Phase 2 predose levels of FTY720 and the ratio of the maximum change in heart rate in Phase 2 over Phase 1**

Parameter	Estimate	CV (%)
Emax	1.31	14.0
EC50 (ng/mL)	1.48	40.1

Figure 7-8 **Individual predose concentration of FTY720 in Phase 2 versus ratio of the baseline heart rate minus nadir heart rate in Period 2 over Period 1**



- The group with the longest washout interval (Group B) had the lowest predose FTY720 levels and also demonstrated the largest change in heart rate.
- Subjects with considerable carryover into Phase 2 were not sensitive to a rechallenge.
- The inhibitory Emax model suggested that if the predose FTY720 concentration from previous treatments had dropped to about 1.5 ng/mL, half of the maximum effect on negative chronotropy will be observed.

Conclusions:

- High-dose loading regimens of FTY720 can be used to expedite steady-state blood levels.
- In Group 2 (FTY720 2.5 mg), FTY720 rechallenge did not affect the mean nadir heart rate after either a 7 or 14 day interdosing interval. This provides evidence that patients who stop taking FTY720 2.5 mg at steady state can restart FTY720 after a 1- to 2-week period without any effect on heart rate.
- In Group 1 (FTY720 5.0 mg), approximately one-half of the acute dynamic effect of FTY720 is present after a 1-month interruption in treatment. The full acute dynamic effect on heart rate is present after a 2-month interruption. That is, the longer the washout period between FTY720 doses, the greater the chance that FTY720 rechallenge may cause a negative effect on heart rate.
- Using an inhibitory Emax model of the relationship of systemic FTY720 concentration and negative chronotropic effect of FTY720 on rechallenge, FTY720 concentrations of 1.5 ng/mL or greater at the time of a rechallenge dose of FTY720 provide protection against clinically significant negative chronotropic effect (i.e., a heart rate decrease <2-3 bpm).

Study FTY720A 2306*:* **A randomized, double-blind, placebo-controlled, timelagged, ascending, multiple-dose, pharmacokinetic, pharmacodynamic, safety and tolerability study of FTY720 in healthy volunteers**

PK, PD: MD FTY720. The study intended to assess dose levels of 5, 10, and 20 mg given once-daily for 14 days but the study was terminated after cohort 1 (5 mg) due to elevated hepatic enzymes.

A brief overview of some essential components of the study design is given below:

Study Design	randomized, double-blind, placebo-controlled, time-lagged, ascending, multipledose study
Study Population	N=12 Age: 20-48 years (mean 35.1 years) Gender: 5 males, 7 females Weight: 60.8-85.7 kg (mean 72.1 kg) Race: 12 Whites
Dosage and Administration	12 subjects were enrolled (9 to receive FTY720 and 3 to receive placebo). Baseline assessments were made on day -2. On run-in day -1, all subjects received a single dose of placebo followed by pretreatment Holter monitoring and exercise oximetry testing. The multiple-dose treatment period with FTY720 or placebo was from days 1 through 14. Study medication was taken with 240 ml water following a light breakfast and fast for at least 4 hours thereafter. FTY720 5 mg (FMI) capsules: batch US03085, package control number 04-1455CH. Placebo: batch AEUS/2004-0219, package control number 04-07540US. Diet: During waking hours, subjects were required to have a fluid intake of at least 200 ml every 4 hours in addition to fluid taken with the medication. Xanthine-containing beverages were to be discontinued 48 hours before dosing of study drug(s) Alcohol was prohibited for 72 hours prior to dosing until study completion.
Sampling: Blood	For FTY720 and FTY720-P (Blood): Predose, and then 12, 16, 24 hours postdose on day 1 for first-dose exposure; predose on days 3, 4, 5, and 7 for drug accumulation; predose and then 1, 2, 4, 6, 8, 12, 16, 24 hours postdose on day 14 for last-dose exposure; and at the clinic visits on days 21, 28, 35, 42, 56, 70, 84 for drug washout.

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.08 ng/mL FTY720-P 1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 9.9% Inter-day accuracy: < 12.5 % FTY720-P: Linear range : 1-50 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 8.7% Inter-day accuracy: < 9.9 %
PK Assessment	FTY720 and FTY720-P in blood: Cmax,b, Tmax, AUCτ,b, Cave, PTF, R, t1/2
PD Assessment	Holter monitoring, telemetry, echocardiography, standard pulmonary function tests, methacoline challenge test, exercise oximetry, Mini-Mental State Examination, and visual-evoked potential studies
Safety Assessment	Assessment of physical examinations standard clinical laboratory parameters, blood lymphocyte and lymphocyte subset counts, Epstein-Bar viral load, vital signs, and ECG recordings.

Results:

Pharmacokinetics Results:

First-dose exposure and accumulation

Mean FTY720 and FTY720-P PK parameters on day 1 and day 14 are summarized below:

Table 7-2 Pharmacokinetic parameters

Parameter	Fingolimod		Fingolimod-phosphate	
	Day 1	Day 14	Day 1	Day 14
$C0_b$ (ng/ml)	0	17.3 ± 6.7	0	8.6 ± 2.8
t_{max} (h)	12 (12-16)	6 (4-12)	12 (12-16)	6 (4-16)
$C_{max,b}$ (ng/ml)	3.6 ± 0.6	22.5 ± 8.5	2.9 ± 0.4	11.8 ± 3.4
$AUC_{\tau,b}$ (ng.h/ml)	59 ± 8	467 ± 163	48 ± 5	246 ± 73
$C_{avg,b}$ (ng/ml)	--	19.4 ± 6.8	--	10.2 ± 3.1
PTF (%)	--	27 ± 6	--	33 ± 9
Accumulation ratio	--	7.8 ± 2.1	--	5.1 ± 1.4
$t_{1/2}$ (days)	--	11.3 ± 3.6	--	7.2 ± 2.4

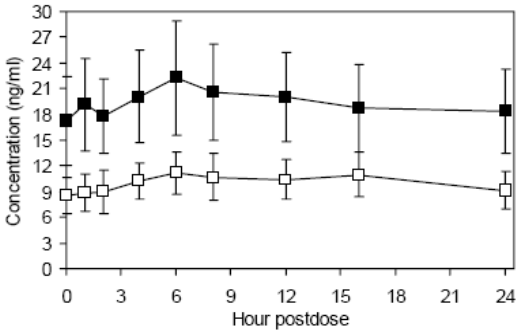
Values are mean ± sd except for tmax which is median (range).

- The mean exposure to fingolimod-P was around 19% lower than fingolimod on day 1and remained lower throughout the accumulation phase to day 14.
- On day 14 fingolimod-P mean predose C0b was half that of fingolimod.
- Over 14 days of dosing fingolimod blood levels accumulated nearly 8-fold and fingolimod-phosphate blood levels around 5-fold.

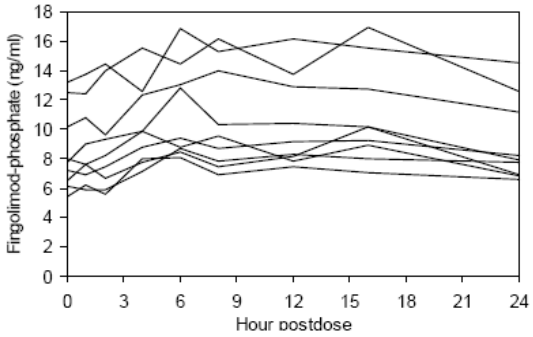
Last-dose exposure and washout

The mean concentration-time profiles of FTY720 and FTY720-P day 14 are shown in the following figure:

Figure 7-2 Drug exposure: last-dose



Mean concentrations of fingolimod (*filled squares*) and fingolimod-phosphate (*open squares*) on day 14. *Bars* represent 95% confidence intervals.



Synoptic view of individual fingolimod-phosphate profiles over the dosing interval on day 14.

- On day 14, peak-trough fluctuations were around 30 percent for both analytes.
- Interindividual variability for AUCτ,b was high with a coefficient of variation of 35 percent for fingolimod and 30 percent for fingolimod-P.
- The mean fingolimod-P AUCτ,b on day 14 was about half that of fingolimod.
- The corresponding molar ratio of fingolimod-P/fingolimod AUCτ on day 14 was 0.42 ± 0.03 with a range from 0.39 to 0.44with low interindividual variability (CV=7 %.
- Fingolimod concentrations on day 70 and 84 were near the assay quantification limit and, in some profiles, appeared flat over time departing from a log-linear decline. This may have biased the mean half-life estimate of 11 days downward compared to the value in earlier studies of 8 days.
- Fingolimod-P half-life estimate averaged 7 days.

<u>Pharmacodynamics Results:</u>

<u>Lymphocyte responses:</u> Absolute lymphocyte counts

- Counts fluctuated over time about 30% around the baseline in the placebo group without any temporal pattern over the study course.
- In the FTY720 treatment group a marked decrease in lymphocytes was apparent by the first postdose assessment on day 3 with near nadir counts.
- The nadir represented an average 90% decrease from predose and was maintained over the 14-day treatment period.
- At the first posttreatment assessment, 3 days after the last dose (day 17), counts were starting to recover and reached predose values generally around day 84.

Lymphocyte subsets

Table 7-4 Lymphocyte subsets in FTY720 treatment group

Subset	Descriptor	Predose	End of treatment	Recovery	
		Day 1	Day 14	Day 28	Day 112
CD3+	T-cell	1.77 ± 0.48	0.12 ± 0.06	0.31 ± 0.12	3.07 ± 2.51
CD3+ / CD16+	Natural killer cell	0.07 ± 0.10	0.01 ± 0.01	0.02 ± 0.02	0.13 ± 0.18
CD4+	T helper	1.11 ± 0.31	0.03 ± 0.02	0.14 ± 0.10	1.81 ± 1.34
CD4+ / CD45RA+	T naïve	0.60 ± 0.23	0.02 ± 0.02	0.07 ± 0.06	0.91 ± 0.58
CD4+ / CD45RO+	T memory	0.73 ± 0.30	0.02 ± 0.02	0.10 ± 0.07	1.41 ± 1.29
CD8+	T suppressor	0.67 ± 0.20	0.11 ± 0.06	0.18 ± 0.07	1.31 ± 1.26
CD14+	Monocyte	0.04 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.49 ± 1.45
CD20+	B-cell	0.30 ± 0.13	0.02 ± 0.01	0.05 ± 0.02	0.44 ± 0.22

Subset units are 10^9/L. Values are mean ± sd. Data source: Appendix 3 Table 3.3.8.1 and following.

- All subsets exhibited a decrease on days 14 and 28.
- The end-of-study values demonstrated recovery back to predose ranges.

Heart rate

- The daytime nadir was 2 hours postdose in placebo-treated subjects in accordance with normal circadian rhythm; whereas, the nadir was shifted to 7 hours postdose in FTY720-treated subjects due to the negative chronotropic effect of the study drug.
- The daytime area under the heart rate-time curve, AUE(0-12), was 25% lower under the influence of FTY720 compared with placebo.
- The first day absolute heart rate nadir occurred in the nighttime in both groups and was 24% lower for FTY720-treated subjects (ratio of geometric means, 0.81, p = 0.025).
- A partial recovery in morning heart rate was apparent by day 7 despite continued dosing of FTY720 through day 14.
- Mean morning heart rates were similar in both groups from day 28 onwards.

Echocardiography

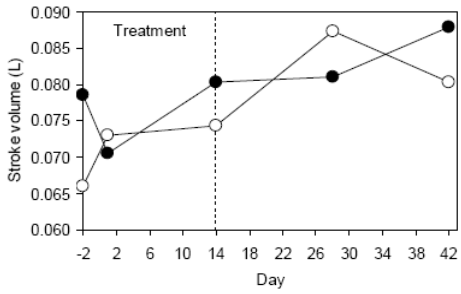
Table 7-6 Echocardiography data

Response	Baseline	During treatment		Post-treatment	
	Day -2	Day 1	Day 14	Day 28	Day 42
Stroke volume (L)					
Placebo	0.066 ± 0.005	0.730 ± 0.015	0.074 ± 0.014	0.087 ± 0.011	0.080 ± 0.015
FTY720	0.079 ± 0.026	0.071 ± 0.009	0.080 ± 0.016	0.081 ± 0.014	0.088 ± 0.018
Cardiac output (L/min)					
Placebo	4.63 ± 0.49	6.37 ± 2.37	5.03 ± 0.68	5.33 ± 0.60	5.00 ± 0.85
FTY720	5.02 ± 0.92	4.42 ± 0.47	4.80 ± 0.74	5.16 ± 0.55	4.87 ± 0.63

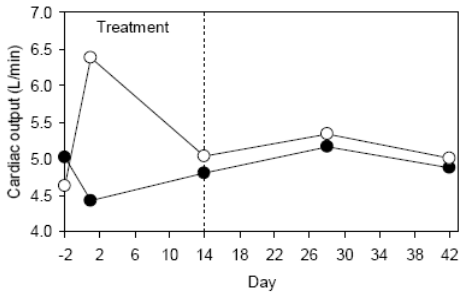
Values are mean ± sd. Source data: Appendix 3 Table 3.7.1; Appendix 6 Table 7.1.2 and following.

Stroke volume and cardiac output trajectories are shown in the figure below:

Figure 7-5 Stroke volume and cardiac output trajectories



Mean stroke volume trajectories in subjects receiving placebo (*open circles*) and FTY720 (*filled circles*).



As in left panel for cardiac output.

- Median stroke volume remained stable over the course of the study.

- Mean stroke volume decreased by 8 ml from baseline to day 1 (time of maximal dynamic effect of FTY720 on the heart), however this decrease was due, in part, to a skewed distribution of stroke volume at baseline.
- The mean day 1 / baseline stroke volume ratio was 1.00 (range, 0.58 – 1.60).
- Mean stroke volume increased above baseline levels by day 14 and remained higher than baseline when measured on days 28 and 42. These data are consistent with FTY720 having little or no negative inotropic effect on resting ventricular function.
- Cardiac output is a function of stroke volume and heart rate. Both mean and median cardiac output decreased from baseline to day 1 by 12% and 8%, respectively.
- The mean day 1 /baseline cardiac output ratio was 0.91 (range, 0.63 – 1.12). This decrease in cardiac output was coincident with the decrease in heart rate which occurred after FTY720 dosing.

Reviewer's note:
The stroke volume value (0.730) on day 1 in the placebo appears to be a typo.

Pulmonary function responses

- Both forced expiratory flow in 1 second (FEV1) and forced vital capacity (FVC) exhibited no clear trends over time nor apparent differences between FTY720-treated and placebo-treated subjects (p = 0.903 and 0.466).
- There was a trend for lower forced mid-expiratory flow (FEF25-75%) in FTY720-treated subjects compared with placebo-treated subjects (p = 0.123).

Methacholine challenge test

Methacholine is an inhaled drug which induces bronchoconstriction in normal lungs. Patients with an underlying tendency for reactive airways (asthmatics, for example) respond more vigorously to methacholine. A methacholine challenge was used to determine if and to what extent FTY720 increases airway reactivity. It was performed at screening and on days 2 and 15 with sequentially rising methacholine doses of 0.025, 0.25, 2.5, 10 and 25 mcg on each testing occasion.

Table 7-8 Methacholine challenge responses

Day	0.025 mcg	0.25 mcg	2.5 mcg	10 mcg	25 mcg
FTY720:					
Screening	-0.9 ± 2.0	-2.9 ± 4.7	-3.3 ± 9.0	-7.0 ± 6.7	-9.8 ± 8.0
Day 2	0.8 ± 8.2	-0.3 ± 8.0	-2.8 ± 8.0	-8.8 ± 3.0	-14.4 ± 14.1
Day 15	-1.1 ± 5.0	-0.8 ± 4.6	-6.6 ± 6.6	-14.9 ± 17.6	-9.5 ± 6.7
Placebo:					
Screening	-2.0 ± 4.0	-3.0 ± 2.0	-4.0 ± 2.7	-5.0 ± 3.5	-5.3 ± 3.5
Day 2	-0.7 ± 4.5	0.0 ± 3.6	-2.0 ± 3.0	-3.0 ± 4.0	-4.0 ± 6.1
Day 15	1.7 ± 5.5	1.7 ± 4.6	-2.0 ± 6.6	-3.3 ± 6.1	-2.0 ± 7.6

Responses are percent change from prechallenge in FEV1 as mean ± sd.
Source data: Appendix 6 Table 5.2.4 and following.

- At screening, mean FEV1 decreased in a dose-dependent manner with rising methacholine dose from -0.9% to -9.8%.
- FTY720 causes a mild to moderate increase in responsiveness to methacholine which appears to remain unchanged during the course of FTY720 dosing while one subject (5106) had a strong response on day 15 with a FEV1 decrease of -52% at a methacholine dose of 10 mcg.

Ability of a beta-agonist to improve FEV1

Albuterol is beta-agonist which reduces bronchoconstriction. The ability of albuterol to improve FEV1 was measured on day 1 approximately 2-4 hours after the first FTY720 dose.

- Albuterol increased FEV1 by 5% from 3.54 ± 0.61 L to 3.71 ± 0.56 L (p = 0.002).
- The ability of albuterol to reverse the bronchoconstriction from methacholine was assessed after each methacholine challenge. Albuterol increased FEV1 by 10.4 ± 9.4% on day 2 and 9.0 ± 6.2% on day 15.

Exercise oximetry

The 12-minute exercise oximetry testing consisted of 3 minutes stepping exercise (*minutes 1-3*), 3 minutes recovery (*minutes 4-6*), and 6 minutes posttest observation (*minutes 7-12*). Oxygen saturation and heart rate were recorded every minute up to 6 minutes and every 2 minutes thereafter to 12 minutes.

Table 7-9 Exercise oximetry responses in FTY720-treatment group

Day	Predose	Exercise		Recovery
	Saturation (%)	Saturation (%)	Heart rate (bpm)	Saturation (%)
-1	98.0 ± 1.3	96.9 ± 2.0	118 ± 27	98.5 ± 1.2
1	98.9 ± 1.5	95.7 ± 3.5	102 ± 23	98.6 ± 1.3
14	97.9 ± 1.5	96.6 ± 2.5	108 ± 20	97.7 ± 1.5
28	97.1 ± 2.0	96.2 ± 2.0	111 ± 21	97.3 ± 1.1
42	97.7 ± 1.5	97.2 ± 1.1	121 ± 16	98.3 ± 0.8

Values are percent oxygen saturation as mean ± sd
Predose = minute 0; *exercise* = minutes 1-3; *recovery* = minutes 4-6.

- On day 1 after the first dose of FTY720, the mean decrease from predose during exercise of -3.2%. Thereafter mean decreases were similar to baseline.
- Six FTY720-treated subjects had an oxygen saturation <95% and/or a decrease from predose of >3% during exercise. Of these, three subjects appeared to have preexisting bronchoreactivity that was slightly exacerbated by FTY720 and three had treatment-emergent oxygen desaturation.

<u>Central nervous system effects</u>

Mini-Mental State Examination

The Mini-Mental State Examination was administered at screening, at the end of treatment on day 14, and 2 weeks after treatment on day 28.

- Total scores throughout the study ranged from 27 to 30 (maximum). There was no trend in individual scores or mean scores in FTY720-treated subjects: 29.1 ± 1.3 at screening, 28.7 ± 1.6 on day 14, and 29.1 ± 1.1 on day 28.

Visual-evoked potential studies

Visual-evoked potentials were measured at screening and on day 15.

- Nothing abnormal was noted by the test evaluator with the exception of FTY720-treated subject 5109 on day 15: borderline prolonged right P-100 characterized by a blunted positive wave response.

- There was no trend in individual or mean results in the FTY720 treatment group. At screening the means were 109 ± 3 (left eye) and 112 ± 7 (right eye); on day 15 the means were 111 ± 2 (left eye) and 110 ± 4 (right eye).

AEs:

Elevated hepatic enzymes

Table 7-1 Summary data for subjects with elevated hepatic enzymes

Subject	AST (8-32 U/L)		ALT (0-39 U/L)		G-glutamyltransferase (6-38 U/L)	
	Days	Maximum	Days	Maximum	Days	Maximum
5103	14-56	369	14-28	521	28	41
5104	14-56	71	14-84	153	17-56	55
5105	56	33	56-84	61	56-84	50
5108	28-EOS	130	28-84	182	--	--
5109	5 and 56	41	28-84	70	--	--
5111	14-28	63	14-EOS	116	17-EOS	84

Parameters are in U/L. Normal ranges are in column headers. *EOS* = end-of-study visit.

- Six subjects in the FTY720 treatment group had elevations in AST, ALT, and/or gammaglutamyltransferase.
- Elevations were generally noted between days 14 to 84 reaching a maximum around day 28 or 56.
- In all cases, the enzymes returned to the normal range by the end-of-study visit with two exceptions: subject 5108 had slightly elevated AST (38 U/L) and subject 5111 had slightly elevated ALT (47 U/L) and gamma-glutamyltransferase (53 U/L) at the end-of-study visit.

Conclusions:

- 6 of the 9 FTY720-treated subjects had mild to moderate elevations in hepatic enzymes which recovered by the end of the study.
- Blood levels of fingolimod-P accumulated 5.1-fold from first dose to day 14.
- Total blood lymphocytes decreased 90% from predose to nadir. The nadir was maintained during the 14-day course of treatment followed by a recovery of lymphocyte counts nearly to pretreatment values by the end of the study. Lymphocyte subsets followed similar temporal patterns.
- Mean heart rate decreased from 74 bpm predose to a daytime nadir of 53 bpm 7 hours after the first dose. The decrease in heart rate was accompanied by a mean 12% increase in cardiac output on day 1 but no apparent change in stroke volume.
- Neither FEV1 nor FVC changed significantly; whereas, $FEF_{25-75\%}$ decreased by a mean 19% by the end of treatment. FTY720 did not negate the capacity of albuterol to decrease bronchoconstriction. FTY720 induced a generally mild-to-moderate increase in responsiveness to methacholine.
- FTY720 had no effect on neural conduction within the central nervous system as assessed by the Mini-Mental State examination and visualevoked potentials.

Study FTY720A 2105*:* **A randomized, parallel group, double-blind, placebo controlled, 14 days multiple-dose treatment to assess the pulmonary and cardiac pharmacodynamics of FTY720 (0.5 and 1.25 mg) in healthy volunteers**

A brief overview of some essential components of the study design is given below:

Study Design	Randomized, parallel-group, double-blind, placebo-controlled, 14-day, multiple-dose study
Study Population	N=39 enrolled, 37 completed
Dosage and Administration	Subjects were randomized equally into 1 of 3 treatment groups: placebo (n=14), FTY720 0.5 mg (n=12), and FTY720 1.25 mg (n=13). Each subject participated in a 21-day screening period, a 14-day treatment period, and a 28-day post-treatment follow-up period. Study medication was taken with 240 mL of water within 5 minutes of a light breakfast. FTY720 1.25 mg capsules: batch number H106DB, expiry date 12/2007 FTY720 0.5 mg capsules: batch number AEUS/2005-0346, expiry date 12/2007 Placebo capsules: batch number H107DB, expiry date 12/2007. <u>Diet:</u> No fluid intake apart from the fluid given at the time of drug intake/ meals is allowed from 2 hours before until 2 hours after dosing. Otherwise, subjects should have a fluid intake of at least 200 ml every 4 hours during waking hours on Day 1, in addition to fluid taken with meals and medication. Xanthine-containing beverages were to be discontinued 48 hours before dosing of study drug(s) Alcohol was prohibited for 48 hours prior to dosing until study completion.
Sampling: Blood	For FTY720 and FTY720-P (Blood): Day 1: predose and at 1, 2, 4, 6, 8, 12, 16, and 24 hours postdose Days 3, 7, 14: predose Days 28, 42: any time during visit
PD sampling	**Holter monitoring** for 24 hours on Days -1, 1, 7 and 14: heart rate (mean, minimum, maximum) by hour, arrhythmia monitoring and frequency and duration of sinus pauses **Echocardiography (2D and Doppler)** on Days -1, 1, 7, 14, 28: cardiac output, stroke volume and systemic vascular resistance **Exercise Oximetry** (3 minute step test) on Days -1, 1, 7, 14 and 28 **Pulmonary function tests** on Days -1, 1, 2, 7, 14 and 28: FEV1, FVC, and FEF 25-75% with methacholine challenge / albuterol reversibility **Absolute lymphocyte count** on Days -1 and 1: pre-dose, 1, 2, 3, 4, 6, 8, 12h and 24 hr post dose and on Days 3, 7, 8, 14, 15, 28, 42

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation FTY720 0.08 ng/mL FTY720-P 0.1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 8.4% Inter-day accuracy: < 2.4 % FTY720-P: Linear range : 0.1-20 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 11.4% Inter-day accuracy: < 8.7 %
PK Assessment	FTY720 and FTY720-P in blood: AUC0-24h,b, Clast,b, Cmax, b, Rac, tlag, tlast, tmax
PD Assessment	Holter monitoring, Echocardiography, Exercise Oximetry, Pulmonary function tests, Absolute lymphocyte count
Safety Assessment	Assessment of physical examinations, electrocardiograms (ECGs), vital signs, standard clinical laboratory evaluations (blood chemistry, urinalysis, hematology) and adverse event monitoring.

Results:

Pharmacokinetics Results: FTY720 and FTY720-P PK on day 1

Table 11-2 Day 1: FTY720 and FTY720-P pharmacokinetics after a 0.5-mg and a 1.25-mg dose in healthy volunteers

PK parameters	0.5 mg (n=12)		1.25 mg (n=12)	
	FTY720	FTY720-P	FTY720	FTY720-P
t_{lag} *(h)	2.00	2.00	1.00	1.50
	(0.00- 2.15)	(0.00- 4.00)	(0.00- 2.02)	(0.00- 2.00)
t_{max} *(h)	12.02	8.00	12.02	8.00
	(12.00- 23.97)	(6.00- 8.85)	(8.00- 23.97)	(6.00- 12.00)
$C_{max,b}$ #(ng/mL)	0.386 ± 0.0683	0.521 ± 0.116	0.958 ± 0.124	1.15 ± 0.268
	[0.381; 18]	[0.51; 21]	[0.95; 13]	[1.12; 25]
$AUC_{0-24h,b}$ #(ng/mL.h)	7.14 ± 1.52	7.33 ± 1.38	17.2 ± 2.50	15.7 ± 2.53
	[7.00; 21]	[7.23; 17]	[17.1; 15]	[15.5; 17]
Rac #	7.72 ± 1.11	4.91 ± 0.853	5.12 ± 1.82	3.97 ± 1.38
	[7.64; 15]	[4.84; 18]	[4.78; 43]	[3.68; 46]
D28 FTY720-P/FTY720 ratio#	0.40 ± 0.08		0.36 ± 0.07	
	[0.40; 19]		[0.36; 17]	
$AUC_{0-24h,b}$ FTY720-P/FTY720 ratio #	0.83 ± 0.12		0.73 ± 0.07	
	[0.82; 15]		[0.72; 11]	

#arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation]; *median (minimum-maximum); R_{ac}: (predose concentration Day 14)/($C_{24h,b}$ Day 1); D28 and $AUC_{0-24h,b}$ analytes ratios computed using molar units.

Source: Post-text Table 16.2.5-2.13 to Post-text Table 16.2.5-2.19

- Intersubject variability for AUC0-24h,b and Cmax,b was small to moderate for both doses (CV= 13% to 21%).
- FTY720 and FTY720-P mean AUC0-24h,b and Cmax,b values for the 1.25-mg dose group are about 2-2.5 times greater than those of the 0.5-mg dose group, consistent with the dose ratio.

- FTY720-P intersubject variability for AUC0-24h,b and Cmax,b was small to moderate for both doses (CV= 17% to 25%).
- FTY720 and FTY720-P blood predose concentrations increased between Day 2 and Day 14 in all subjects from both dose groups with 2 exceptions in the 1.25-mg dose group. In Subjects 5109 and 5131, FTY720 and FTY720-P predose concentrations were smaller on Day 14 than on Day 7, which may be due to poor compliance of these subjects to the treatment.
- The accumulation ratio computed as Day 14 predose/C24h, b Day 1 was greater for FTY720 than for FTY720-P in both dose groups.
- The geometric mean FTY720-P/FTY720 predose concentration ratio (in molar units) was greatest on Day 2 and declined on Day 3 and Day 7, remaining nearly similar between Day 7 and Day 28.
- These ratios were also comparable between the 2 doses, with Day 28 values of 0.36-0.40 (CV= 17-19%). These values are consistent with those from previous studies.

Pharmacodynamics Results:

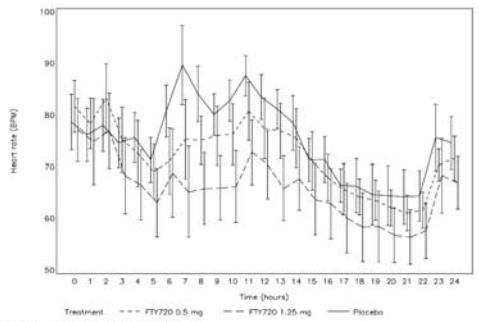
Heart rate

Mean heart rate on day -1:

- On Day -1, the heart rate profiles of the 3 treatment groups were similar with no apparent differences between the 3 treatment groups at any time point.
- The mean heart rate in the first 12 hours (defined as the mean of AUCE0-12 divided by 12) for the 3 treatment groups ranged from 80-83 BPM.

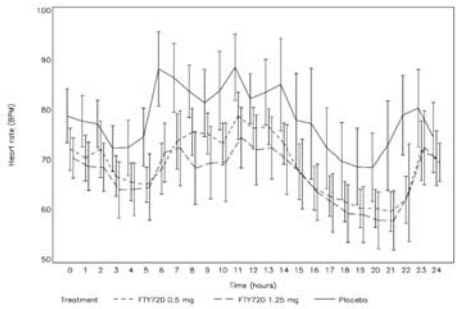
Mean heart rate trajectories on day 1 (left), and Day 7 (right) are shown in the figures below:

Figure 11-2 Day 1: Hourly average heart rate (mean plus minus 95% CI) post placebo dose



Note: Time =0 hours is approximately 8-9 AM.
Source: Post-text Figure 14.2-2.2

Figure 11-3 Day 7: Hourly average heart rate (mean plus minus 95% CI) post placebo dose



Note: Time =0 hours is approximately 8-9 AM.
Source: Post-text Figure 14.2-2.2

Day 1

- The 1.25 mg treatment resulted in a significant decrease in heart rate. In the first 12 hours postdose, the adjusted mean of the average heart rate was 69.6 BPM, compared to 81.5 BPM in the placebo group ($p<0.0001$); the adjusted mean nadir0-12 heart rate was 60.8 BPM compared to 71.6 BPM in the placebo group ($p<0.0001$).
- The 0.5 mg treatment also resulted in a significant decrease in heart rate. The adjusted mean of the average heart rate during the first 12 hours postdose in this treatment group was 73.6 BPM compared to 81.5 BPM in the placebo group ($p<0.0001$); the mean nadir0-12 was 65.4 BPM compared to 71.6 BPM in the placebo group ($p=0.0023$). The

adjusted mean nadir0-12 in the 0.5-mg treatment group was 4.5 BPM higher than that in the 1.25-mg treatment group (p=0.0210).

- The 0.5-mg treatment cohort manifested a decrease in heart rate which was approximately halfway between the placebo and 1.25-mg cohorts. The difference between placebo and 0.5-mg cohorts became evident at approximately 5 hours postdose and persisted for approximately 8 hours, after which these 2 groups had similar heart rates. The trajectory of average heart rate in the first 12 hours in the 0.5-mg treatment group was similar to the placebo group, with a nadir average heart rate for both groups of approximately 55 BPM, occurring at 5 hours postdose.

Day 7

- Both FTY720 treatment groups had persistent lower heart rates compared to placebo by approximately 10-15 BPM. The adjusted mean of the average heart rate and nadir during the first 12 hours postdose on Day 7 was significantly smaller in the 0.5 and 1.25 mg treatment groups than in placebo (p<0.0001). The negative chronotropic effect present during the first 12 hours postdose on Day 7 for both FTY720 treatment groups was not significantly different from the negative chronotropic effect present on Day 1 (p>0.08).

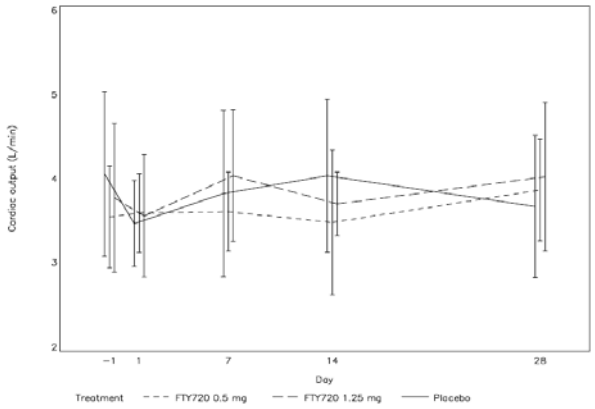
Day 14

- Similar patterns were observed as seen on day 7 so not repeating the data.

Hemodynamics

The mean cardiac output between day -1 to day 28 are shown in the figure below:

Figure 11-8 Cardiac output (mean plus minus 95%CI) for the 3 treatment groups



Source: Post-text Figure 14.2-2.4

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Similar patterns were also observed for the mean stroke volume and the mean systemic vascular resistance between day -1 to day 28, therefore data are not shown to avoid redundancy.

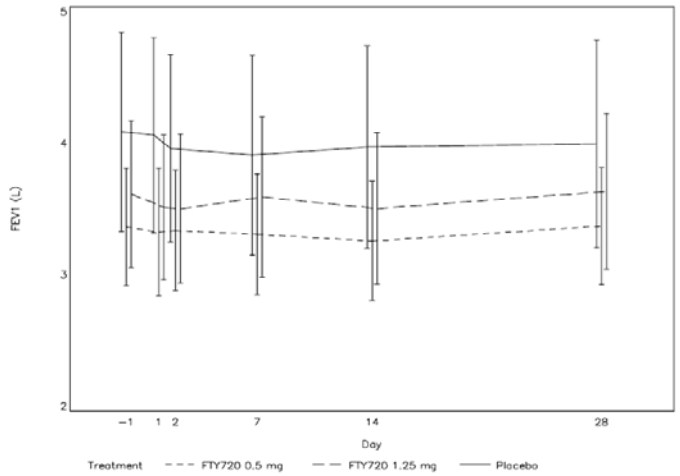
- No systematic treatment differences were observed over time or on any particular day for these data.

Pulmonary function

Spirometry

Mean FEV1 data are shown in the figure below:

Figure 11-11 FEV_1 (mean plus minus 95%CI) for the 3 treatment groups



Source: Post-text Figure 14.2-2.10

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- Relative to Day -1, neither dose of FTY720 appeared to have any effect on FEV1 compared to placebo.
- Similar findings were also observed for FEF_{25-75}, FVC and FEV1/FVC.

Methacholine challenge

- The maximum of the mean percent changes in FEV1 for the placebo, FTY720 0.5-mg, and FTY720 1.25-mg dose groups was equal to -12.4% , -14.6% , and -16.9% , respectively.
- For the remaining time-points the intermediate doses of methacholine had a similar degree of effect in the three treatment groups when compared to Day -1.
- These data suggested that FTY720 treatment at doses of 0.5 mg or 1.25 mg did not alter the pulmonary response to methacholine.

Albuterol challenge

- The expected effect of albuterol would be, in part, to reverse the bronchoconstrictive effect of methacholine; however, no consistent bronchodilatory response to albuterol was apparent on Day -1 for the three treatment groups, or for the placebo group throughout the study. Therefore, it is difficult to assess if FTY720 reduced the capacity of albuterol to increase airflow.
- The effect of albuterol during active FTY720 treatment was similar to placebo and, as such, these data indicate that FTY720 treatment did not result in paradoxical increase in airway resistance to albuterol treatment.

Oxygen saturation

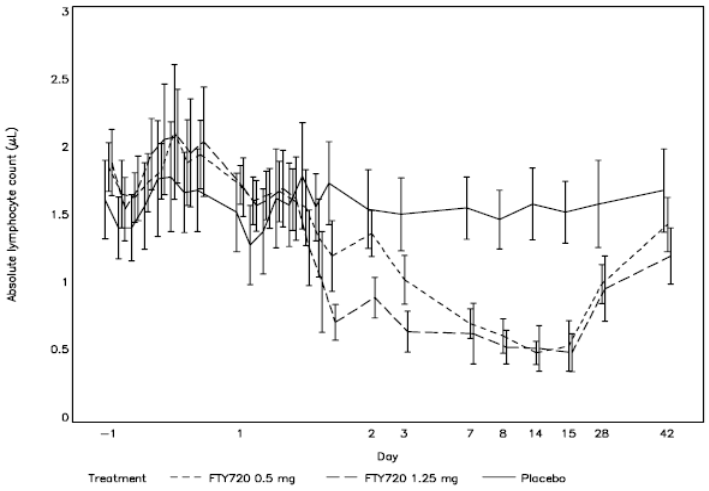
- These data indicate no FTY720 treatment effect on oxygen saturation at rest or during exercise stress over the course of the study.

<u>Lymphocyte responses</u>

Absolute lymphocyte counts

Lymphocyte trajectories during 14 day dosing period and recovery period are shown in the figure below:

Figure 11-13 Lymphocyte count (mean plus minus 95%CI) by treatment group



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Source: Post-text Figure 14.2-2.18

- On Day 1, both FTY720 treatment groups manifested a significant decrease in lymphocyte count AUEC0-12 ($p \leq 0.0004$) compared to placebo.
- Approximately six hours post FTY720 dose on Day 1, both FTY720 treatment groups manifested a decrease in mean lymphocyte, which persisted for the remaining 14 days of treatment.
- In the FTY720 1.25-mg treatment group, the lymphocyte count at 12 hours postdose on Day 1 was approximately 600 cells/uL, which was similar to the Day 14 count of approximately 500 cells/uL while the FTY720 0.5-mg treatment group manifested a more gradual decrease reaching a similar nadir after one week of daily dosing
- Two weeks after dosing stopped, the mean lymphocyte count for the two FTY720 treatment groups was approximately 1000 cells/uL. The mean lymphocyte count for the two FTY720 treatment groups continued to increase from Day 28 to Day 42.

Conclusions:

- Over the 14 days of treatment, both FTY720 0.5 mg and 1.25 mg had significant negative chronotropic effect. The onset of negative chronotropic effect was detectable approximately 3-4 hours post Day 1 dosing.
- During the first half day of treatment, the mean negative chronotropic effect for FTY720 0.5 mg was 7.9 BPM lower than placebo and for FTY720 1.25 mg it was 11.9 BPM lower than placebo.
- The negative chronotropic effect present on Day 14 for both FTY720 treatment groups was not significantly different from the negative chronotropic effect present on Day 1.
- FTY720 treatment had no effect on ventricular function as measured by cardiac output or stroke volume.
- FTY720 treatment had no effect on pulmonary function as measured by spirometry, methacholine challenge, and exercise oximetry.
- Over the 14 days of treatment, both FTY720 0.5 mg and 1.25 mg resulted in a significant decrease in peripheral lymphocyte count, which was first detectable six hours postdose on Day 1.
- The observed FTY720 and FTY720-P pharmacokinetics in this study are consistent with that from previous studies.

Study FTY720D 2106*:* **An open-label single-dose single-sequence study to evaluate the effect of oral inhaled salmeterol administration on the negative chronotropic effect of 1.25 mg oral FTY720 in healthy subjects**

A brief overview of some essential components of the study design is given below:

Study Design	open-label single dose single sequence study
Study Population	N=16 enrolled, 14 completed
Dosage and Administration	Period 1: Salmeterol 250 mcg (25 mcg base/inhalation x 2 at 0, 30, 60, 90 and 120 minutes after first dose started approximately at 1200) Period 2: FTY720 1.25 mg + Salmeterol 250 mcg (25 mcg base/ inhalation x 2 at 4.0, 4.5, 5, 5.5 and 6 hours after the FTY720 dose administered approximately at 0800)

Periods 1 and 2 were separated by a 7-day washout

Study medication was administered with 240 ml of water immediately after a standard breakfast.

	Batch No.
FTY720 1.25 mg	H106DB
Salmeterol (Serevent®)25 mcg Evohaler	0039R, 0043R

Diet:
No fluid intake apart from the fluid given at the time of drug intake / meals is allowed from 2 hours before until 2 hours after dosing. Otherwise, subjects should have a fluid intake of at least 200 ml every 4 hours during waking hours on Day 1, in addition to fluid taken with meals and medication.

Xanthine-containing beverages were allowed but was to be discontinued 48 hours before dosing of study drug(s)

Alcohol was prohibited for 48 hours prior to dosing until study completion.

Sampling: Blood	For FTY720 and FTY720-P (Blood): At pre-dose and at 2, 4, 6, 8, 12h postdose on Period 2 day 1
Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation

	Blood
FTY720	0.08 ng/mL
FTY720-P	0.1 ng/mL

PK Assessment	FTY720 and FTY720-P in blood: Cmax, b, tlag, tmax, AUC0-tlast, b, AUC0-8h, b, AUC4-8h, b
PD Assessment	Continuous heart rate monitoring via telemetry. Area under HR curve (AUE[4-8]) and nadir heart rate (Emax)
Safety Assessment	Assessment of adverse events, vital signs, 12-lead ECGs performed, safety laboratory assessments (hematology, blood chemistry, and urinalysis), serum potassium, and physical examinations.

Results:

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 and FTY720-P are summarized in the following table:

Table 11-2 FTY720 and FTY720-P pharmacokinetics after a single 1.25 mg dose in healthy volunteers (n=14)

PK parameters	FTY720	FTY720-P
t_{lag} *	0.00	0.00
(h)	(0.00-2.05)	(0.00-2.05)
t_{max} *	12.00	6.09
(h)	(2.05- 12.05)	(6.00- 12.05)
$C_{max, b}$ #	1.06 ± 0.229	1.10 ± 0.213
(ng/mL)	[1.03; 24]	[1.09; 18]
$AUC_{0\text{-}tlast, b}$ #	8.68 ± 1.63	8.83 ± 1.55
(ng/mL.h)	[8.53; 20]	[8.72; 17]

$AUC_{0\text{-}tlast, b}$ can also be considered as $AUC_{0\text{-}12h, b}$*: median (minimum-maximum); #: arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation]

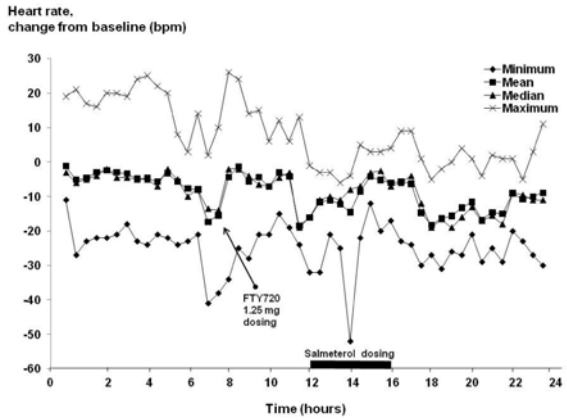
- Blood samples were collected over 12 hours only, which is insufficient to characterize accurately the pharmacokinetics of FTY720 and FTY720-P.
- Compared to the previous measured concentrations (8 hours postdose), blood concentrations were still increasing at 12 hours postdose for FTY720 and FTY720-P.
- Cmax, b and tmax may not have been well captured. Despite these limitations, FTY720 and FTY720-P computed Cmax, b and AUC0-tlast, b (equal to AUC0-12h, b) are consistent with previous studies.
- AUC0-12h, b interindividual variability is moderate for both analytes (about 20%). This is also consistent with previous studies.

Pharmacodynamics Results:

Heart rate response : Effect of salmeterol alone on heart rate

- During this 4-hour period of salmeterol treatment mean heart rate increased approximately 6 bpm compared to baseline (Day -1).
- Integrating over the salmeterol-dosing period, the AUEC1200-1600 for heart rate increased 26 beats hr/min (p=0.0125).

Effect of salmeterol on FTY720-induced negative chronotropism



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- Immediately after the FTY720 dose, from 0800 to 1100, the change in heart rate from baseline ranged from -1.2 to -7.1 bpm. However, at 1130 and 1200, the change in heart

rate from baseline was -18.4 and -16.1, respectively. This further negative inflection of the change in heart rate from baseline is consistent with a significant FTY720-induced negative chronotropic effect (p=0.0187 comparing hours 0800 and 1200).

- With salmeterol dosing 4 hours post FTY720 dose, the negative chronotropic effect of FTY720 began to attenuate, with the change in heart rate from baseline significantly increasing from -16.1 at hour 1200 to -6.0 bpm at 1600, a difference of approximately 10 bpm (p=0.0025).
- Salmeterol dosing ended at 1600 and the positive chronotropic effect of salmeterol diminished substantially by 1630 and appeared to be gone by 1730.

Conclusions:

- Despite limitations in the sampling times which preclude accurate PK assessment, the FTY720 and FTY720-P pharmacokinetics in this study were consistent with that observed in previous studies.
- Salmeterol had a mild, positive chronotropic effect on heart rate of approximately 6 beats per minute.
- Salmeterol was active in significantly reversing the negative chronotropic effect of FTY720. After 3 hours of salmeterol treatment, the negative heart rate effect of FTY720 treatment was almost completely reversed, with the heart rate returning to a level measured pre-FTY720 dose.
- Inhaled salmeterol treatment may be considered a possible means to clinically manage the negative chronotropic effect of FTY720.

Study FTY720D 2110*:* **A double-blind, placebo controlled, dose titration study to assess the effect of small doses of FTY720 on the negative chronotropic effect of FTY720 in healthy volunteers**

A brief overview of some essential components of the study design is given below:

Study Design	Double-blind, placebo controlled, dose titration study
Study Population	**Table 11-2 Demographics – safety population** Source: Post-text Table 14.1-3.1 MD = multiple dosing, N = number of subjects, SD = standard deviation, BMI=Body Mass Index
Dosage and Administration	The subjects were divided in three groups: 1. Placebo: once daily for 10 days (n=9), 2. 1.25 mg FTY720: once daily for 9 days (n=12) and 3. Titration (n=15): ascending doses of FTY720 with 0.125 mg on Day 1 to 3, 0.25 mg on Days 4 and 5, 0.5 mg on Days 6 and 7, and 1.25 mg on Days 8 and 9 Study medication was administered with 240 ml of water immediately after a standard breakfast. Batch No. FTY720 0.125 mg: AEUS/2007-0345 FTY720 0. 5 mg: AEUS/2006-0274 FTY720 1.25 mg: H271HC Matching placebo: H107DB Diet: No fluid intake apart from the fluid given at the time of drug intake and breakfast is allowed from 2 h before until 2 h after dosing Xanthine-containing beverages were allowed but was to be discontinued 48 hours before dosing of study drug(s) Alcohol was prohibited for 48 hours prior to dosing until study completion.
Sampling: Blood	For FTY720 and FTY720-P (Blood): Day 1 at pre-dose and at 3, 6, 9, 12, and 24 hours post-dose (Day 2 pre-dose), on Day 2 at 3, 6, 9, 12, and 24 hours post-dose, on Day 4 at pre-dose and at 3, 6, 9, 12, and 24 hours post-dose, on Day 6 at pre-dose and at 3, 6, 9, 12, and 24 hours, on Day 8 at pre-dose and at 3, 6, 9, 12, and 24 hours post-dose
Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation Blood FTY720 0.0635 ng/mL FTY720-P 0.1 ng/mL

Demographic variable		FTY720 dose titration N=15	1.25 mg FTY720 (MD) N=12	Placebo (MD) N=9	Total N=36
Age (years)	Mean (SD)	39.9 (8.35)	39.0 (8.78)	41.9 (4.91)	40.1 (7.67)
Height (cm)	Mean (SD)	174.5 (11.36)	174.7 (12.12)	174.8 (6.30)	174.6 (10.34)
Weight (kg)	Mean (SD)	74.23 (11.476)	74.34 (15.128)	76.08 (9.973)	74.73 (12.164)
Sex:	Male	10 (66.7 %)	7 (58.3 %)	7 (77.8 %)	24 (66.7 %)
	Female	5 (33.3 %)	5 (41.7 %)	2 (22.2 %)	12 (33.3 %)
Race:	Caucasian	14 (93.3 %)	12 (100 %)	9 (100 %)	35 (97.2 %)
	Other	1 (6.7 %)			1 (2.8 %)
Ethnicity:	Mixed ethnicity	1 (6.7 %)			1 (2.8 %)
	Other	14 (93.3 %)	12 (100 %)	9 (100 %)	35 (97.2 %)
BMI (kg/m^2)	Mean (SD)	24.36 (2.798)	24.09 (2.363)	24.81 (2.011)	24.38 (2.426)

	FTY720: Linear range : 0.0635-63.5 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 4.7% Inter-day accuracy: < 4 % FTY720-P: Linear range : 0.1-20 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 18.4% Inter-day accuracy: < 7.3 %
PK Assessment	FTY720 and FTY720-P in blood: Cmax,b, and AUC(0-tz),b (surrogate for AUC0-24h,b) tmax, tlag and tlast, AUC(0-tz),b, Cmax,b
PD Assessment	24-hour telemetry, 24-hour Holter-ECG monitoring
Safety Assessment	Assessment of adverse events, hematology, blood chemistry, and urine (performed at the clinical study site and the central laboratory), and vital signs, physical condition, and body weight.

Pharmacokinetics Results:

The pharmacokinetic parameters of FTY720 and FTY720-P are summarized in the following table:

Table 11-6 FTY720 and FTY720-P pharmacokinetics in the two treatment groups (n=12 in the 1.25 mg dose group and n=15 in the titration group)

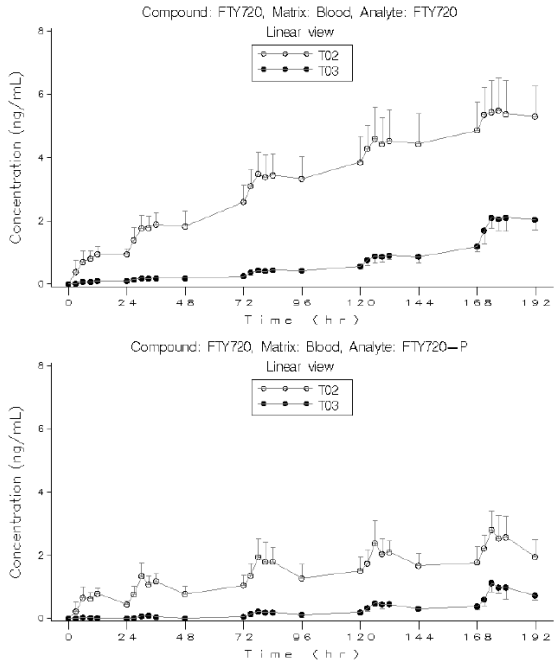
Source: Post text Table 14.2-1.6, and Post text Table 14.2-1.7)

Profile day	PK parameters	FTY720		FTY720-P	
		Dose titration	1.25 mg	Dose titration	1.25 mg
1	t_{lag} *	3.00	0.00	3.03+	1.5
	(h)	(0.00- 9.00)	(0.00- 3.00)	(2.98- 12.00)	(0.00- 6.02)
	t_{max} *	12.02	12.03	9.03+	12.00
	(h)	(5.98- 24.00)	(6.00- 24.00)	(6.00- 23.9)	(6.00- 12.00)
	$C_{max,b}$ #	0.102 ± 0.019	0.985 ± 0.228	0.05 ± 0.0653+	0.873 ± 0.190
	(ng/mL)	[0.1004; 20]	[0.9606; 24]	[a; a]	[0.853; 23]
	$AUC_{(0-tz),b}$ #	1.806 ± 0.518	18.3 ± 4.83	0.205 ± 0.333+	13.01 ± 3.076
	(ng/mL.h)	[1.73; 33]	[17.8; 26]	[a; a]	[12.6; 27]
2	t_{max} *	12.07	12.00	6.02++	6.03
	(h)	(6.00- 23.9)	(6.00- 23.9)	(3.00- 12.1)	(6.00- 12.1)
	$C_{max,b}$ #	0.193 ± 0.0362	1.99 ± 0.471	0.136 ± 0.212++	1.42 ± 0.363
	(ng/mL)	[0.190; 19]	[1.94; 25]	[a; a]	[1.38; 26]
	$AUC_{(0-tz),b}$ #	4.12 ± 0.81	41.2 ± 8.62	0.547 ± 0.856++	23.6 ± 5.39
	(ng/mL.h)	[4.051; 19]	[40.3; 23]	[a ; a]	[22.9; 25]
4	t_{max} *	12.00	9.01	9.00	6.05
	(h)	(6.00- 24.00)	(3.00- 12.00)	(6.00- 12.1)	(5.98- 12.1)
	$C_{max,b}$ #	0.453 ± 0.0864	3.58 ± 0.702	0.249 ± 0.0519	2.047 ± 0.578
	(ng/mL)	[0.446; 19]	[3.51; 21]	[0.244; 21]	[1.97; 29]
	$AUC_{(0-tz),b}$ #	9.77 ± 1.84	79.3 ± 15.4	3.64 ± 1.57	37.8 ± 10.4
	(ng/mL.h)	[9.62; 18]	[77.9; 20]	[3.17; 69]	[36.6; 28]
6	t_{max} *	9.05	9.01	6.07	6.03
	(h)	(3.00- 23.9)	(3.00- 24.00)	(3.02- 12.0)	(3.00- 12.1)
	$C_{max,b}$ #	0.951 ± 0.205	4.75 ± 0.991	0.53 ± 0.1034	2.48 ± 0.68
	(ng/mL)	[0.932; 21]	[4.65; 22]	[0.5203; 20]	[2.39; 30]
	$AUC_{(0-tz),b}$ #	20.3 ± 3.54	105.9 ± 21.4	9.22 ± 1.35	46.4 ± 9.35
	(ng/mL.h)	[20.05; 17]	[103.8; 21]	[9.13; 15]	[45.4; 22]
8	t_{max} *	12.00	7.53	6.03	6.07
	(h)	(6.00- 24.00)	(3.00- 12.00)	(5.98- 12.0)	(6.00- 12.00)
	$C_{max,b}$ #	2.204 ± 0.35	5.77 ± 1.04	1.204 ± 0.258	3.00 ± 0.665
	(ng/mL)	[2.18; 16]	[5.68; 19]	[1.18; 20]	[2.93; 24]
	$AUC_{(0-tz),b}$ #	47.2 ± 8.30	128 ± 23.0	20.4 ± 4.69	56.2 ± 13.1
	(ng/mL.h)	[46.6; 18]	[126; 19]	[19.9; 23]	[54.7; 25]

#: arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation]; *: median (minimum-maximum); +: n=6; ; ++: n=10; a: parameter can not be computed

The mean concentration-time profiles of FTY720 and FTY720-P are shown in the following figure:

Figure 11-7 **Arithmetic mean (+SD) blood concentration-time profiles over 8 days of FTY720 (upper graph) and FTY720-P (lower graph) per dose group (T02: fixed dose group; T03: titration group)**



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FTY720 pharmacokinetics

- Cmax,b and AUC(0-tz),b on day 1 and day 2 were about 10 times smaller in the titration group than in the fixed dose group, consistent with the ratio of the administered doses.
- Every time a new dose increment occurred in the titration group, FTY720 AUC(0-tz),b and Cmax,b increased more, compared to the previous assessment, in this group than in the fixed dose group.
- The inter-subject variability for AUC(0-tz),b and Cmax,b was small to moderate (CV=16 to 33%).
- FTY720 Cmax,b and AUC(0-tz),b values observed on day 1, in the fixed dose regimen, were reached on day 6 in the dose titration group. The geometric mean Cmax,b and AUC(0-tz),b on day 8 in the titration group represented 37-38% of those of the fixed dose group.

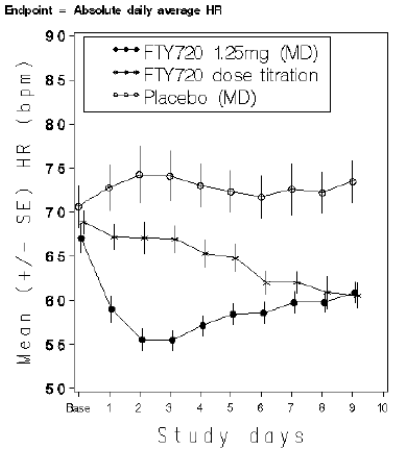
FTY720-P pharmacokinetics

- Every time a new dose increment occurred in the titration group, FTY720-P AUC(0-tz),b and Cmax,b increased more, compared to the previous assessment, in this group than in the fixed dose group.
- Apart from day 4 AUC(0-tz),b in the titration group, FTY720-P inter-subject variability for AUC(0-tz),b and Cmax,b was small to moderate (CV= 15 to 29%).
- FTY720-P Cmax,b and AUC(0-tz),b values observed on day 1, in the fixed dose regimen, were likely reached on day 7 in the dose titration group.
- The geometric mean Cmax,b and AUC(0-tz),b on day 8 in the titration group represented 36-40% of those of the fixed dose group.
- The geometric mean FTY720-P/FTY720 Cmax,b, AUC(0-tz),b and predose concentration (C0,b) ratios (in molar units) all decreased throughout the study in the fixed dose group, especially on the initial days and much less from day 4, to reach values of 0.41, 0.34 and 0.28, respectively, on day 8.

Pharmacodynamics Results:

Heart rate response : Daily average heart rate

Figure 11-1 **Mean (+/-SE) daily average heart rate on Days 1 to 9, by treatment regimen group (PD population)**



Source data: Post-text Figure 14.2-2.4, MD multiple dose, PD=pharmacodynamic

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- The FTY720 1.25 mg treatment group manifested a significant decrease in heart rate of heart rate of approximately 8 BPM from Day -1 to Day 1 and an additional decrease in heart rate approximately 3 BPM from Day 1 to Day 2. After Day 3, there was a significant increase in heart rate of approximately 5 BPM.
- The FTY720 titration group manifested a gradual decrease in heart rate of approximately 1-2 BPM per day over eight day course of the dose titration. The initiation of the 1.25 mg FTY720 on Day 8 did not result in dip in heart rate compared to the preceding days.
- Similar result was shown for the daily minimum heart rate and daily absolute minimum heart rate therefore data and detail description is not repeated again.

Conclusions:

- The maximal negative chronotropic effect of the standard FTY720 1.25 mg regimen, a decrease in heart rate of approximately 8 BPM, was seen on Day 1.
- After Day 2, no additional negative chronotropic effect was detected in the FTY720 1.25 mg group. Rather mean heart rate increased by approximately 3-4 BPM between Day 3 and 9.
- The FTY720 titration regimen resulted in a gradual accumulation of negative chronotropic effect over the nine day course of the study. The maximal negative chronotropic effect on any particular day was approximately 2-3 BPM.
- In the FTY720 titration regimen, the first 1.25 mg dose administered on Day 8, resulted in a decrease in heart rate of only 1-2 BPM. Thus, the acute, negative chronotropic effect of a 1.25 mg dose was almost completely abolished when compared to Day 1 of the FTY720 1.25 mg regimen.
- At no time in the study did the FTY720 titration group manifest mean heart rates as low as those detected in the FTY720 1.25 mg dose group on Days 1 and 2.
- By the end of the study, both FTY720 treatment groups had nearly identical heart rates.
- The FTY720 and FTY720-P Cmax,b and AUC(0-tz),b Day 1 values in the fixed dose group were reached on days 6-7 in the titration group.
- On Day 8, FTY720 and FTY720-P Cmax,b and AUC(0-tz),b in the dose titration group represented 36-40% of the values observed in the fixed dose group (Versus 10% on Day 1 for FTY720 PK parameters).

Study FTY720D 2113*:* **An exploratory, randomized, blinded, placebo-controlled, parallel group, multiple-dose study to assess the effect of FTY720 on mean flow velocity in cerebral vessels, platelet function, and macular thickness following once daily dosing for 4 weeks in healthy subjects**

A brief overview of some essential components of the study design is given below:

<table>
<tr><td>Study Design</td><td>randomized, blinded, placebo-controlled, parallel group, multiple-dose study</td></tr>
<tr><td>Study Population</td><td>N=88
Table 11-2 Summary of demographics and baseline characteristics by treatment group
<table>
<tr><th></th><th></th><th>FTY720 0.5mg N = 30</th><th>FTY720 1.25mg N = 29</th><th>Placebo N = 29</th></tr>
<tr><td>Age (years)</td><td>Mean</td><td>37.3</td><td>38</td><td>38.6</td></tr>
<tr><td></td><td>SD</td><td>9.24</td><td>10.92</td><td>7.37</td></tr>
<tr><td></td><td>Median</td><td>37</td><td>41</td><td>40</td></tr>
<tr><td></td><td>Range</td><td>20-51</td><td>19-51</td><td>23-49</td></tr>
<tr><td>Gender – n (%)</td><td>Male</td><td>27 (90%)</td><td>24 (82.8%)</td><td>26 (89.7%)</td></tr>
<tr><td></td><td>Female</td><td>3 (10%)</td><td>5 (17.2%)</td><td>3 (10.3%)</td></tr>
<tr><td>Race – n (%)</td><td>Caucasian</td><td>30 (100%)</td><td>28 (96.6)</td><td>29 (100%)</td></tr>
<tr><td></td><td>Hispanics</td><td></td><td>1 (3.4)</td><td></td></tr>
<tr><td>Weight (kg)</td><td>Mean</td><td>79</td><td>78.9</td><td>81.1</td></tr>
<tr><td></td><td>SD</td><td>11.32</td><td>11.03</td><td>8.95</td></tr>
<tr><td></td><td>Median</td><td>78</td><td>78</td><td>82</td></tr>
<tr><td></td><td>Range</td><td>57-102</td><td>60-104</td><td>64-108</td></tr>
<tr><td>Height (cm)</td><td>Mean</td><td>179.8</td><td>178.3</td><td>180.3</td></tr>
<tr><td></td><td>SD</td><td>6.48</td><td>9.76</td><td>7.02</td></tr>
<tr><td></td><td>Median</td><td>180</td><td>180</td><td>178</td></tr>
<tr><td></td><td>Range</td><td>168-190</td><td>160-194</td><td>166-192</td></tr>
<tr><td>BMI (kg/m^2)</td><td>Mean</td><td>24.4</td><td>24.9</td><td>25.0</td></tr>
<tr><td></td><td>SD</td><td>2.75</td><td>2.90</td><td>2.43</td></tr>
<tr><td></td><td>Median</td><td>24.9</td><td>25.3</td><td>24.7</td></tr>
<tr><td></td><td>Range</td><td>18.4-29.5</td><td>18.1-29.8</td><td>20.4-29.9</td></tr>
</table>
BMI = body mass index, SD = standard deviation
Source: Post-text Table 14.1-3.1</td></tr>
<tr><td>Dosage and Administration</td><td>Table 1 Dosing schedule
<table>
<tr><th>Group</th><th>Treatment Description</th></tr>
<tr><td>A (n=29)</td><td>Placebo</td></tr>
<tr><td>B (n=30)</td><td>FTY720 0.5 mg</td></tr>
<tr><td>C (n=29)</td><td>FTY720 1.25 mg</td></tr>
</table>
In the first week of treatment, a loading regimen of escalating doses of FTY720 was administered over a four-day period in order to achieve pharmacokinetic steady state at the end of this time period.

After this four-day loading period, daily dosing of FTY720 was used to maintain subjects at steady-state for the remainder of the four-week active treatment period.</td></tr>
</table>

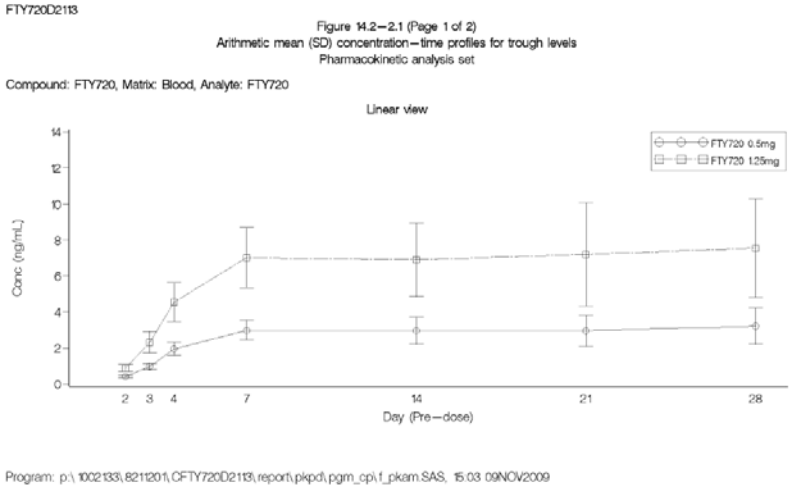
<table>
<tr><td></td><td>
Table 9-2 FTY720 loading regimens
<table>
<tr><th>Day</th><th>Dose for 0.5 mg/day</th><th>Dose for 1.25 mg/day</th></tr>
<tr><td>1</td><td>0.5</td><td>1.25</td></tr>
<tr><td>2</td><td>1.0</td><td>2.5</td></tr>
<tr><td>3</td><td>1.5</td><td>3.75</td></tr>
<tr><td>4</td><td>2</td><td>5</td></tr>
<tr><td>5</td><td>0.5</td><td>1.25</td></tr>
<tr><td>6</td><td>0.5</td><td>1.25</td></tr>
<tr><td>7</td><td>0.5</td><td>1.25</td></tr>
</table>
Study medication was administered with 240 ml of water. The subjects should eat a light breakfast but there is no restriction of the timing of the dose and meal times.
Table 2 FTY720 dosings and formulations
<table>
<tr><th>Group</th><th>Dose form & strength</th><th>Dosage</th><th>Mode of administration</th><th>Batch & formulation numbers</th></tr>
<tr><td>B (n=29)</td><td>FTY720 0.5 mg capsules</td><td>Once a day between 8:00 and 12:00 (with allowed window of ± 30 min), Day 1 to 28</td><td>Orally with 240 ml of water</td><td>FTY720 0.5mg batch: H444KD 3752920.006</td></tr>
<tr><td>C (n=29)</td><td>FTY720 1.25 mg capsules</td><td>Once a day between 8:00 and 12:00 (with allowed window of ± 30 min), Day 1 to 28</td><td>Orally with 240 ml of water</td><td>FTY720 1.25mg batch: H445KD 3761319.006</td></tr>
</table>
Table 3 Reference (placebo) dosing and formulation
<table>
<tr><th>Group</th><th>Dose form & strength</th><th>Dosage</th><th>Mode of administration</th><th>Batch & formulation numbers</th></tr>
<tr><td>A (n=29)</td><td>Placebo matched capsules</td><td>Once a day between 8:00 and 12:00 (with allowed window of ± 30 min), Day 1 to 28</td><td>Orally with 240 ml of water</td><td>Placebo global batch: H107DB 3755667.032</td></tr>
</table>
<u>Diet:</u>
Normal intake of xanthine-containing beverages was continued.
Normal intake of alcohol was continued.
</td></tr>
<tr><td>Sampling: Blood</td><td>For FTY720 and FTY720-P (Blood):
Pre-dose on days 2, 3, 4, 7, 14, 21, and 28</td></tr>
<tr><td>Analysis (Blood)</td><td><u>Method</u>
LC/MS/MS
<u>Lower Limits of Quantitation</u>
<table>
<tr><th></th><th><u>Blood</u></th></tr>
<tr><td>FTY720</td><td>0.08 ng/mL</td></tr>
<tr><td>FTY720-P</td><td>0.1 ng/mL</td></tr>
</table>
<u>FTY720:</u>
Linear range : 0.08-16 ng/mL in blood
Inter-day Precision
(%CV for Quality Controls) : < 8.5%
Inter-day accuracy: < 7.9 %

<u>FTY720-P:</u>
Linear range : 0.1-20 ng/mL in blood
Inter-day Precision
(%CV for Quality Controls) : < 9.1%
Inter-day accuracy: < 7.3 %</td></tr>
<tr><td>PK Assessment</td><td>FTY720 and FTY720-P in blood: C0 on days 2, 3, 4, 7, 14, 21, and 28</td></tr>
<tr><td>PD Assessment</td><td>Cerebral blood flow rate:
Mean blood flow velocity (Vm) in the middle cerebral artery (MCA)</td></tr>
</table>

	Platelet function: Platelet adhesion time in response to epinephrine (PFA100® assay) **Macular thickness:** Central foveal thickness
Safety Assessment	Assessment of physical examination, ECGs, 24h telemetry, vital signs, standard clinical laboratory evaluations hematology, blood chemistry, urinalysis along with AE and serious adverse event (SAE) monitoring.

Pharmacokinetics Results:

The FTY720 concentration-time profiles at both dose levels are shown in the figure below (FTY720-P concentration-time profiles were similar therefore not shown):



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Comparison of Cmins in the present study in healthy subjects to that of a pivotal study in MS patients:

- Steady state concentrations were reached already on day 7.
- The Day 28 predose concentrations are close to the ones observed at steady state in the first phase III pivotal study [CFTY720D2302] where multiple sclerosis patients received a once daily treatment for 12 months.

Reviewer's note:

- *The sponsor stated two dose groups did not show deviations from dose-proportionality, which is in line with other studies. However, slightly lower than dose proportional was observed from this data and also from the reviewer's analysis, this finding is consistent through many studies.*

Pharmacodynamics Results:

Blood flow velocity in the MCA

The results of the mean blood flow velocity measurements in the middle cerebral artery (mean ± SD) as measured by MCA are summarized in the table below:

Table 11-3 Change in mean flow velocity in the MCA from baseline to Day 28

		FTY720 0.5 mg (cm/s) N = 30	FTY720 1.25 mg (cm/s) N = 29	Placebo (cm/s) N = 29
Analysis Variable				
Baseline	n	30	29	29
	Mean	58.3	55.6	55.9
	SD	12.57	10.93	12.55
Day 28	n	30	29	29
	Mean	54.3	54.6	52.2
	SD	10.60	9.41	10.88

MCA = middle cerebral artery, n= evaluable subjects SD = standard deviation, Source: Post-text Table 14.2-1.1.1

- Blood flow measurement in the MCA showed that there were only minor changes in the blood flow velocity from baseline to Day 28 in all treatment groups.

Platelet adhesion time by PFA100®

Platelet adhesion time measurements using PFA100® with the epinephrine/collagen cartridge are summarized in the table below:

Table 11-4 Change in mean platelet adhesion time (seconds) in response to epinephrine (PFA100® assay) from baseline to Day 28

		FTY720 0.5 mg (s) N = 30	FTY720 1.25 mg (s) N = 29	Placebo (s) N = 29
Analysis Variable				
Baseline	n	30	29	29
	Mean	113.3	110.4	115.3
	SD	16.05	19.19	16.44
Day 28	n	30	29	29
	Mean	121.1	117.9	125.7
	SD	23.53	21.78	17.85

n = evaluable subjects, SD = standard deviation
Source: Post-text Table 14.2-1.2.4

- Platelet adhesion time resulted in a 7 to 10% increase of adhesion time from baseline to Day 28 in all treatment groups.

Statistical analysis

The summary of the statistical analysis for the three primary endpoints is shown below:

Table 11-6 Summary of analysis of the change from placebo in primary endpoints at Day 28

Treatment	Statistic	Mean blood flow velocity MCA (cm/s)	Platelet adhesion time (s)	Central foveal thickness Left side (µm)	Central foveal thickness Right side (µm)
	Reference value	11.32	22.60	34.62	35.21
FTY720 0.5 mg	N	30	30	30	30
	LS Mean,	53.23	120.94	176.29	174.00
	Difference	0.58	-3.03	3.04	-3.58
	95% CI	-2,83, 3.99	-11.99, 5.94	-1.66, 7.75	-10.28, 3.11
	Conclusion	Non-Inferior	Non-Inferior	Non-Inferior	Non-Inferior
FTY720 1.25 mg	N	29	29	29	29
	LS Mean,	55.25	119.75	177.53	178.66
	Difference	2.60	-4.22	4.29	1.08
	95% CI	-0.83, 6.03	-13.31, 4.88	-0.41, 8.99	-5.65, 7.81
	Conclusion	Non-Inferior	Non-Inferior	Non-Inferior	Non-Inferior

Reference value: 20% of mean baseline value
CI = confidence interval, LS mean = least squares mean, MCA = middle cerebral artery, n = evaluable subjects
Source: Post-text Table 14.2-1.4.1

- The table shows that for all 3 primary parameters, the 2-sided 95% confidence intervals for the mean difference versus placebo fall within the pre-specified limit of non-inferiority ('reference value' = 20% of the overall average baseline value), and were thus considered non-inferior to placebo.

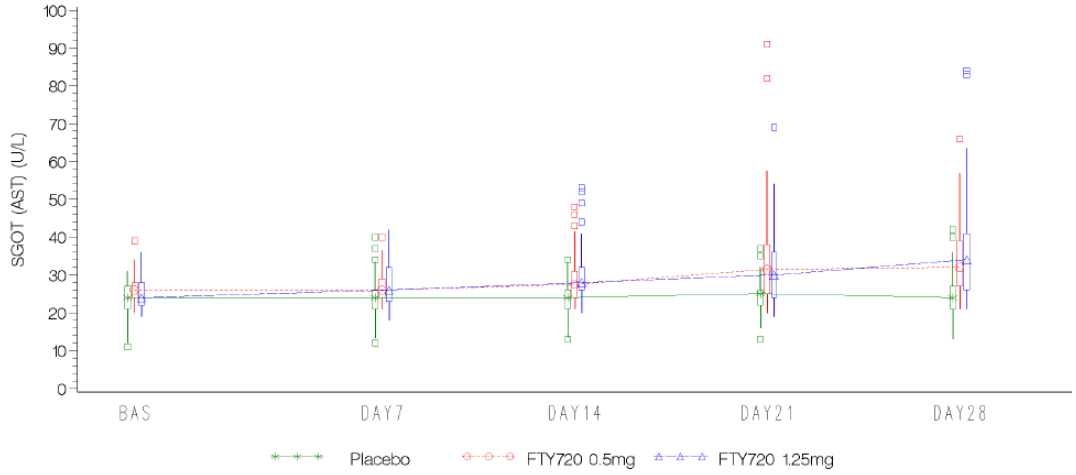
Abnormal biochemistry examinations:

Compared to no subjects in the placebo group, five subjects on FTY720 treatment (one on 0.5 mg and four on 1.25 mg) had ALT/SGPT elevations of 3-5 fold above baseline over the course of the study.

Box plot of SGOT over study period in all treatments are shown in the figure below (Similar patterns were observed for SGPT therefore plot not shown):

CFTY720D2113

Figure 14.3—1.3 (Page 2 of 5)
Box Plot of blood chemistry: TBIL SGOT SGPT GGT ALKPHS
Safety analysis set



The medians at each time point are joined by a line; the upper (lower) edge of the box represents the 75th (25th) percentile; a whisker is drawn from the upper (lower) edge of the box to the largest (smallest) value within 1.5xinterquartile range above (below) the edge of the box; values outside the whiskers are identified with a square.
Program: P:\ 1002133\ 8211201\ CFTY720D2113\ report\ pgm_saf\ F_BIO.SAS, 16:24 10NOV2009

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- The liver enzymes AST/SGOT, ALT/SGPT and GGT increased from baseline to study completion in both FTY720 treatment groups.
- Dose-dependent increases were observed for AST/SGOT and ALT/SGPT.
- The most pronounced mean increase was seen in ALT/SGPT in both FTY720 treatment groups.
- The mean values increased over the treatment period with the highest mean value at study completion.
- All three liver parameters manifested increasing outlier subjects in both FTY720 treatment group by day 14.

Conclusions:

- This study confirmed that 0.5 mg and 1.25 mg doses of FTY720 administered over 4 weeks at steady-state do not alter cerebrovascular blood flow or platelet function and do not increase the thickness of the macula in healthy volunteers.
- A 3-5 fold increase in liver enzymes over baseline was seen in five subjects receiving FTY720 treatment.
- The FTY720 and FTY720-P observed blood concentrations reached steady state by day 7, were close to those observed in multiple sclerosis patients and most subjects were well exposed to FTY720 and FTY720-P over the 28-day treatment.

4.1-4. HUMAN PD STUDIES
4.1-4.2 Patient PD

Study FTY720D 2102*:* **A double-blind, randomized, placebo-controlled, parallel, time-lagged, ascending, multi-centre, multiple-dose study to measure the magnitude and time course of the effect of FTY720 on FEV1 and other pulmonary function tests (FVC, FEF25-75%, and FEV1/FVC) in patients with moderate asthma**

PD in moderate asthma patients: MD FTY720 on pulmonary functions.

A brief overview of some essential components of the study design is given below:

Study Design	double-blind, randomized, placebo-controlled, parallel, time-lagged, ascending, multi-centre, multiple-dose study
Study Population	N=36 enrolled, 34 completed **Table 11-1 Demographic summary by treatment (Safety population)** FTY720 0.5mg N=9; FTY720 1.25mg N=9; FTY720 2.5mg N=9; Placebo N=9; All subjects N=36 **Age** (years) Mean 43 38 37 38 39 SD 11.1 15.0 13.0 9.5 12.1 Range 24, 61 21, 56 23, 58 26, 51 21, 61 **Gender** – n (%) Male 4 (44%) 2 (22%) 4 (44%) 4 (44%) 14 (39%) Female 5 (56%) 7 (78%) 5 (56%) 5 (56%) 22 (61%) **Race** – n (%) Caucasian 8 (89%) 6 (67%) 9 (100%) 8 (89%) 31 (86%) Black 0 1 (11%) 0 0 1 (3%) Asian 1 (11%) 1 (11%) 0 1 (11%) 3 (8%) Other 0 1 (11%) 0 0 1 (3%) **Weight** (kg) Mean 77.4 67.9 76.8 77.3 74.8 SD 17.12 7.73 13.38 11.08 12.90 **Height** (cm) Mean 170 165 172 170 169 SD 13.8 8.6 6.2 10.6 10.1 **BMI** (kg/m^2) Mean 26.5 25.2 25.8 26.6 26.0 SD 3.10 3.79 3.71 2.49 3.22 Source: Post-text Table 14.1-3.1
Dosage and Administration	Subjects were randomized equally into 1 of 3 treatment groups (n=12, 3:1): FTY720 0.5 mg, FTY720 1.25 mg and FTY720 2.5 mg for 10 days. Study medication was taken with water. FTY720 1.25 mg capsules: Batch #: H389ED FTY720 0.5 mg capsules: Batch #: AEUS/2006-0274 Placebo capsules: Batch number: H107DB <u>Restriction:</u> Alcohol was prohibited for 24 hours prior to dosing until study ompletion.
Sampling: Blood	For FTY720 and FTY720-P (Blood): Day 1 at pre-dose and at 1, 2, 4, 6, 8, 12, 16, and 24h postdose, Days 2, 3 and 7 at 6 hours post-dose and Day 10 at pre-dose and at 1, 2, 4, and 6h post-dose.

Analysis (Blood)	Method LC/MS/MS Lower Limits of Quantitation FTY720 0.08 ng/mL FTY720-P 0.1 ng/mL FTY720: Linear range : 0.08-30 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 5.8% Inter-day accuracy: < 2 % FTY720-P: Linear range : 0.1-20 ng/mL in blood Inter-day Precision (%CV for Quality Controls) : < 23.8% Inter-day accuracy: < 10 %
PK Assessment	FTY720 and FTY720-P in blood: Day 1: Cmax, b, Tmax, Tlag, AUC0-6h, b, AUC0-24h, b,; Day 10: Cmax, b, Tmax, AUC0-6h, b; Rac: AUC0-6h,b day10/ AUC0-6h,b day1.
PD Assessment	Forced Expiratory Volume in 1 second (FEV1), Forced Vital Capacity (FVC), Forced mid-Expiratory Flow (FEF 25-75%), and FEV1/FVC, Reversibility, Peak Expiratory Flow Rate (PEFR)
Safety Assessment	Assessment of physical examinations, ECGs, vital signs, spirometry assessments, standard clinical laboratory evaluations (hematology, blood chemistry, urinalysis), adverse event and serious adverse event monitoring

Results:

Pharmacokinetics Results:

FTY720 and FTY720-P pharmacokinetics observed in the different treatment groups on Day 1 and Day 10 are summarized in the table below:

Table 11-3 FTY720 and FTY720-P pharmacokinetics in the three treatment groups (n=9 in the 0.5 and 2.5 mg dose groups and n=8 in 1.25 mg dose group)

Day	Parameters	FTY720			FTY720-P		
		0.5 mg	1.25 mg	2.5 mg	0.5 mg	1.25 mg	2.5 mg
1	T_{lag} * (h)	0.00 (0.00- 1.05)	0.00 (0.00- 0.00)	0.00 (0.00- 0.00)	1.02 (0.00- 2.05)	0.00 (0.00- 0.00)	1.05 (0.00- 1.05)
	T_{max} * (h)	12.05 (11.95- 15.93)	12.08 (8.00- 23.75)	12.05 (6.05- 12.17)	6.05 (4.12- 8.00)	6.05 (4.05- 12.05)	6.12 (6.00- 8.05)
	$C_{max,b}$ # (ng/mL)	0.421±0.101 [0.411; 22.5]	1.04 ± 0.2 [1.023; 19.9]	2.099± 0.455 [2.057; 21.4]	0.534± 0.114 [0.524; 21.0]	1.64± 0.517 [1.58; 28.7]	2.50 ± 0.492 [2.46; 19.4]
	$AUC_{0-6h,b}$ # (ng/mL.h)	1.39 ± 0.498 [1.31; 36.7]	3.87 ± 1.094 [3.75; 26.7]	7.67 ± 1.35 [7.57; 17.2]	1.57 ± 0.616 [1.47; 40.9]	4.82 ± 1.62 [4.62; 30.3]	6.88 ± 1.055 [6.81; 15.4]
	$AUC_{0-24h,b}$ # (ng/mL.h)	8.20 ± 2.20 [7.97; 24.5]	20.1 ± 3.66 [19.8; 18.5]	40.3 ± 7.89 [39.7; 19.1]	7.18 ± 1.71 [7.00; 24.7]	21.9 ± 5.12 [21.4; 23.2]	35.07 ± 7.18 [34.4; 20.4]
10	T_{max} * (h)	4.00 (1.03- 6.05)	4.02 (0.98- 6.05)	4.25 (4.05- 6.05)	5.98 (2.05- 6.08)	5.03 (1.07- 6.05)	6.05 (4.05- 6.12)
	$C_{max,b}$ # (ng/mL)	2.52 ± 0.675 [2.45; 25.3]	5.71 ± 1.76 [5.47; 32.9]	10.4 ± 2.073 [10.15; 22.4]	2.01 ± 1.39 [1.76; 51.5]	4.096± 0.903 [4.011; 22.2]	6.54 ± 1.44 [6.41; 20.8]
	Predose # (ng/mL)	2.021 ± 0.572 [1.95; 28.7]	5.051 ± 1.80 [4.75; 40.4]	8.52 ± 2.15 [8.22; 30.9]	0.971± 0.211 [0.951; 21.8]	2.77 ± 0.779 [2.67; 30.4]	4.01± 1.34 [3.77; 40.7]
	$AUC_{0-6h,b}$ # (ng/mL.h)	13.9 ± 3.69 [13.5; 26.1]	32.7 ± 10.3 [31.2; 33.4]	57.9 ± 12.3 [56.6; 24.7]	8.34 ± 3.023 [7.96; 31.6]	21.2 ± 4.29 [20.8; 22.2]	31.1 ± 7.25 [30.3; 25.0]
	Rac#	10.8 ± 3.77 [10.3; 33.8]	8.57 ± 2.26 [8.33; 25.9]	7.78 ± 2.20 [7.47; 31.9]	6.045± 3.204 [5.43; 50.6]	4.63 ± 1.13 [4.50; 26.0]	4.62 ± 1.25 [4.46; 30.5]

#: arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation]; *: median (minimum-maximum)

Source: Post-text Table 14.2-1.1, Post-text Table 14.2-1.2, and Post-text Table 14.2-1.3.

Day 1 FTY720 and FTY720-P pharmacokinetics

- The median Tmax was about 12 hours for the 3 groups (range: 6.05- 23.75 hours).
- The variability in AUC0-24h,b, AUC0-6h,b and Cmax,bwas small to moderate for all doses (CV= 17.2% to 36.7%).
- The variability in AUC0-24h,b and AUC0-6h,b decreased with increasing doses.
- FTY720 mean AUC0-24h,b, AUC0-6h,b and Cmax,b increased in an apparent proportional manner with the dose.
- FTY720-P variability in AUC0-24h,b, AUC0-6h,b and Cmax,b, as measured by the coefficient of variation of the geometric mean, ranged from 15.4% to 40.9% for all doses.
- FTY720-P mean AUC0-24h,b, AUC0-6h,b and Cmax,b also increased in an apparent proportional manner with the dose.

Day 10 FTY720 and FTY720-P pharmacokinetics

- The variability in FTY720 and FTY720-P Day 10 AUC0-6h,b was moderate for all doses with values ranging from 22.2% to 33.4%.
- FTY720 and FTY720-P mean AUC0-6h,b and predose concentrations mean values increased in an apparent proportional manner with the dose from 0.5 mg to 1.25 mg but in an under-proportional manner from 1.25 mg to 2.5 mg.

- The accumulation ratio, Rac, was greater for FTY720 than for FTY720-P and decreased slightly with increasing doses (except for FTY720-P, between 1.25 mg and 2.5 mg where it remained constant).

Pharmacodynamics Results:

Primary pharmacodynamic results: AUEC0-6h FEV1

The primary PD variable was the baseline-adjusted AUEC0-6h FEV1 on Day 1 which was calculated as the AUEC FEV1 over the 6-hour PFT profile on Day 1 divided by the AUEC0-6h FEV1 on Day -1. The secondary variable was the baseline-adjusted AUEC0-6h FEV1 on Day 10 which was similarly calculated as the primary PD variable.

Descriptive statistics by treatment and the results of the statistical analysis of the effect of FTY720 treatment on those variables is summarized in table below:

Table 11-2 Summary of statistical analysis of baseline-adjusted /AUEC0-6h FEV1 days 1 and 10 (PD analysis set)

Day	Treatment	% change from baseline Geometric mean (95% CI)	% change from placebo Geometric mean (95% CI)	P-value
1	Placebo	-4.24 (-6.79, -1.62)		
	FTY720 0.5 mg	-3.41 (-5.99, -0.76)	0.87 (-2.93, 4.81)	0.65
	FTY720 1.25 mg	-5.03 (-7.56, -2.42)	-0.82 (-4.55, 3.05)	0.66
	FTY720 2.5 mg	-4.97 (-7.52, -2.35)	-0.76 (-4.48, 3.09)	0.68
10	Placebo	-4.25 (-7.49, -0.90)		
	FTY720 0.5 mg	-2.96 (-6.26, 0.46)	-1.35 (-3.49, 6.44)	0.58
	FTY720 1.25 mg	-10.10 (-13.33, -6.75)	-6.11 (-10.72, -1.25)	0.016
	FTY720 2.5 mg	-7.53 (-10.69, -4.27)	-3.43 (-8.02, 1.40)	0.15

CI = confidence interval
Source: Post-text Table 14.2-2.2

- On day 1, a mean reduction from baseline in AUEC0-6h FEV1 of between -3 and -5% was observed for all treatments with no evidence of any difference between placebo and active treatment.
- By day 10 the response was more variable ranging from -3 to -10%. The mean reduction of -10.1% observed in the FTY720 1.25 mg group was significantly larger than that observed for placebo on day 10 (P=0.016). An increased magnitude of effect, -7.5%, was also measured for the FTY720 2.5 mg group compared to placebo (P=0.15).
- No effect was seen for the FTY720 0.5 mg group when compared to placebo.

Emax1-6h versus AUEC0-6hr FEV1

- Maximal effect of FTY720 on FEV1 over the six hours post dose, Emax1-6h FEV1 showed a pattern of response to FTY720 treatment almost identical to that of AUEC0-6h FEV1.
- FEF25-75, FVC, and FEV1/FVC are similarly analyzed as FEV1 described earlier.
- FEF25-75 is a measure of airway resistance which is closely related to FEV1 but more closely detects changes in small airway resistance. Although there were no significant effects of FTY720 treatment on either AUEC0-6h FEF25-75 or Emax1-6h FEF25-75, there was a trend on day 10 for the two highest doses of FTY720, 1.25 mg and 2.5 mg, to result in -11% to -12% change in AUEC0-6h FEF25-75.
- Neither FVC nor FEV1/FVC, as measured by either Emax1-6h or AUEC0-6hr, was affected by FTY720 treatment.

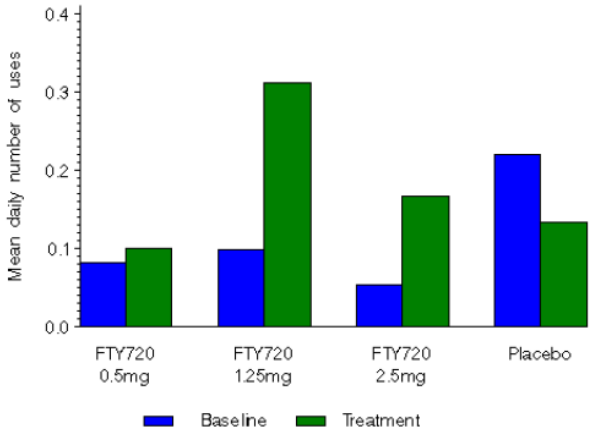
Secondary pharmacodynamic results

Secondary pulmonary parameters, FEV1, FEF25-75, FVC, FEV1/FVC over time post dose were analysed by the sponsor. However, since detail pulmonary function changes by FTY720 will be reviewed by safety reviewer and medical officer, therefore the data are not presented and discuss here.

Rescue SABA intake

Mean daily number of the use of rescue medication in all treatments is shown in the figure below:

Figure 11-2 Mean daily number of use of rescue medication (PD analysis)



Source: Post-text Table 14.3-6.4

- There was a significant, approximately 500% increase in mean rescue SABA use in either the FTY720 1.25 or 2.5 mg treatment groups when compared to placebo treatment (p=0.032 and p=0.063, respectively).
- Mean rescue SABA use in the FTY720 0.5 mg treatment group was not significantly different from placebo, although there was a numerical increase of 190% (p=0.26).
- The placebo group manifested a decrease in mean rescue SABA use during the treatment.
- The FTY720 0.5 mg treatment group had similar mean rate of rescue SABA use in both the run-in and treatment phase of the study.
- Both the 1.25 and 2.5 mg FTY720 treatment groups had clearly increased rescue SABA use.

Conclusions:

- The observed FTY720 and FTY720-P pharmacokinetics in this study are consistent with that from previous studies.
- FTY720 treatment can be safely started and maintained for at least ten days in patients with moderate asthma.
- FEV1 AUEC0-6h, the primary endpoint of this study, was identical for the FTY720 0.5 mg, the planned clinical dose, and placebo treatment groups on days 1 and 10.
- FEF25-75 AUEC0-6h, a secondary endpoint of this study and a more sensitive measure of small airway resistance was also identical for both FTY720 0.5 mg and placebo treatment groups on days 1 and 10.
- At FTY720 doses at 2.5 fold (1.25 mg) and 5 fold (2.5 mg) the planned clinical dose, a mild decrease (<20%) of FEV1 AUEC0-6h and FEF25-75 AUEC0-6h was measured.
- There was no significant effect of FTY720 0.5 mg on rescue SABA use compared to placebo over the course of the study.
- At FTY720 dose of 1.25 mg and 2.5 mg there was a significant increase in the rate of rescue SABA use of approximately 5 fold.

4.2 APPENDIX II

CLINICAL PHARMACOLOGY FILING FORM

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	N 22-527	**Brand Name**	Gilenia®
OCP Division (I, II, III, IV, V)	DCP-I	**Generic Name**	Fingolimod (FTY720)
Medical Division	HFD-120	**Drug Class**	sphingosine 1-phosphate (S1P) receptor modulator
OCP Reviewer	Ju-Ping Lai Jagan Parepally	**Indication(s)**	Relapsing Remitting Multiple Sclerosis (RRMS)
OCP Team Leader	Angela Men	**Dosage Form**	Capsule (0.5 mg)
Pharmacometrics Reviewer	Joo-Yeon Lee	**Dosing Regimen**	0.5 mg once daily
Date of Submission	12/21/2009	**Route of Administration**	Oral
Estimated Due Date of OCP Review	4/19/2010	**Sponsor**	Novartis
Medical Division Due Date	4/26/2010	**Priority Classification**	Priority
PDUFA Due Date	6/21/2010		

Clin. Pharm. and Biopharm. Information

The sponsor submitted this original NDA 22527 (NME) on December 21st, 2009 seeking for approval of Gilenia® (Fingolimod, FTY720) for the treatment of Relapsing Remitting Multiple Sclerosis (RRMS). This NDA is under the priority review classification.

Fingolimod is a sphingosine-1-phosphate (S1P) receptor modulator. Fingolimod phosphorylated to the active moiety, *S*-enantiomer fingolimod-P. Fingolimod-P is a functional antagonist of sphingosine-1-phosphate (S1P) receptor which reduces the peripheral lymphocyte count mediated by down-modulation of the S1P1 receptor on lymphocytes. This results in a reduced egress of lymphocytes from the lymph nodes; in particular auto-aggressive T-cells that perform a central role in the MS inflammatory disease process are prevented from recirculating to the CNS. Fingolimod-P reversibly dephosphorylated back to the inactive form fingolimod. At steady state, fingolimod and fingolimod-P are in dynamic equilibrium.

The proposed product is hard capsule, with only one proposed strength of 0.5 mg. The recommended dosing regimen is 0.5 mg once-daily administered orally.

There are 56 human study reports, including 31 clinical pharmacology studies, which include 1 exposure-response report, 1 population PK report and 1thorough QT study, and for efficacy and safety, 4 controlled trials, 5 cross study reports and 16 other study reports. A clinical pharmacology study (D2109) will be submitted at 120 day safety update under the agreement with the Agency at the Pre-NDA meeting. The submission also contains 15 in vitro studies for information regarding protein binding (4 reports), hepatic metabolism and drug interactions (8 reports) and transporters (3 reports) and 7 bioanalytical validation and assay reports for fingolimod, its only active metabolite, fingolimod-P, and two inactive metabolites, M2 and M3. In addition, one model based report characterizing the effect of FTY720 on heart rate in healthy volunteers was provided as an analysis from more than one study.

All clinical studies were conducted with hard gelatin capsule formulations. Early clinical studies utilized formulations that included (b) (4) (clinical service form, CSF). Subsequent clinical trials, including all phase II and phase III studies of the MS program, used the formulation intended for the market with yellow/white capsule shells (final market image, FMI). The CSF and the FMI formulations are bioequivalent. Absolute bioavailability of fingolimod was assessed for the FMI.

This NDA consists of

- **Biopharmaceutics studies (5 studies):**
  1. BA: (3 studies)
     FTY720A0106: Food effect, SD 1 mg CSF, fasted vs high fat, n=14 (No effect on AUC and Cmax for FTY720, FTY720-P not measured)
     FTY720A0108: Absolute BA, 1 mg IV infusion 2h vs 1.25 PO FMI, n=11 (mean apparent absolute oral BA= 93%)
     FTY720A0107: Food effect, SD 1.25 mg FMI, fasted vs fed with high-fat, n=29 (No effect on AUC and Cmax for FTY720 and AUC for FTY720-P but Cmax↓34%, no clinical impact?)
  2. Comparative BA/BE: (2 study)
     FTY720A0116: Relative BA, SD 1.25 and 2.5 mg, CSF vs FMI, n=25 (BE)
     FTY720A2309: BE, SD 1.25x2 vs 2.5 mg FMI, n=34 (BE for FTY720, BE for FTY720-P Cmax but not AUC)
  3. Analytical methods: (7 Methods)

- **In vitro studies pertinent to PK using human biomaterials (15 studies):**
  1. Plasma protein binding: (4 studies) (Fingolimod and fingolimod-P are highly protein bound (>99.7%))
  2. Hepatic metabolism and drug interaction: (8 studies) (mainly via CYP4F2, not inducers or inhibitors of major cytochrome P450 isoenzymes)
  3. Studies using other human biomaterials: (3 studies)

- **Human pharmacokinetic studies (12 studies):**
  1. Healthy subject PK and tolerability: (2 studies)
     (dose-proportional SD 0.25-40 mg, MD QD 0.125-5 mg)
     FTY720A 2215: SAD high dose (5-40 mg), n=56
     FTY720A 2217: Mass balance, SD 4.47 mg, n=4 (Urinary and faecal excretion of radioactivity reached 81 % and 11 %)
  2. Patient PK and initial tolerability study reports: (3 studies)
     FTY720A B101: SAD (0.125 – 3.5 mg)in renal transplant patients with cyclosporine, n=20 (dose-proportional)
     FTY720A B102: MAD (0.125 – 5 mg)in renal transplant patients with cyclosporine, n=65
     FTY720A 0115: SD 0.07 mg/kg pediatric renal transplant patients with cyclosporine, n=7
  3. Intrinsic factors: (4 studies)
     FTY720A 0112: SD 5 mg, in stable cirrhotic liver disease patients with mild (n=8) or moderate (n=8) hepatic insufficiency (AUCb was increased by 12% and 44% in mild and moderate)
     FTY720A 2204: SD 1.25 mg in severe hepatic insufficient patient
     FTY720D 2108: SD 1.25 mg in severe renal insufficient patient
     FTY720A 2304: SD and MD 1.25 and 2.5 mg, Japanese vs Caucasien
  4. Extrinsic factors: (2 studies)
     FTY720A 0107: DDI, FTY720 1 mg SD vs steady state cyclosporine 200 mg BID
     FTY720A 2311: DDI, FTY720 5 mg SD vs ketoconazole 200 mg BID x 9 days.
  5. **Population PK (1 report)**

- **Human pharmacodynamic studies (14 studies):**
  1. Healthy PD and PK/PD: (12 studies)
     FTY720A 0114: DDI, FTY720 SD vs atenolol or diltiazem.
     FTY720A 0118: DDI, FTY720 SD vs i.v. atropine.
     FTY720A 0119: DDI, FTY720 SD vs isoproterenol.
     FTY720A 2213: effects of FTY720 (1.25 and 5 mg) on heart rate and rhythm.
     FTY720A 2213E1: effects of a desensitizing dosing regimen of FTY720 on heart rate.
     FTY720A 2305: effect of various inter-treatment intervals on the heart rate.
     FTY720A 2306: MAD, safety and tolerability
     FTY720D 2101: QT
     FTY720D 2105: MD, pulmonary and cardiac pharmacodynamics of FTY720 (0.5 & 1.25 mg)
     FTY720D 2106: DDI, SD oral inhaled salmeterol on the negative chronotropic effect
     FTY720D 2110: small doses of FTY720 on the negative chronotropic effect of FTY720
     FTY720D 2113: effect of FTY720 on mean flow velocity in cerebral vessels, platelet function, and macular thickness
  2. Patient PD and PK/PD: (2 studies)
     FTY720D 2102: magnitude and time course of the effect of FTY720 on FEV1 and other pulmonary function tests
     FTY720D PKPD: Modeling of the exposure-response relationship

- **Efficacy and safety studies (25 studies):**
  1. Controlled trials: (4 studies)
  2. Cross study reports: (5 reports) Including one modeling report on heart rate in healthy subjects
  3. Other study reports: (16 reports)

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x	7		
I. Clinical Pharmacology				
Mass balance:	x	1		
Isozyme characterization:	x	8		
Transporters:	x	3		
Blood/plasma ratio:	x	(1)		
Plasma protein binding:	x	4		
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	x	1		
multiple dose:				
Patients-				
single dose:	x	2		
multiple dose:	x	1		
Dose proportionality -				
fasting / non-fasting single dose:	x	2		
fasting / non-fasting multiple dose:	x	1		
Drug-drug interaction studies -				
In-vivo effects on primary drug:	x	2PK+(4PD)		
In-vivo effects of primary drug:				
In-vitro:				

Subpopulation studies -				
ethnicity:	x	1		
gender:				
pediatrics:				
geriatrics:				
renal impairment:	x	3		
hepatic impairment:	x	2		
Obese subject:				
PD -				
Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:	x	14		12 in healthy/2 in patients
Phase 3 clinical trial:				
Population Analyses -		1		
Data rich:	x	(3)		
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability	x	1		
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:	x	1		
Bioequivalence studies -				
traditional design; single / multi dose:	x	1		
replicate design; single / multi dose:				
Food-drug interaction studies	x	2		
Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References	271			
Total Number of Studies				
	16 PK + 1 Pop PK+ 1 Exp.-Res.+ 12 PKPD+ 1 QTc+ 15 in vitro+ 7 Assay+ Literature			

Filability and QBR comments		
I.	**"X" if yes**	Comments
II. Application filable?		Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
III. Comments sent to firm?		
QBR questions (key issues to be considered)	• Is dose proportionality established in the therapeutic range? • Is there any food effect? • Is dose adjustment necessary for concomitant use of other drugs? • Is dose adjustment necessary for renal or hepatic dysfunction patients? • Are there exposure(dose)-response(efficacy and safety) relationships?	
Other comments or information not included above		
Primary reviewer Signature and Date	Ju-Ping Lai	
Secondary reviewer Signature and Date	Angela Men	

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	x			
2	Has the applicant provided metabolism and drug-drug interaction information?	x			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	x			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?				Would be a review issue
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	x			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	x			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x			
General					
18	Are the clinical pharmacology and biopharmaceutics studies of	x			

	appropriate design and breadth of investigation to meet basic requirements for approvability of this product?				
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			x	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- Please provide all datasets and programs with outputs for population PK abnd exposure-response analyses as following direction below;
"All datasets used for model development and validation should be submitted as a SAS transport files (*.xpt). A description of each data item should be provided in a Define.pdf file. Any concentrations and/or subjects that have been excluded from the analysis should be flagged and maintained in the datasets.

Model codes or control streams and output listings should be provided for all major model building steps, e.g., base structural model, covariates models, final model, and validation model. These files should be submitted as ASCII text files with *.txt extension (e.g.: myfile ctl.txt, myfile out.txt)."

- If all datasets / programs already have been submitted with valid format, please provide an exact location.

Reviewing Clinical Pharmacologist Date

Team Leader/Supervisor Date

4.3 APPENDIX III
QT Consult

Interdisciplinary Review Team for QT Studies Consultation: Thorough QT Study Review

IND	70,139
Generic Name	FTY 720 (Fingolimod)
Sponsor	Novartis
Indication	Treatment of Multiple Sclerosis
Dosage Form	Capsule
Drug Class	Immuno-modulator
Therapeutic Dosing Regimen	1.25 mg q.d.
Duration of Therapeutic Use	Acute or Chronic
Maximum Tolerated Dose	40 mg q.d. single dose, 5 mg q.d. multiple dose
Submission Number and Date	SDN 133, 30 Jul 2008
Review Division	DNP / HFD 120

1 SUMMARY

1.1 OVERALL SUMMARY OF FINDINGS

This study failed to exclude a 10 ms prolongation of the QT interval for both doses of FTY720 (1.25 and 2.5 mg). At 6 hours post-dosing on Day 7, the maximum mean ΔΔQTcI for both 1.25-and 2.5-mg doses was 10 ms with an upper one-sided 95% CI of ~14 ms (see Table 1).

We do not have confidence in the accuracy of the estimated effect of administering FTY720 on the QTc interval for the following reasons:

1. The positive control, a single oral dose of 400 mg moxifloxacin, failed to have the expected effect on ΔΔQTcI (change from baseline and placebo corrected); the largest ΔΔQTcI for moxifloxacin was about 10.5 ms and occurred at 6 and 8 hours post-dose. This profile is not likely since the T_{max} of moxifloxacin observed in this study was 3 hours (see Figure 5). This is especially relevant, since the largest ΔΔQTcI for FTY720 was of the same magnitude and occurred at the same time points as that observed for moxifloxacin (see Figure 12).
2. Despite a 2-fold increase in the exposure to FTY720 plasma concentrations, there was no dose-response relationship for QT prolongation. There was also not a concentration-QTc relationship for FTY720 and its metabolite FTY720-P. This does not, however, rule out the existence of a positive exposure-response relationship because of the small range of steady-state concentrations observed on Day 7 (see Figure 3 and Figure 4).

We recommend baseline and periodic on-therapy ECGs are collected for safety assessments in clinical trials irrespective of the results of the TQT study because bradycardia and conduction defects have been noted in the clinical program (although

1

there have been no cases of Mobitz II or 3rd degree blocks). According to the guidance to investigators in the current IB, vitals signs (including BP, HR and ECG) are being monitored pre-dosing and following a 6-hour observation period after administration of FTY720.

The study was a randomized, multiple-dose, four-arm parallel study in which a total of 199 subjects were administered 1.25 mg steady-state FTY720, 2.5 mg steady-state FTY720, 400 mg moxifloxacin or placebo. Overall findings are summarized in the Table 1.

Table 1: The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for FTY720 (1.25 mg and 2.5 mg) and the Largest Lower Bound for Moxifloxacin (FDA Analysis)

Treatment	Time (hour)	ΔΔQTcI (ms)	90% CI (ms)
FTY720 1.25 mg	6	10.0	13.6
FTY720 2.50 mg	6	10.5	14.0
Moxifloxacin 400 mg*	6	10.5	5.7

* Multiple endpoint adjustment is not applied. The largest lower bound after Bonferroni adjustment for 3 timepoints is 4.3 ms. Note: The sponsor specified times 1.5, 3, and 6 as the times to be tested for Moxifloxacin. At 8 hours the estimated ΔΔQTc was 11.0 ms and the unadjusted lower bound of the 90% C.I. was 7.5 ms.

The supratherapeutic dose (2.5 mg) produces mean C_{max} values 2-fold higher than the mean C_{max} for the therapeutic dose (1.25 mg). These concentrations are similar to the predicted worst case scenario (drug interaction with ketoconazole). It is expected from drug interaction studies that co-administration of FTY270 with ketoconazole can elevate FTY720's mean C_{max} as much as 1.2-fold higher than the C_{max} of the 1.25 mg dose. Hepatic impairment increases FTY720's exposure by 2-fold without altering C_{max}. The supratherapeutic dose (2.5 mg) produces AUC values 2-fold higher than mean AUC for therapeutic dose (1.25 mg).

1.2 QT Interdisciplinary Review Team's Comments

The sponsor used the time average of the subjects' baseline measurements as the baseline. We recommend a time-matched baseline adjustment for parallel studies to account for within subject diurnal variation. The use of time-matched baseline adjustment in this study resulted in a few more significant increases in QT according to the categorical analysis and an additional time-point in the 1.25-mg group in which ΔΔQTcI was greater than 10 ms.

2 BACKGROUND

FTY720, a synthetic sphingosine 1 phosphate (S1P) receptor modulator, has been previously evaluated in renal transplantation and is currently in clinical development by Novartis for multiple sclerosis (MS). The renal transplant program has been discontinued.

2.1 Market Approval Status

FTY720 is not approved for marketing in any country

2.2 PRECLINICAL INFORMATION

Source: IB, 4 June 2007

> "No effect of FTY720 treatment on heart rate or electrocardiograms (ECGs) was seen upon multiple dosing (subchronic to chronic) in animal toxicology studies (dogs and monkeys). FTY720 did not show the potential to induce QT prolongation when tested in vitro (human ether-a-go-go-related gene: HERG assay in HEK293 cells; Purkinje fibers of sheep or rabbits) or in vivo.
>
> "Using telemetric devices in rats, dogs, or monkeys, it could be shown that FTY720 induced a moderate decrease in heart rate and an increase in blood pressure after a single p.o. dose.
>
> "Short periods of sinus arrest were seen in rats or dogs. In vitro experiments in the rabbit Langendorff model confirmed an effect of FTY720-P on the level of the sinoatrial (SA) node. The effects of FTY720 on the heart rate were not influenced by the circadian rhythm (vagotonus) in dogs, and could be reversed in vitro or in vivo by atropine or isoproterenol."

Reviewer's Comments: Data provided in vivo and in vitro do not suggest an effect on QT. However, it is difficult to make an assessment since no doses or in vitro concentrations tested are provided in the IB. Bradycardia reported may be due to a direct effect on the sinus node or through the activation of an inward-rectifying G-alpha-protein regulated potassium channel (GIRK/IKAch). The bradycardia is reverted by atropine.

2.3 PREVIOUS CLINICAL EXPERIENCE

Source: IB, 4 June 2007

> "As of end of April 2007, with more than 1000 patients randomized in the phase III program, the following 15 SAEs have been reported after first dose administration (please note that study medication remains blinded -FTY 720, placebo or Interferon β-1a, in these cases):
>
> - Three cases of second degree Mobitz I (Wenckebach) AV block in the first 24 hour, of whom two cases were symptomatic (one was successfully treated with atropine and the other did not require treatment) and one asymptomatic case. Study drug was discontinued in these patients. The AV block resolved within the first 24 hours in all patients.
> - One case of symptomatic chest pain (chest pain thought not to be of cardiac origin by the investigator) and five cases of asymptomatic bradycardia. None of these cases required treatment.
> - Three cases of first degree AV block (prolonged PR interval) detected on ECG. In all of these cases, the first degree AV block had resolved within 24 hours of onset.
> - One case of tachycardia secondary to anxiety and one case of asymptomatic EKG changes ("different" QRS morphology in lead V2 which the local cardiologist believes was not indicative of a conduction abnormality and which had resolved within 24 hours).

- One case of marginal QT interval prolongation which both the local cardiologist and DSMB cardiologist assessed to be within the normal range of physiologic variation.

"No SAE of high grade AV block (Mobitz II or third degree) have been reported as of end April 2007.

"In summary, FTY720 causes a reduction in heart rate (by an average of 13-16 bpm at 1.25 mg) that is maximal upon treatment initiation and attenuates over time, despite increasing blood levels with continued dosing. From all studies completed to date, it appears that this is an expected pharmacodynamic effect that is clinically benign, well tolerated and manageable.

"In healthy volunteers (study A2213) treated with 1.25 and 5 mg FTY720 for 7 days showed that FTY720 treatment was associated with 5-12 mm Hg decrease in systolic blood pressure on Day 1 of FTY720 dosing. Over the remaining 6-day course of the study, this difference in systolic blood pressure between placebo- and FTY720-treated groups diminished to approximately 2-3 mm Hg.

"No clear data suggesting an increase in blood pressure values has been observed in patients treated with FTY720 in the renal transplant studies, i.e. the changes in blood pressure seen in the phase II and III trials were balanced between all treatment arms. Interpretation of BP data in transplant patients is complicated by the fact that the majorities of patients undergoing transplantation has hypertension and are on multiple drugs known to affect BP.

"In the phase II MS study, there was an initial reduction in mean blood pressure (5 to 6 mm Hg lower than the baseline value) within 4 to 5 hours after the administration of FTY720, followed by a sustained elevation (4 to 6 mm Hg higher than the baseline value) after 2 months of treatment, with no further increase during the extension study."

Reviewer's Comments: Five deaths due to cardiac arrest are reported in renal transplant patients (with other co-morbidities) although there was no report of associated QT prolongation. There are no reports of TdP or seizures.

Consistent with the depressant effect on the sinus node causing bradycardia noted in vitro, conduction defects have been noted in the clinical program. However, there have been no reports of Mobitz II or third degree heart blocks. Leukopenia, elevated LFTs, increased airway resistance and twofold increased risk for macular edema have also been noted.

2.4 CLINICAL PHARMACOLOGY

Appendix 5.1 summarizes the key features of FTY720's clinical pharmacology.

3 SPONSOR'S SUBMISSION

3.1 OVERVIEW

The sponsor submitted the study report for FTY720D 2101 including electronic datasets and waveforms to the ECG warehouse.

3.2 TQT STUDY

3.2.1 Title

A randomized, parallel group, multiple-dose study to evaluate the effects of FTY720 on cardiac safety in healthy subjects vs. placebo with positive control (moxifloxacin hydrochloride)

3.2.2 Protocol Number

FTY720D 2101

3.2.3 Study Dates

Study initiation date: 23-Jan-2007 (first subject screened)

Study completion date: 22-Jun-2007 (last subject completed)

3.2.4 Objectives

Primary objective:

- To measure the effect of FTY720 on the QTcI interval compared to placebo on Day 7 in healthy subjects.

Secondary objective:

- To measure the effect of FTY720 on cardiac intervals (PR, QRS, RR) and QTc corrected by Fridericia and Bazett's formulae.
- To explore the PK/PD relationship of FTY720 and FTY720-phosphate (FTY720-P) blood concentrations (and/or PK parameters) and QTcI changes in healthy subjects, should an effect on the QTcI be found.
- To assess the pharmacokinetics of FTY720 and FTY720-P after multiple FTY720 administrations

3.2.5 Study Description

3.2.5.1 Design

This was a randomized, placebo-controlled, multiple oral dose study conducted in parallel groups of healthy adult male and female subjects. Moxifloxacin was administered under open-label conditions. FTY720 and matching placebo were administered under double-blind conditions.

3.2.5.2 Controls

The Sponsor used both placebo and positive (moxifloxacin) controls.

3.2.5.3 Blinding

Moxifloxacin was administered under open-label conditions.

3.2.6 Treatment Regimen

3.2.6.1 Treatment Arms

Participating subjects were to be randomized to one of the four treatment groups, moxifloxacin treatment group, placebo group, FTY720 1.25-mg group, or FTY720 2.5-mg group (21 to Moxifloxacin and 56 to each of the other groups). For the FTY720 treatment groups, a loading dose regimen was used over a 4 day period to achieve steady state before the 1.25-mg and 2.5-mg doses were administered from Day 5 to Day 7.

3.2.6.2 Sponsor's Justification for Doses

"The FTY720 2.5 mg dose was chosen based on the following:

1. This dose is a 2-fold multiple of the highest planned clinical dose (i.e. 1.25 mg)
2. Based on the fact that FTY720 is metabolized predominately by CYP450 4F, there are no clinically significant drug-drug interactions anticipated in view of possible increased FTY720 systemic exposure. Based on *in vitro* studies, ketoconazole was identified as the drug which would potentially have the greatest effect on FTY720. This was then verified in a well designed healthy volunteer study in which ketoconazole increased mean FTY720 exposure by 2-fold.
3. Regarding organ dysfunction, renal insufficiency had no effect on FTY720 systemic exposure. In the setting of severe hepatic insufficiency, mean FTY720 exposure in patients increased 2-fold.
4. In the scenario in which a subject on chronic dosing of 1.25 mg FTY720 daily inadvertently takes three doses of 1.25 mg on a particular day, the FTY720 concentration that day has been estimated to increase by about 20%. In fact, it has been estimated that a subject would have to take 10 additional FTY720 1.25 mg doses in one day to increase FTY720 steady state concentration by 2-fold on this particular day.
5. Finally, a steady-state loading regimen for 5.0 mg (4-fold multiple) was found to cause transient, mild shortness of breath or chest tightness in 5 of 12 healthy volunteers receiving this regimen (Study FTY720A2305). However, in this same study using a steady-state loading regimen for 2.5 mg (2-fold multiple)
6. It is not possible to quickly load subjects to steady state over a day or two because doses of FTY720 ≥20 mg are associated with symptomatic increases in airway resistance (FTY720A2215).
7. It is not possible to use doses at higher multiples of the projected therapeutic dose/exposure of FTY720 as recommended in the ICH E14 guideline because subjects would remain at biologically active levels of drug for many weeks post end of study."

Reviewer's Comment: The supratherapeutic dose of 2.5 mg is 2-fold higher than the therapeutic dose of 1.25 mg. The increase in exposure over the clinical dose is shown in Table 4. *The current expected high clinical exposure scenario is either severe hepatic insufficiency which would increase exposure 2-fold or drug interaction with ketoconazole which would increase C_{max} and exposure less than 2-fold. The increase in exposure and*

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C_{max} observed in this study just meet this scenario. Given the experimental limitations in achieving greater than 2-fold changes in C_{max} described by the sponsor, the dose is acceptable. With such a small margin between the C_{max} observed in this study and that from the high clinical exposure scenario, close attention must be paid in future development to yet undiscovered factors which may increase exposure, such as age.

3.2.6.3 Instructions with Regard to Meals

Doses were administered within 5 minutes after a standard breakfast. Meals were to be consumed and doses taken between 07:30 and 09:00 on each occasion.

Reviewer's Comment: A high-fat breakfast had no effect on C_{max} or AUC. Administration after a standard breakfast is therefore acceptable.

3.2.6.4 ECG and PK Assessments

Triplicate ECG measurements were obtained on Day -1 at 0, 1.5, 3, 4, 6, 8 10, 12, 14, 16, 18, 20, 22 and 34 hours post-dose and on Day 7 at 0, 1.5, 3, 6, 8 and 12 hours post-dose. Blood samples for measurement of FTY720 and FTY720-P were obtained pre-dose on Days 2, 4, 5 and 6 and on Day 7 pre-dose, 0.75, 1.5, 3, 6, 8, 12, 16 and 24 hours post-dose. Moxifloxacin concentrations were measured on Day 7 predose, 0.75, 1.5, 3, 6, 8, 12, 16 and 24 hours post-dose.

Reviewer's Comment: The timing of the ECG and PK assessments was adequate. Since steady state was achieved by Day 7 and fluctuations in concentrations of FTY720 and FTY720-P were small, the exact timing of the ECG and PK assessments were not critical.

3.2.6.5 Baseline

The sponsor used the time average of the subjects' baseline measurements as the baseline values for assessing changes from baseline at each post-baseline assessment time.

3.2.7 ECG Collection

Continuous 12-Lead ECGs were collected using the Mortara H-12(+) High Fidelity 12-Lead Digital Holter Recorder at the time points specified above. The ECG signal was recorded on a compact flash memory cards (flash cards) provided to the sites. eRT (core lab) generated three 10-second, 12-Lead ECG tracings, approximately 1 minute apart, at each time point specified in the protocol.

12-lead ECGs were monitored at regular times during the study.

Reviewer's Comments: Further details about ECG interpretation and acquisition were unavailable.

3.2.8 Sponsor's Results

3.2.8.1 Study Subjects

Overall 199 subjects (male and female with a normal baseline ECG, 18-50 yrs of age, BMI between 18-30 kg/m^2) were randomized to the study; 189 subjects completed the study. Ten subjects were discontinued and replaced.

Two subjects discontinued due to the adverse events during the treatment period and one subject withdrew consent. Seven subjects discontinued during the placebo baseline period on Day-1.

3.2.8.2 Statistical Analyses

3.2.8.2.1 Primary Analysis

Analysis Plan

The primary analysis endpoint for this study was "change from mean baseline in QTcI on Day 7" (=Y). This endpoint was obtained for each of the post baseline time points by subtracting the time-averaged baseline values from the Day 7 values of QTcI at the following post-dose time points:

For FTY720 and placebo treatment groups: 0, 1.5, 3, 6 and 12 hr.

For moxifloxacin treatment group: 1.5, 3 and 6 hr.

All subjects were to have ECGs in triplicate at the following time points on Day 7: 0, 1.5, 3, 6 and 12 hr. For time-averaged changes, the baseline value was obtained as follows: 1) the mean of the 3 values at each time point at baseline was obtained, and 2) these resulting values were averaged to obtain the time-averaged baseline value.

Denote $\Delta(t)$ as the mean difference of primary endpoint of two treatments at time point t. For the primary endpoints, the hypotheses

$$H_o: \Delta(t) \geq 10 \text{ ms versus } H_1: \Delta(t) < 10 \text{ ms}$$

were to be tested for each of the specified time points separately. The one-sided t-test (α=5%) was to be used for each time point. It would be concluded that there is no effect on the QTcI interval induced by FTY720 if all tests reject the null hypotheses simultaneously or, equivalently, if the upper bound of 95% one-side confidence interval (based upon the t-statistic) at each time point lies below 10 ms.

Results

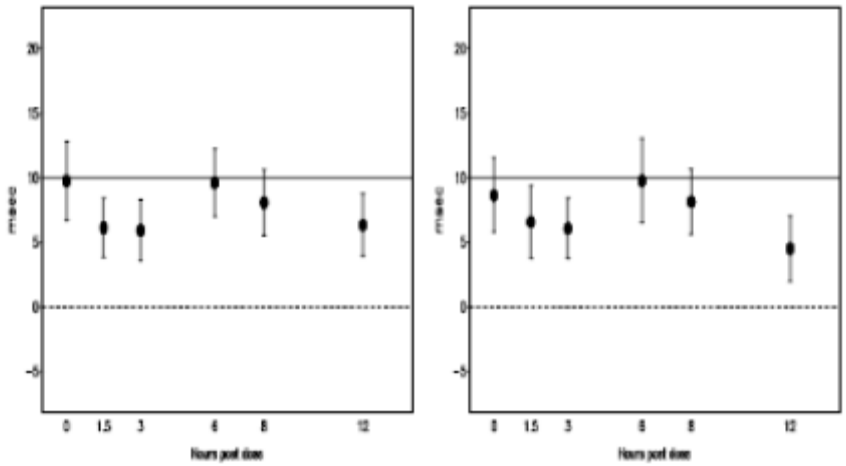
The mean change of QTcI on Day 7 from baseline is shown in Table 2. This calculation of change from baseline is sometimes referred to as the "single subtraction" or "single delta". In the placebo treatment group, at all six time points, there is a net negative change on mean QTcI of approximately 5-7 ms. In contrast, for the other three treatment groups there is a net positive change of mean QTcI of approximately 2-4 ms.

Table 2: Mean QTcI Change From Baseline On Day 7 By Treatment Group

	PLACEBO		MOXIFLOXACIN				FTY720 1.25 MG				FTY720 2.5 MG			
Hour	N	Δ QTcI Mean (SE)	N	Mean (SE)	Δ from placebo	90% CI	N	Mean (SE)	Δ from placebo	90% CI	N	Mean (SE)	Δ from placebo	90% CI
0.000	55	-8.1(1.5)	21	-5(2.4)	3	-0.9 ,7	52	1.7(1.6)	9.8	6.8 ,12.7	61	0.6(1.4)	8.7	5.8 ,11.5
	54	-2.8(1.4)	21	2(2.3)	4.8	1.1 ,8.5	52	3.3(1.5)	6.1	3.3 ,8.9	61	3.8(1.3)	6.6	3.9 ,9.3
3.000	55	-4.6(1.3)	21	1.8(2.1)	6.5	3.1 ,9.8	52	1.3(1.3)	5.9	3.4 ,8.5	61	1.5(1.2)	6.1	3.7 ,8.5
6.000	55	-7.6(1.6)	21	2.5(2.6)	10.2	5.9 ,14.5	52	2(1.7)	9.6	6.4 ,12.9	61	2.2(1.6)	9.8	6.7 ,12.9
8.000	55	-6.1(1.3)	21	4.7(2)	10.8	7.5 ,14.1	51	2(1.3)	8.1	5.6 ,10.6	61	2.1(1.2)	8.2	5.8 ,10.5
12.000	54	-2.7(1.3)	21	4.9(2.1)	7.5	4.1 ,11	51	3.7(1.4)	6.4	3.7 ,9	61	1.9(1.3)	4.5	2 ,7

In Figure 1, the data from two FTY720 treatment groups are seen; the 1.25 mg group in the right panel and the 2.5 mg group in the left panel. A line is placed at +10 ms to represent a "threshold of interest" typically set by health authorities. Both FTY720 treatment groups manifest an almost identical time pattern of the "double delta" mean QTcI. The point estimates for both FTY720 treatment groups are net positive changes which range from approximately 5 to 10 ms. For both of these treatment groups, at 3 out of the 6 time points the 90% confidence interval of the QTcI "double delta" is greater than or equal to the 10 ms criterion. These data are consistent with a positive effect of FTY720 on the QTcI interval.

Figure 1: Mean QTcI change in FTY720 treatment groups on Day 7 corrected by mean baseline and placebo group (with 90% confidence interval)



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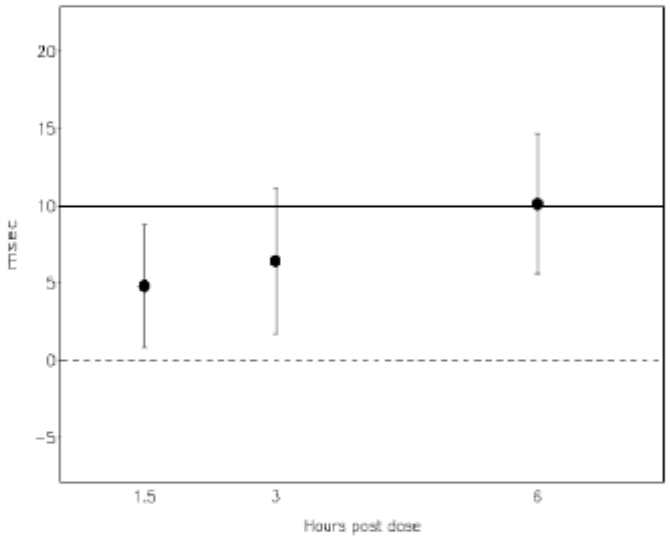
Reviewer's Comments: For a parallel study, we recommend using a time-matched baseline adjustment at each time point instead of averaging all the baseline measurements. This reviewer found that the upper 90% CI limit exceeded 10 ms at the same times using the time-matched baseline, except that the 1.25 mg group vs. placebo difference also exceeded 10 ms at 1.5 hours using the time-matched baseline.

As shown in

Figure **2** below, the study detected a maximal effect of moxifloxacin of approximately 10 ms and at hours 3 and 6, the 96% confidence intervals of the point estimates included 10 ms. In addition, none of the 96% confidence intervals at all three time points included 0 ms.

Figure 2 mean QTcI change in moxifloxacin treatment groups corrected by mean baseline and placebo (with 96% confidence interval)



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Reviewer's Comments: The sponsor appears to have used a 96% confidence interval for the moxifloxacin comparisons after considering multiple endpoint adjustment which we agree. The statistical reviewer verified the sponsor's analyses which were based on the change from the time averaged baseline. Additional analyses based on time-matched baseline are presented in Section 4.2.

3.2.8.2.2 Categorical Analysis

In Table 3, the outlier analysis by treatment group on Day 7 is shown.

There were no subjects who had a QTcI > 480 ms. There was one subject (7174) in the FTY720 2.5 mg treatment group who had two out of six ECGs with a QTcI between 450 and 465 ms. The remaining four Day 7 ECGs for this subject had a QTcI <450 ms. There was one subject in the placebo treatment group with one ECG with a QTcI between 450 and 460 ms. The remaining five Day 7 ECGs for this subject had a QTcI <450 ms. There was one subject, again 7174, in the FTY720 2.5 mg treatment group who had one out of

six ECGs with a change from baseline QTcI >60 ms. This same subject also had one out of six ECGs with a change from baseline QTcI of 30-60 ms. The remaining four ECGs for this subject on Day 7 were negative changes from baseline.

Table 3 Outlier Analysis of QTcI on Day 7

Treatment	Hours postdose	Increase* > 30 msec % (n/N)**	Increase* > 60 msec % (n/N)**	New* > 450 msec % (n/N)**	New* > 480 msec % (n/N)**	New* > 500 msec % (n/N)**
Placebo	0	-	-	-	-	-
	1.5	-	-	-	-	-
	3	-	-	-	-	-
	6	-	-	-	-	-
	8	-	-	-	-	-
	12	-	-	1.9 (1/54)	-	-
FTY720 1.25mg	0	-	-	-	-	-
	1.5	-	-	-	-	-
	3	-	-	-	-	-
	6	-	-	-	-	-
	8	-	-	-	-	-
	12	-	-	-	-	-
FTY720 2.5mg	0	-	-	-	-	-
	1.5	1.8 (1/61)	-	1.8 (1/61)	-	-
	3	-	-	-	-	-
	6	-	1.6 (1/61)	1.6 (1/61)	-	-
	8	-	-	-	-	-
	12	-	-	-	-	-
Moxifloxacin	0	-	-	-	-	-
	1.5	-	-	-	-	-
	3	-	-	-	-	-
	6	-	-	-	-	-
	8	-	-	-	-	-
	12	-	-	-	-	-

* As compared to the mean baseline measurements. A notable value is only counted once, in the highest category;
** % is based on number of subjects with both baseline and post-dose measurements. A dash (-) represents 0 events.

Reviewer's comments: The statistical reviewer verified the preceding table created by the sponsor. The increases in the preceding table were based on the changes from time averaged baseline. The statistical reviewer found the following increases based on changes from time matched baseline.

At hour 0, increase > 30 for 1 (1.9%) subject in 1.25 mg and 3 (5%) in 2.5 mg FTY720.

1.5, increase > 30 for 2 (3.3%) subjects in 2.5 mg FTY720

6, increase > 30 for 1 (1.9%) in 1.25 and increase > 60 for 1 (1.6%) in 2.5 mg

8, increase > 30 for 1 (1.6%) in 2.5 mg group

12, increase > 30 for 1 (4.8%) in moxifloxacin group

Thus, there appear to be a few more significant increases when the analysis is based on the time-matched baseline rather than the time-averaged baseline.

3.2.8.3 Safety Analysis

There were no deaths or SAEs. Two subjects discontinued due to AEs of rash following FTY720 1.25 mg and intermittent dyspnea.

There were no ventricular tachycardia or ventricular fibrillation adverse events, nor were these ventricular arrhythmias noted on ECG.

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Four female subjects experienced vasovagal syncope. Two subjects were assigned to FTY720 1.25 mg, one to FTY720 2.5 mg and one to placebo. These were not associated with QT prolongation.

3.2.8.4 Clinical Pharmacology

3.2.8.4.1 Pharmacokinetic Analysis

The PK results are presented in Table 4 (FTY720 and FTY720-P) and Table 5 (moxifloxacin). C_{max} and AUC values for FTY720 and FTY720-P in the thorough QT study were 2-fold higher following administration of the 2.5 mg FTY720 supratherapeutic dose compared with 1.25 mg FTY720, the intended clinical dose.

Table 4: FTY720 and FTY720-P pharmacokinetics on Day 7 after a loading dose regimen in healthy volunteers

PK parameters	FTY720		FTY720-P	
	1.25 mg	2.5 mg	1.25 mg	2.5 mg
$C_0^{SS},_b$ # (ng/mL)	5.09 ± 1.46 [4.88; 29]	10.1 ± 2.93 [9.71; 31]	2.66 ± 0.781 [2.55; 30]	5.18 ± 1.45 [5.00; 28]
$C_{24}^{SS},_b$ # (ng/mL)	5.38 ± 1.66 [5.13; 32]	10.8 ± 3.5 [10.3; 32]	2.57 ± 0.781 [2.46; 31]	5.00 ± 1.57 [4.78; 31]
t_{max} * (h)	8.00 (0.75- 16.0)	8.00 (3.00- 24.0)	6.00 (6.00- 8.08)	6.00 (5.92- 8.02)
$C_{max}^{SS},_b$ # (ng/mL)	6.73 ± 1.82 [6.49; 28]	13.4 ± 3.87 [12.9; 29]	4.11 ± 0.951 [4.00; 24]	7.94 ± 1.79 [7.75; 22]
$C_{av}^{SS},_b$ # (ng/mL)	5.83 ± 1.63 [5.61; 29]	11.7 ± 3.41 [11.2; 29]	3.13 ± 0.832 [3.03; 27]	6.12 ± 1.59 [5.93; 26]
$AUC_{\tau},_b$ # (ng/mL.h)	140 ± 39.1 [135; 29]	280 ± 81.7 [269; 29]	75.1 ± 20.0 [72.6; 27]	147 ± 38.2 [142; 26]
PTF # (%)	28 ± 10 [26; 41]	28 ± 11 [26; 45]	48 ± 14 [46; 33]	47 ± 13 [45; 30]
Rac #	7.0 ± 1.5 [6.9; 21]	6.8 ± 2.1 [6.5; 36]	5.0 ± 1.0 [4.9; 21]	5.2 ± 1.3 + [5.1; 25]
$RC_{max}^{SS},_b$ #			0.5 ± 0.08 [0.49; 17]	0.48 ± 0.08 [0.48; 16]
$RAUC_{\tau},_b$ #			0.43 ± 0.05 [0.43; 13]	0.42 ± 0.05 [0.42; 12]

#: arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation]

*: median (minimum-maximum); +: n=60; Rac: $C_{24h}^{SS},_b$ Day7/ $C_{24h}^{SS},_b$ Day1; RC_{max}^{SS}, $_b$ and $RAUC_{\tau},_b$ are FTY720-P/FTY720 ratios for $C_{max}^{SS},_b$ and $AUC_{\tau},_b$ in molar units, respectively

b: blood matrix

Source: Clinical Study Report P-7 Table 3.

Table 5. Moxifloxacin pharmacokinetics on Day 7 after a 400 mg single dose in healthy volunteers

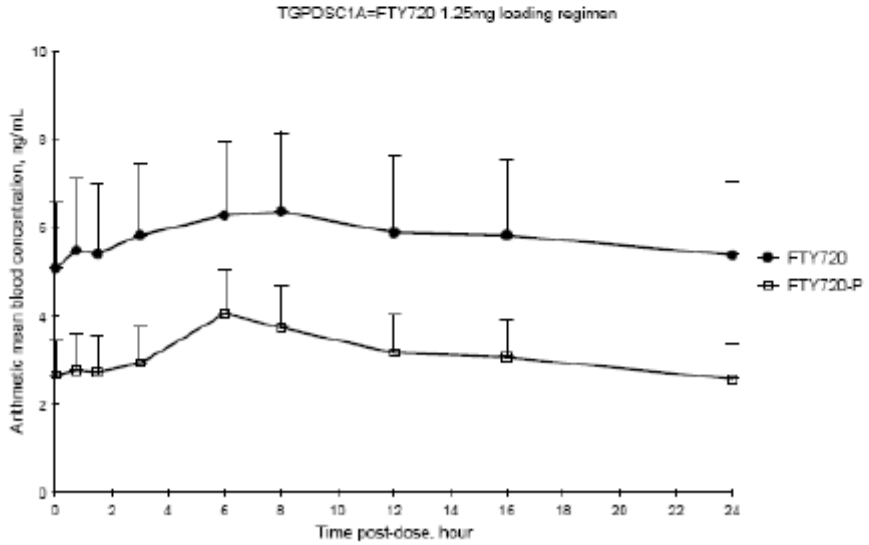
PK parameters	
t_{lag} * (h)	0.00 (0.00-0.75)
t_{max} * (h)	3.00 (0.75- 6.02)
C_{max} # (mg/L)	2.62 ± 0.742 [2.53; 28]
AUC_{0-24h} # (mg/L.h)	30.6 ± 5.05 [30.2; 17]
$AUC_{0-\infty}$ # (mg/L.h)	40.8 ± 8.05 [40.0; 20]
$t_{1/2}$ # (h)	11.4 ± 3.0 [11.0; 27]

*: median (minimum-maximum); #: arithmetic mean ± standard deviation [geometric mean, % geometric mean coefficient of variation]

Source: Clinical Study Report P-8 Table 4.

The concentration time profiles of FTY720 and FTY720-P for the 1.25 mg and 2.5 mg dose regimens are shown in Figure 3 and Figure 4, respectively. The concentration-time profile for moxifloxacin is shown in Figure 5.

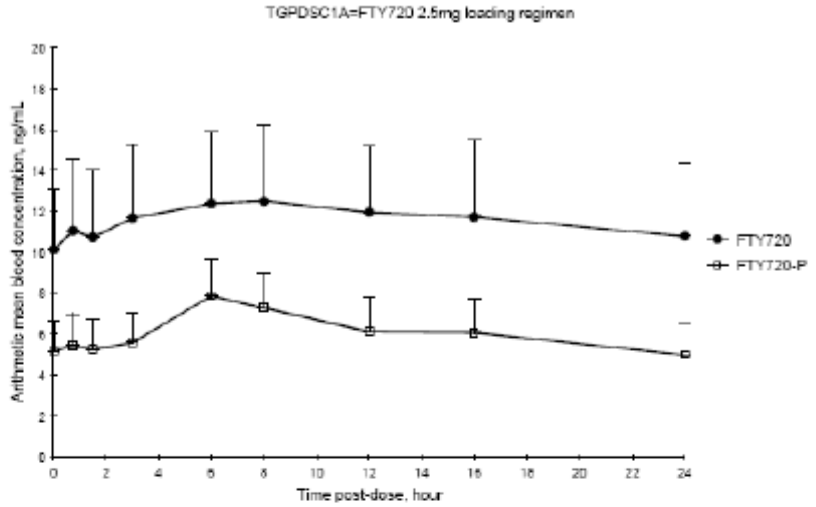
Figure 3: FTY720 and FTY720-P arithmetic mean (+SD) concentration-time profile (ng/ml) on Day 7 following 1.25 mg dose



Source: Clinical Study Report P-1491 Figure 16.2.5-2.1.

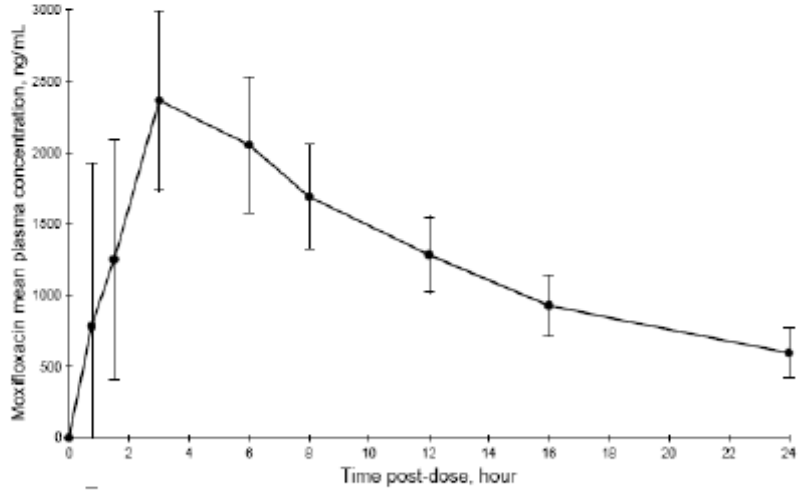
13

Figure 4: FTY720 and FTY720-P arithmetic mean (+SD) concentration-time profile on Day 7 following 2.5 mg dose.



Source: Clinical Study Report P-1493 Figure 16.2.5-2.5

Figure 5: Moxifloxacin single dose arithmetic mean (+SD) plasma concentration-time profile on Day 7 following 400 mg moxifloxacin



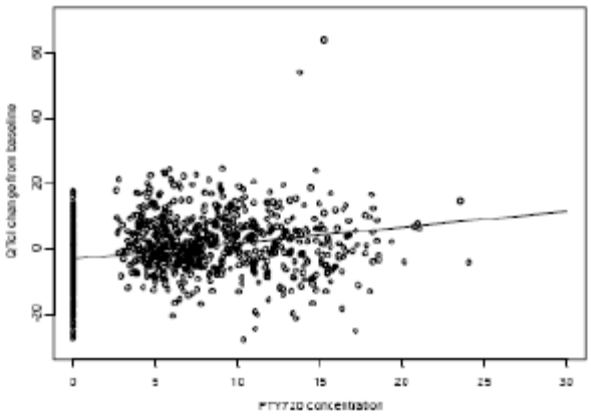
Source: Clinical Study Report P-1613 Figure 16.2.5-2.19.

3.2.8.4.2 Exposure-Response Analysis

The relationship between the time-matched change from baseline in QTcI and plasma concentrations of FTY720 and FTY720-P was explored graphically. When placebo data was included, a regression line with a positive slope was observed for FTY720 and

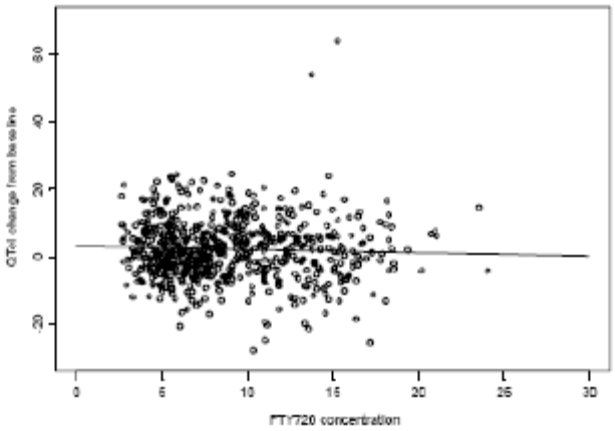
FTY70-P concentrations as seen in Figure 6 and Figure 8, respectively. When placebo data was excluded, no discernible relationship was observed between FTY720 or FTY720-P and time-matched change from baseline in QTcI, as shown in Figure 7 and Figure 9, respectively.

Figure 6: QTcI change from mean baseline vs. FTY720 blood concentration (ng/mL) including placebo data on Day 7 with regression line.



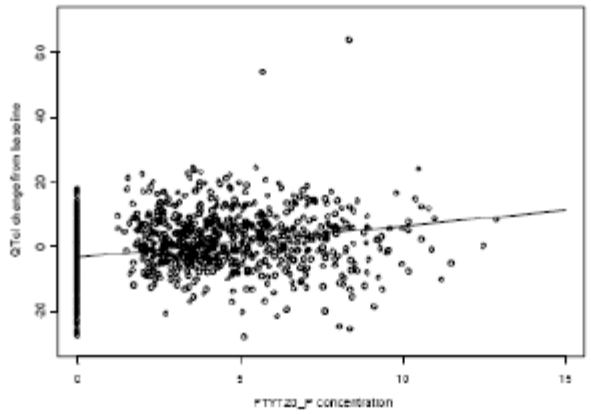
Source: Clinical Study Report P-79 Figure 11-17

Figure 7: QTcI change from mean baseline vs. FTY720 blood concentration (ng/mL)excluding placebo data on Day 7 with regression line



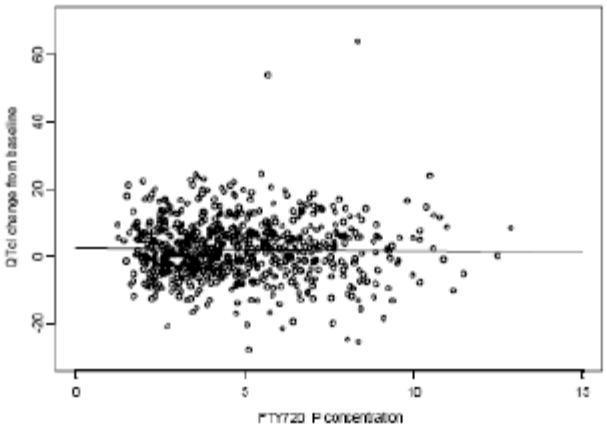
Source: Clinical Study Report P-81 Figure 11-19

Figure 8: QTcI change from mean baseline vs. FTY720-P blood concentration (ng/mL) including placebo data on Day 7 with regression line



Source: Clinical Study Report P-80 Figure 11-18

Figure 9: QTcI change from mean baseline vs. FTY720-P blood concentration (ng/mL) excluding placebo data on Day 7 with regression line



Source: Clinical Study Report P-81 Figure 11-20

Reviewer's Analysis: The positive slopes of the regression lines in Figure 6 *and* Figure 8 *are due to the negative change from baseline QTcI observed in placebo subjects on Day 7. Even though* Figure 7 *and* Figure 9 *do not show a positive slope when the placebo subjects are removed, the range of observed concentrations of FTY720 and FTY720-9 is too small to rule out a positive concentration-response relationship. Plots of ΔΔQTc vs. FTY720 and FTY720-P concentrations are presented in* Figure 13 *and* Figure 14, *respectively.*

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4 REVIEWERS' ASSESSMENT

4.1 EVALUATION OF THE QT/RR CORRECTION METHOD

The observed QT-RR interval relationship is presented in Figure 10 together with the Bazett's (QTcB), Fridericia (QTcF), and individual correction (QTcI).

Figure 10: QT, QTcB, QTcF, and QTcI vs. RR (Each Subject's Data Points are Connected with a Line)

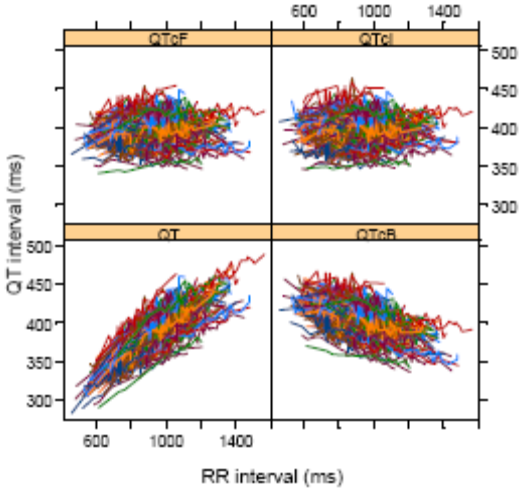
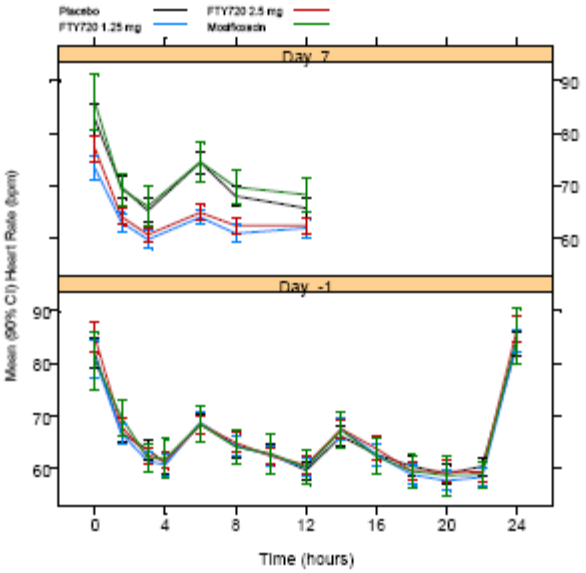


Table 6: Average Sum of Squared Slopes for Different QT-RR Correction Methods

		TRT								
		FTY720 1.25mg loading regimen		FTY720 2.5mg loading regimen		FTY720 matched placebo		Moxifloxacin hydrochloride 400mg		All Trt
		n	msss	n	msss	n	msss	n	msss	msss
method	sex									
QTcB	F	25	0.0073	31	0.0059	28	0.0059	11	0.0041	0.0061
	M	27	0.0060	30	0.0056	27	0.0063	10	0.0065	0.0060
	All	52	0.0066	61	0.0057	55	0.0061	21	0.0053	0.0060
QTcF	F	25	0.0040	31	0.0016	28	0.0022	11	0.0040	0.0027
	M	27	0.0015	30	0.0021	27	0.0011	10	0.0019	0.0016
	All	52	0.0027	61	0.0018	55	0.0016	21	0.0030	0.0021
QTcI	F	25	0.0035	31	0.0013	28	0.0017	11	0.0029	0.0022
	M	27	0.0013	30	0.0019	27	0.0010	10	0.0023	0.0015
	All	52	0.0024	61	0.0016	55	0.0014	21	0.0026	0.0018

The time course of heart rate is presented in Figure 11

Figure 11: Time course of heart rate on Day -1 and Day 7 for placebo, 1.25 mg FTY720, 2.5 mg and moxifloxacin groups.



The FTY720 treatment groups have mean heart rates consistently about 5 to 10 beats per minute lower than both the placebo and moxifloxacin groups. The lower heart rates observed in the FTY720 treatment groups on Day 7 are within the range of heart rates observed during Day -1 which are used to calculate the individual correction. Therefore the QTcI correction can adequately be calculated using drug-free data on Day -1.

4.2 STATISTICAL ASSESSMENTS

4.2.1 QTc Analysis

4.2.1.1 The Primary Analysis for FTY720

Overall 199 subjects were randomized to the study; 189 subjects completed the study and

10 subjects were discontinued and replaced. This can be broken down by treatment group as follows.

Moxifloxacin treatment group: 21 planned, 21 entered, 21 completed

Placebo treatment group: 56 planned, 57 entered, 55 completed

FTY720 1.25 mg group: 56 planned, 57 entered, 52 completed

FTY720 2.5 mg group: 56 planned, 64 entered, 61 completed

As shown in Table 7, two subjects discontinued (#'s: 5110 and 7156) due to the adverse events during the treatment period, and one subject withdraw consent.

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Seven subjects discontinued during the placebo baseline period on Day-1.

Table 7 Subjects Discontinuing Treatment during the study

Subject	Finding	Day of last dosing
5110	Skin rash	3
7151	Withdrew consent due to personal reasons	6
7156	Intermittent dyspnea	4
5116	ALT out of standard range	-1
5121	Amylase increased over the standard range	-1
5147	Lipase out of standard range	-1
5158	Bilirubin > 27 umol/L	-1
5179	Significant illness prior to dosing (cutaneous lesion)	-1
7126	WBC increased out of standard range	-1
7141	Withdrawal of consent	-1

All 189 subjects that completed the study were included in the analysis.

The sponsor designated the QTcI as the primary QT assessment. $QTcI = QT/(RR)^{\beta}$

The estimated coefficient β was derived on the basis of a regression of log QT on log RR which assumed random effects for subject intercept and slope. This model was fit using only the baseline ECG data.

At 0, 6, and 8 hours post-dose, the 1-sided 95% upper bound for the difference between the placebo group and both of the 1.25 and 2.5 mg groups exceeded 10 ms in terms of the change in QTcI. This was true for both the time averaged baseline as well as the time matched baseline. There were no statistically significant differences found using QTcB, but the same results computed for QTcI were confirmed using QTcF. Therefore, FTY720 appears to increase the QTc interval.

The statistical reviewer used t-tests of the change from time-matched baseline to analyze the ΔQTcI effect because the sponsor had specified t-tests as the analysis method. The analysis results are listed in the following tables.

Table 8 Analysis Results of ΔQTcI and ΔΔQTcI for Treatment Group = FTY720 1.25 mg x 7 days

	ΔQTCI: PLACEBO		ΔQTCI: FTY720 1.25 MG		ΔΔQTCI	
Time/(hr)	N	Mean(S.E.)	N	Mean(S.E.)	Estimate (S.E.)	90% CI
0.0	55	-2.5(1.9)	52	6.2(1.9)	8.7(2.2)	5 ,12.4
1.5	54	-4.1(1.6)	52	3.1(1.6)	7.2(1.9)	4 ,10.4
3.0	55	-2.4(1.6)	52	2.1(1.6)	4.5(1.9)	1.4 ,7.6
6.0	55	-7(1.8)	52	3(1.9)	10(2.2)	6.4 ,13.6
8.0	55	-3.1(1.3)	51	6.4(1.4)	9.5(1.6)	6.8 ,12.1
12.0	54	-3.6(1.6)	50	2.6(1.6)	6.1(1.9)	3 ,9.3

Table 9 Analysis Results of ΔQTcI and ΔΔQTcI for Treatment Group = FTY720 2.5 mg x 7 days

	ΔQTCI: PLACEBO		ΔQTCI: FTY720 2.5 MG		ΔΔQTCI	
Time/(hr)	N	Mean(S.E.)	N	Mean(S.E.)	Estimate (S.E.)	90% CI
0.0	55	-2.5(1.9)	60	6(1.8)	8.5(2.2)	5 ,12.1
1.5	54	-4.1(1.6)	60	2.7(1.5)	6.9(1.9)	3.8 ,9.9
3.0	55	-2.4(1.6)	60	3.1(1.5)	5.5(1.8)	2.5 ,8.5
6.0	55	-7(1.8)	61	3.5(1.7)	10.5(2.1)	7.1 ,14
8.0	55	-3.1(1.3)	61	4.5(1.3)	7.6(1.5)	5.1 ,10.2
12.0	54	-3.6(1.6)	61	0.8(1.5)	4.4(1.8)	1.4 ,7.4

The largest upper bounds of the 2-sided 90% CI for the mean difference between FTY720 1.25 mg and placebo, and between FTY720 2.5 mg and placebo were 13.6 and 14.0, respectively.

4.2.1.2 Assay Sensitivity Analysis

The statistical reviewer used the same statistical model to analyze moxifloxacin and placebo data. The results are presented in Table 10. The largest moxifloxacin effect after baseline correction is 11 ms at hour 8 post dose which is not likely since the T_{max} of moxifloxacin observed in this study was 3 hours.

Table 10: Analysis Results of ΔQTcI and ΔΔQTcI for Moxifloxacin

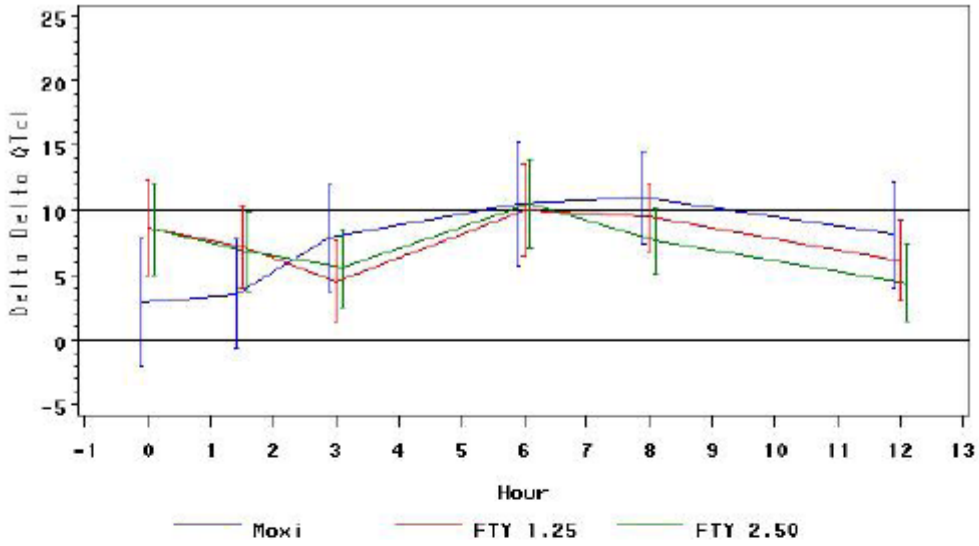
	ΔQTCI: PLACEBO		ΔQTCI: MOXIFLOXACIN		ΔΔQTCI		
Time/(hr)	N	Mean(S.E.)	N	Mean(S.E.)	Estimate (S.E.)	90% CI (unadjusted)	90% CI (adjusted)*
0.0	55	-2.5(1.9)	21	0.4(3)	2.9(3)	-2 ; 7.8	
1.5	54	-4.1(1.6)	21	-0.6(2.6)	3.5(2.6)	-0.7 , 7.7	-1.9 , 9
3.0	55	-2.4(1.6)	21	5.5(2.6)	7.9(2.5)	3.7 , 12	2.5 , 13.2
6.0	55	-7(1.8)	21	3.4(2.9)	10.5(2.9)	5.7 , 15.2	4.3 , 16.6
8.0	55	-3.1(1.3)	21	7.9(2.2)	11(2.1)	7.5 , 14.5	
12.0	54	-3.6(1.6)	21	4.6(2.5)	8.1(2.5)	4 , 12.2	

* **Bonferroni method was applied for multiple endpoint adjustment for 3 time points (the sponsor specified times 1.5, 3, and 6 as the key times for testing moxifloxacin).**

4.2.1.3 Graph of ΔΔQTcI Over Time

The following figure displays the time profile of ΔΔQTcI for different treatment groups.

Figure 12 Mean and 90% CI ΔΔQTcI Time Course on Day 7



4.2.1.4 Categorical Analysis

Table 11 lists the number of subjects as well as the number of observations whose absolute QTcI values are ≤ 450 ms, between 450 ms and 480 ms. No subject's QTcI was above 480 ms.

Table 11: Categorical Analysis for QTcI

	Value<=450 ms		450 ms<Value<=480 ms	
Treatment Group	# Subj. (%)	# Obs. (%)	# Subj. (%)	# Obs. (%)
Baseline	187 (98.9)	1512 (99.9)	2 (1.1)	2 (0.1)
Placebo	54 (98.2)	327 (99.7)	1 (1.8)	1 (0.3)
Moxifloxacin	21 (100.0)	126 (100.0)	0 (0.0)	0 (0.0)
FTY720 1.25 mg	52 (100.0)	310 (100.0)	0 (0.0)	0 (0.0)
FTY720 2.50 mg	60 (98.4)	364 (99.5)	1 (1.6)	2 (0.5)

Table 12 lists the categorical analysis results for ΔQTcI. One FTY720 2.5 mg subject's change from time-matched baseline was above 60 ms. No other subjects had changes from baseline above 60 ms.

Table 12: Categorical Analysis of ΔQTcI

	Value <=30		30<= Value <60		Value >60	
Treatment Group	# Subj. (%)	# Obs. (%)	# Subj. (%)	# Obs. (%)	# Subj. (%)	# Obs. (%)
Placebo	55 (100.0)	328 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Moxifloxacin	20 (95.2)	125 (99.2)	1 (4.8)	1 (0.8)	0 (0.0)	0 (0.0)
FTY720 1.25 mg	50 (96.2)	307 (99.4)	2 (3.8)	2 (0.6)	0 (0.0)	0 (0.0)
FTY720 2.50 mg	56 (91.8)	356 (98.1)	4 (6.6)	6 (1.7)	1 (1.6)	1 (0.3)

4.2.2 PR Analysis

The same statistical analysis was performed based on PR interval. The point estimates and the 90% confidence intervals are presented in Table 14 and Table 14. The largest upper limits of 90% CI for the PR mean differences between FTY720 1.25 mg and placebo and FTY720 2.5 mg and placebo are 6.1 ms and 9.9 ms, respectively.

Table 13: Analysis Results of ΔPR and ΔΔPR for Treatment Group = FTY720 1.25 mg x 7 days

	ΔPR: PLACEBO		ΔPR: FTY720 1.25 MG		ΔΔPR	
Time/(hr)	N	Mean(S.E.)	N	Mean(S.E.)	Estimate (S.E.)	90% CI
0.0	55	-3.2(1.9)	52	-0.8(1.9)	2.4(2.2)	-1.3 ,6.1
1.5	54	-2.1(1.5)	52	-2.7(1.5)	-0.6(1.8)	-3.5 ,2.2
3.0	55	-1.3(1.4)	52	-2.4(1.4)	-1(1.7)	-3.8 ,1.8
6.0	55	-1.7(1.3)	52	-2.2(1.4)	-0.5(1.6)	-3.1 ,2.1
8.0	55	-2.4(1.4)	51	-0.9(1.4)	1.5(1.6)	-1.2 ,4.2
12.0	54	-0.7(1.5)	50	-3(1.5)	-2.3(1.8)	-5.2 ,0.6

Table 14: Analysis Results of ΔPR and ΔΔPR for Treatment Group = FTY720 2.5 mg x 7 days

	ΔPR: PLACEBO		ΔPR: FTY720 2.50 MG		ΔΔPR	
Time/(hr)	N	Mean(S.E.)	N	Mean(S.E.)	Estimate (S.E.)	90% CI
0.0	55	-3.2(1.9)	60	3.1(1.8)	6.3(2.2)	2.7 ,9.9
1.5	54	-2.1(1.5)	60	-1(1.4)	1.1(1.7)	-1.7 ,3.9
3.0	55	-1.3(1.4)	60	-1.8(1.3)	-0.5(1.6)	-3.2 ,2.2
6.0	55	-1.7(1.3)	61	-1.1(1.3)	0.5(1.5)	-2 ,3.1
8.0	55	-2.4(1.4)	61	-2.3(1.3)	0.1(1.6)	-2.5 ,2.7
12.0	54	-0.7(1.5)	61	-3.6(1.4)	-2.8(1.7)	-5.6 ,-0.1

4.2.3 QRS Analysis

The same statistical analysis was performed based on QRS interval. The point estimates and the 90% confidence intervals are presented in Table 15 and Table 16. The largest

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upper limits of 90% CI for the QRS mean differences between FTY702 1.25 mg and placebo and FTY720 2.5 mg and placebo are 0.7 ms and 1.7 ms, respectively.

Table 15: Analysis Results of ΔQRS and ΔΔQRS for Treatment Group = FTY720 1.25 mg x 7 days

	ΔQRS: PLACEBO		ΔQRS: FTY720 1.25 MG		ΔΔQRS	
Time/(hr)	N	Mean(S.E.)	N	Mean(S.E.)	Estimate (S.E.)	90% CI
0.0	55	-0.2(0.7)	52	-1.4(0.8)	-1.2(0.9)	-2.7 ,0.2
1.5	54	-0.6(0.6)	52	-2.1(0.6)	-1.4(0.7)	-2.6 ,-0.2
3.0	55	0.2(0.6)	52	-2.1(0.6)	-2.2(0.7)	-3.4 ,-1.1
6.0	55	-1.9(0.6)	52	-2.3(0.6)	-0.5(0.7)	-1.7 ,0.7
8.0	55	-0.8(0.6)	51	-2(0.6)	-1.3(0.7)	-2.5 ,0
12.0	54	-1.7(0.6)	50	-2.8(0.6)	-1.1(0.7)	-2.3 ,0.1

Table 16: Analysis Results of ΔQRS and ΔΔQRS for Treatment Group = FTY720 2.5 mg x 7 days

	ΔQRS: PLACEBO		ΔQRS: FTY720 2.50 MG		ΔΔQRS	
Time/(hr)	N	Mean(S.E.)	N	Mean(S.E.)	Estimate (S.E.)	90% CI
0.0	55	-0.2(0.7)	60	-2.1(0.7)	-1.9(0.9)	-3.3 ,-0.5
1.5	54	-0.6(0.6)	60	-1.5(0.6)	-0.9(0.7)	-2 ,0.2
3.0	55	0.2(0.6)	60	-1.1(0.6)	-1.3(0.7)	-2.4 ,-0.1
6.0	55	-1.9(0.6)	61	-1.3(0.6)	0.5(0.7)	-0.6 ,1.7
8.0	55	-0.8(0.6)	61	-1.8(0.6)	-1(0.7)	-2.2 ,0.2
12.0	54	-1.7(0.6)	61	-2.6(0.6)	-1(0.7)	-2.1 ,0.2

4.3 CLINICAL PHARMACOLOGY ASSESSMENTS

The relationship between ΔΔQTcI and FTY720 and FTY720-P concentrations are visualized in Figure 13 and Figure 14, respectively with no evident exposure-response relationship. This, however, does not rule out the existence of an exposure-response relationship due to the small range of observed concentrations observed at steady state on Day 7.

Figure 13: ΔΔQTcI vs. FTY720 concentration

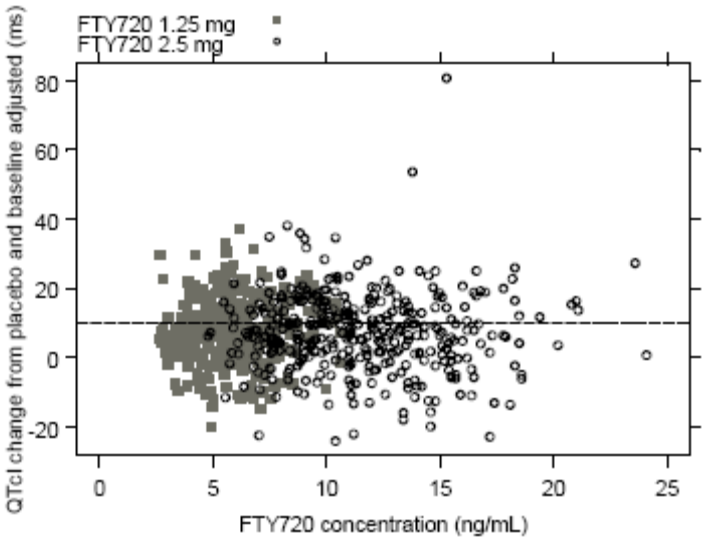
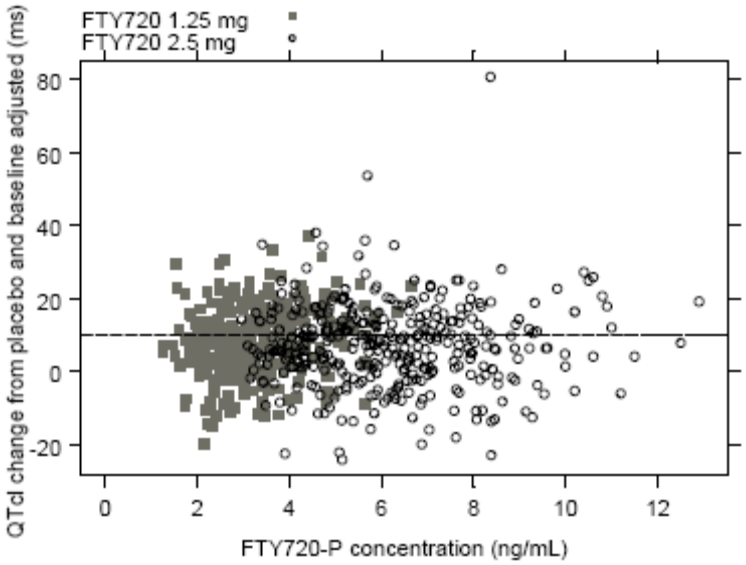


Figure 14: ΔΔ QTcI vs. FTY720-P concentration



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4.4 CLINICAL ASSESSMENTS

5.4.1 Safety assessments

None of the events identified to be of clinical importance per the ICH E 14 guidelines i.e. syncope, seizure, significant ventricular arrhythmias or sudden cardiac death occurred in this study.

No subject had an absolute QTcI increase of over 500 ms. One subject in the FTY720 2.5-mg group had an increase of over 60 ms from baseline.

Although there are few reports of conduction defects in previous clinical experience, there were no clinically significant effects in the PR and QRS intervals in the TQT study. The largest upper limits of 90% CI for the PR mean differences between FTY720 1.25 mg and placebo and FTY720 2.5 mg and placebo are 6.1 ms and 9.9 ms, respectively. There were reports of first degree AV block and IRBBB in the CSR but these were present at baseline as well.

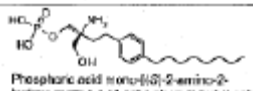
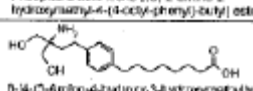
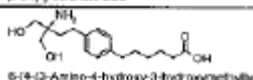
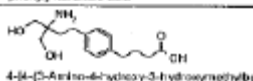
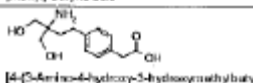
5.4.2 ECG Assessments

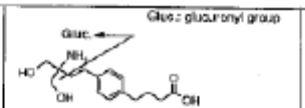
Waveforms from the ECG warehouse were reviewed. According to ECG warehouse statistics over 90% of the ECGs were annotated in the primary lead II, with less than 0.5% of ECGs reported to have significant QT bias, according to the automated algorithm. Overall ECG acquisition and interpretation in this study appear acceptable.

5 APPENDIX

5.1 HIGHLIGHTS OF CLINICAL PHARMACOLOGY

Therapeutic dose	1.25 mg qd	
Maximum tolerated dose	40 mg single dose, 5 mg qd multiple dose	
Principal adverse events	Bradycardia at doses ≥1.25 mg, chest tightness and decreased FEV_1/FEF_{25-75} at doses >2.5 mg, increased transaminases at doses ≥5 mg.	
Maximum dose tested	Single Dose	40 mg
	Multiple Dose	5 mg qd for >1 year
Exposures Achieved at Maximum Tested Dose	Single Dose	FTY720: Mean C_{max}: 26.9 ng/mL (%CV: 24) and AUC: 5753 ng/mL.h (%CV: 50) FTY720-Phosphate: Mean C_{max}: 18.3 ng/mL (%CV: 26) and AUC: 2871 ng/mL.h (%CV: 55)
	Multiple Dose	Kidney transplant patients (n=5), day 28 parameters, 5 mg qd: Mean C_{max}: 27.5 ng/mL (%CV: 31) and AUC_{0-24h}: 568 ng/mL.h (%CV: 28)
Range of linear PK	0.25 to 40 mg single dose (0.25 to 3.5 mg in maintenance renal transplant patients and 5 to 40 mg in healthy volunteers)	
Accumulation at steady state	Kidney transplant patients, accumulation calculated using day 28 and day 1 (pooling results from 0.25, 0.5, 1, 2.5, 5 mg doses qd): Mean 10.5 (%CV: 31)	
Metabolites		

	Abbreviation	Structure, chemical name	Occurrence	Pharmacological activity
	FTY720-P ((*S*)-enantiomer, AML629)	 Phosphoric acid mono-[(*S*)-2-amino-2-hydroxymethyl-4-(4-octyl-phenyl)-butyl] ester	blood, feces	pharmacological principle
	M1	 6-[4-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-phenyl]-octanoic acid	feces	no immuno-suppressant properties
	M2	 6-[4-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-phenyl]-hexanoic acid	blood (traces), urine, feces	no immuno-suppressant properties
	M3	 4-[4-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-phenyl]-butyric acid	blood, urine, feces	no immuno-suppressant properties
	M4	 [4-(3-Amino-4-hydroxy-3-hydroxymethyl-butyl)-phenyl]-acetic acid	urine	no immuno-suppressant properties

	M22	Gluc: glucuronyl group  Glucuronide of 4-[4-(3-amino-4-hydroxy-3-hydroxymethylbutyl)-phenyl]-butyric acid	urine (traces)	unknown
Absorption	Absolute/Relative Bioavailability	FTY720: AUC-ratio oral/IV: Mean: 0.98 (%CV: 31); Oral dose of 1.25 mg and IV dose of 1 mg		
	T_{max}	• FTY720: median 12 h (range: 8-36 h) • FTY720-Phosphate: median 8 h (range: 6-24 h)		
Distribution	Vd/F or Vd	FTY720: Vd: Mean: 1200 L (%CV: 22)		
	% bound	FTY720: Plasma protein binding: Mean: 99.85% (%CV: 11) FTY720-Phosphate: Plasma protein binding: 99.7-99.9% (%CV: not available)		
Elimination	Route	• Renal excretion: 81% (%CV: 4) of the dose as metabolites (FTY720 and FTY720-Phosphate not detected in urine) • Fecal excretion: 11% (%CV: 18) of the dose (mainly FTY720 and FTY720-Phosphate, but other metabolites measured)		
	Terminal t½	• FTY720: Healthy volunteers, 5 mg qd for 14 days: Mean: 11.3 days (%CV: 32) FTY720: Kidney transplant patients, 5 mg qd for 28 days: Mean: 9.3 days (%CV: 28) • Metabolites: (FTY720-Phosphate, M2 and M3): Estimated terminal t½ close to that of FTY720		
	CL/F or CL	CL: Mean 6.3 L/h (%CV: 36)		
Intrinsic Factors	Age	•Limited information in the elderly (>65 years) •Results of population PK studies in kidney transplant patients showed: FTY720 and FTY720-Phosphate: Age effect (up to 2-fold age change of a typical 44 years old patient) on CL/F was of smaller size than the inter-subject variability		
	Sex	Results of population PK studies in kidney transplant patients showed: •FTY720: No Gender effect on CL/F •FTY720-Phosphate: Gender effect on CL/F was of smaller size than the inter-subject variability		
	Race	No clinically relevant differences in the dose-C_{max} or dose-AUC relationships between Asian and white subjects for either FTY720 or FTY720-phosphate after 1.25, 2.5 or 5 mg single dose or 5 mg qd for 7 days. For other races the evaluation is ongoing.		

	Hepatic & Renal Impairment	-Mild hepatic impairment (Child-Pugh class A): C_{max} unchanged, AUC increased by 12% -Moderate hepatic impairment (Child-Pugh class B): C_{max} unchanged, AUC increased by 44% -Severe hepatic impairment (Child-Pugh class C): C_{max} unchanged, AUC increased by 100% -Renal impairment: Results of population PK studies in kidney transplant patients showed: FTY720: Creatinine clearance effect (at month 1) on CL/F was of smaller size than the inter-subject variability. FTY720-Phosphate: No Creatinine clearance effect on CL/F.
Extrinsic Factors	Drug interactions	-FTY720 (single dose)-Cyclosporine A (CYP3A substrate and inhibitor, steady state): The disposition of both drugs in psoriatic patients was not altered. -FTY720 (single dose)-Ketoconazole (CYP3A and CYP4F inhibitor, steady state): In healthy volunteers, FTY720 C_{max} increased by 1.24-fold and AUC increased by 1.78-fold; FTY720-phosphate C_{max} unchanged, its quantifiable $AUC_{(0-t)}$ increased by 1.73. -FTY720 (single dose)-Isoproterenol: FTY720 and FTY720-Phosphate exposures not altered in presence of isoproterenol in healthy subjects. -FTY720 (single dose)-Atropine: Administration of atropine 4 hours after FTY720 did not influence exposure to FTY720 or FTY720-Phosphate in healthy subjects. -FTY720 (single dose)- atenolol (steady state): atenolol, FTY720, and FTY720-Phosphate pharmacokinetics unchanged. -FTY720 (single dose)- diltiazem (steady state): diltiazem, FTY720, and FTY720-Phosphate pharmacokinetics unchanged.
	Food Effects	High-fat breakfast (1 mg single dose): C_{max} and AUC unchanged.
Expected High Clinical Exposure Scenario	Severe hepatic insufficiency: FTY720 exposure increased 2-fold on average. Ketoconazole (inhibition of CYP4F2): FTY720 exposure increased by less than 2-fold on average.	

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5.2 TABLE OF STUDY ASSESSMENTS

	Screening	Baseline	Day								End of study
Procedure / event		Day-1	1	2	3	4	5	6	7	9	(EoS)
Informed consent	X										
Medical history	X										
Eligibility	X	X									
Vital signs	X	X	X[1]	X					X[1]	X	X
Physical examination	X	X									X
ECG	X	X[2]	X[2]						X[2]		X
Safety lab	X	X								X	X
Drug administration		X	X	X	X	X	X	X	X		
PK sampling[3, 4]				X		X	X	X	X	X	

[1] Vital signs measurements taken at pre-dose, 2, 6, 24h post-dose

[2] Triplicate ECG measurements: Day-1: 0, 1.5, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24hr post-dose; Day7: 0, 1.5, 3, 6, 8, and 12 hr post-dose

[3] Blood collection for FTY720 and FTY720-P: Day 2, 4, 5, 6: predose; Day 7: predose, 0.75, 1.5, 3, 6, 8, 12, 16, and 24 hours post-dose

[4] Blood collection for moxifloxacin: Day 7: predose, 0.75, 1.5, 3, 6, 8, 12, 16, and 24 hours post-dose

Eos = End of study

Source Table 9.4 from CSR for FTY720D 2101, page 49

Linked Applications	Sponsor Name	Drug Name
IND 70139	NOVARTIS PHARMACEUTICALS CORP	FTY 720D CAPSULES

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/s/

CHRISTINE E GARNETT
10/20/2008

KEVIN M KRUDYS
10/20/2008

SUCHITRA M BALAKRISHNAN
10/20/2008

JOANNE ZHANG
10/20/2008
Dr. Tristan Massie was the primary statistical reviewer for this QT study.

NORMAN L STOCKBRIDGE
10/20/2008

4.4 APPENDIX IV

PHARMACOMETRICS CONSULT

office of clinical PHarmacology:
Pharmacometric review

Summary of Findings

Key Review Questions

The purpose of this review is to address the following key questions.

Is there any covariate which affects fingolimod PK?

There is no covariate which affects fingolimod PK in a clinically meaningful manner.

Due to the sparse sampling (pre-dose sampling), long-half life of FTY720-p (fingolimod phosphate ; active metabolite, 6-9 days) and the high expected inter-individual variability, the sponsor used linear mixed effect modeling with a random effect on the intercept parameter, a residual variance with an auto-regressive 1st order (AR1) structure and three independent covariates affecting the intercept: gender, weight and race.
Although, the male and weight covariates were associated with significant p-values, the magnitude of the effects associated with these covariates was deemed to be not clinically relevant; a male would have on average a 10.4% lower concentration than a female, and a gain in weight of 14 kg (from 70 to 84 kg) would be on average associated with a 6.2% decrease in concentration. For 70 (b) (4) -kg female who were assigned to 0.5mg, estimated mean concentrations at SS in Asian and Black population are 62% higher and 14% lower than Caucasian patients. However, it should be noted that there were only 14 Asian subjects.

Due to their potential risk of interaction with FTY720-P concentration, the effect of the following concomitant medication on the FTY720-P average concentration was investigated: fluoxetine and paroxetine, itraconazole and ketoconazole, carbamazepine, clarithromycin, corticosteroids and oral contraceptive. Besides, due to their high frequency of use in the studied population, the effect of the most frequent concomitant medication on FTY720-P average concentration was also checked. The most frequent concomitant medications include: baclofen, gabapentin, oxybutin, amantadine, amitriptyline, pregabalin and modafinil. For all these concomitant treatments, no unexpected effect was observed on FTY720-P concentration.

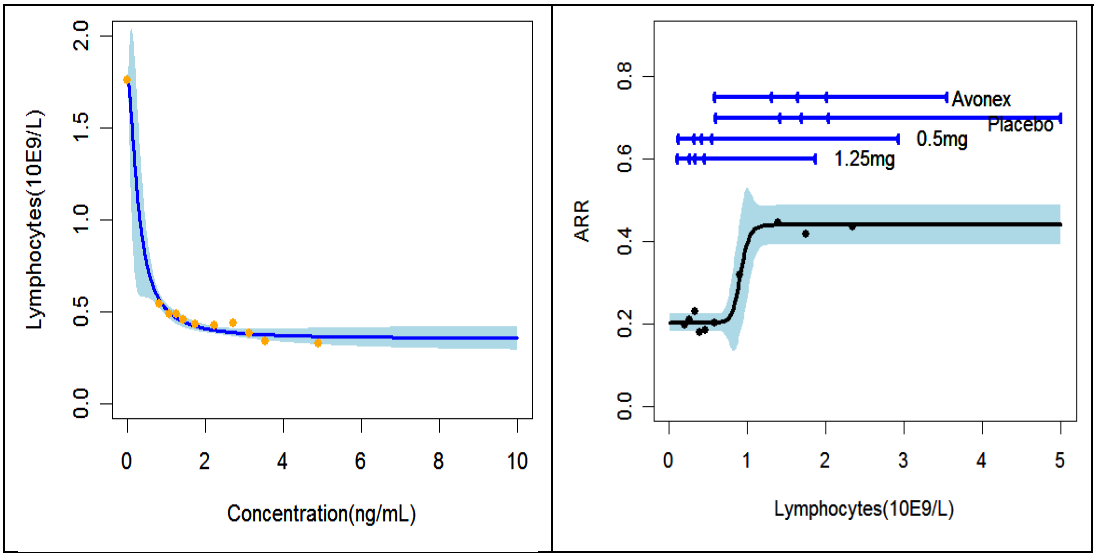
Is there any significant exposure-response relationship? And does the relationship support the proposed dose (0.5mg QD)?

Yes, there is a significant relationship between exposure (FTY720-p average concentration at steady state (ng/mL)) and all efficacy endpoints including aggregate annualized relapse rate(ARR) and MRI lesion count when the placebo group was included in the analysis. However, the relationship is flat without placebo within the observed exposure range.
Two phase III studies (CFTY720D2301, CFTY720D2302) were included in the sponsor's exposure-response analyses for both efficacy and safety; CFTY720D2301 was a 24-month double blind, multicenter, parallel-group study comparing the efficacy and safety of 0.5 mg and 1.25mg fingolimod QD versus placebo; CFTY720D2302 was a 12-month double-blind, randomized, multicenter, parallel-group study comparing the efficacy and safety of 0.5 mg and 1.25 mg fingolimod QD versus interferon β-1a i.m. (Avonex®) administered i.m. once weekly in patients with relapsing-remitting multiple sclerosis.
Primary endpoint was the aggregate annualized relapse rate (ARR) which was computed as total number of confirmed relapses for all the patients divided by the total number of days on the study for all patients, multiplied by 365.25. Fingolimod at both dose groups (0.5mg and 1.25mg) showed superior effectiveness (study 2301: 18%, 16%, study 2302: 26%, 20%) compared to placebo (40%) and active control (interferon β-1a, 33%) in the efficacy analysis with little difference between two dose groups. The similar results were shown in other secondary endpoints such as disability-related endpoint (EDSS score) or MRI measures of inflammation (MRI lesion count).
The sponsor conducted exposure-response analyses to characterize the relationship between FTY720-p concentrations at steady state and key efficacy (MRI lesion count, relapses) and safety endpoints (lymphocyte reduction, liver enzyme, FEV1 and HR), focusing on estimating potency parameter at each dose group to justify the proposed dose of 0.5mg QD. The sponsor's exposure-response (E-R) analyses using lymphocyte counts, new T2 lesions and ARR showed that there was significant exposure-response relationship across all three endpoints and the dose of 0.5mg would achieve about 80-88% of maximum response.
Due to the presence of S1P receptors in multiple tissues, fingolimod manifests a number of other biological effects in addition to the reduction in circulating lymphocytes. These include a transient reduction in heart rate and atrio-ventricular conduction on treatment initiation, a dose-dependent mild increase in airway resistance, macular edema, a mild increase in blood pressure, and asymptomatic elevation in serum levels of hepatic transaminases. The sponsor's exposure-safety analyses showed that most safety endpoints including FEV1, heart rate (HR) and infection rate were not related to FTY720-p concentration within the studied exposure range but liver enzyme level such as AST and ALT slightly increases with increasing concentration especially in male population.

In addition to the sponsor's E-R analyses, the reviewer performed independent analyses to examine whether the sponsor's proposed dose is appropriate or not. Given the benefit and risk profile of fingolimod the reviewer evaluated whether lower doses than 0.5 mg can provide acceptable effectiveness based on the primary endpoint (ARR). The reviewer's analysis indicates that 0.25 mg may provide comparable effectiveness as 0.5 mg.
First the reviewer tried to quantify the relationship between ARR and FTY720-p concentration directly. However, due to the lack of data at lower exposure range, there was uncertainty on the shape of relationship, making it impossible to predict ARR at unstudied lower dose such as 0.25 mg precisely (refer to section 4. reviewer's analysis).
Hence, given the availability of lymphocyte counts data and the presumed relationship between lymphocyte counts and ARR, the reviewer used lymphocyte counts (PD biomarker) as a bridge

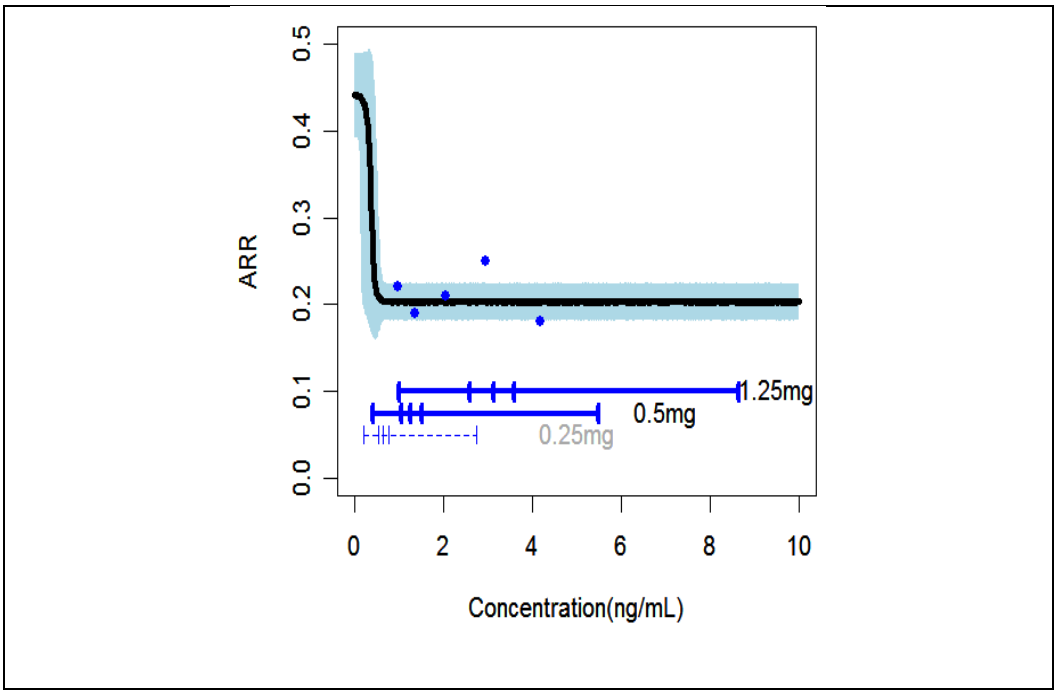
to link ARR and FTY720-p concentration as it was expected that lymphocyte counts-concentration relationship and the ARR- lymphocyte counts relationship would be quantified more precisely.
The left panel in Figure 1 displays the relationship between lymphocyte counts at steady state (SS) and FTY720-p concentration, and the right panel represents the relationship between ARR and lymphocyte counts at SS. Both models describe the observed data reasonably well. ARR-lymphocyte counts relationship suggest that mean lymphocyte reduction below 1E9/L would be necessary to get mean ARR of about 0.2 and further lower lymphocyte counts does not provide additional ARR benefit.

Figure 6. The model predicted relationship for lymphocyte counts at SS and FTY720-p concentration relationship (left) and the relationship for ARR and lymphocyte counts at SS (right) with 95% prediction interval (blue shaded area). The orange dots on the left are observed absolute number of lymphocyte at SS at decile of exposure range. The black dots on the right panel indicate the observed ARR at decile of lymphocyte counts at SS. Also blue vertical bars on the right panel shows the distribution of lymphocyte counts for each treatment group.



To link concentration with ARR, the lymphocyte counts were predicted over the observed concentration range (lymphocyte-concentration model) and then the predicted lymphocyte counts were used to predict ARR (ARR- lymphocyte model). **Error! Reference source not found.** shows the results for ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge. The shape of ARR-concentration relationship was quantified with improved precision. Based on this relationship, the model predicted average ARR of 0.26 (95%CI: 0.22-0.30) at 0.25 mg, which could be almost as effective as 0.5mg.

Figure 7. The predicted ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge with 95% confidence interval. Four dots represent observed ARR at quintile of exposure range.



Recommendations

The Division of Pharmacometrics has reviewed the submission (NDA 22527) and has the following recommendation:

- A lower dose than 0.5mg QD such as 0.25mg, should be studied if the current safety profile at 0.5 mg QD is not acceptable.

PERtinent Regulatory Background

The sponsor is seeking the approval for fingolimod (FTY720) 0.5mg QD for a disease modifying therapy for the treatment of patients with relapsing forms of multiple sclerosis (RRMS) to reduce the frequency of clinical exacerbations and to delay the accumulation of physical disability. Fingolimod is the first sphingosine-1-phosphate (S1P) receptor modulator, in development for the treatment of MS. The key pharmacodynamic effect of fingolimod is dose-dependent reduction of peripheral lymphocyte count mediated by down-modulation of the S1P1 receptor on lymphocytes.
The clinical development program of fingolimod in MS was pursued based on both (1) the hypothesis that restricting lymphocytes to peripheral lymphoid tissue could be of benefit and (2) data from experimental models of MS in animal showing efficacy.
In the Phase III program, fingolimod has demonstrated a reduction in the frequency of relapse by 54-60 % relative to placebo in a 2-year study in patients with RRMS and also demonstrated superior efficacy over a current standard of care, interferon (IFN β-1a, Avonex®) in a 1-year study.

Due to the presence of S1P receptors in multiple tissues, fingolimod manifests a number of other biological effects in addition to the reduction in circulating lymphocytes. These include a transient reduction in heart rate and atrio-ventricular conduction on treatment initiation, a dose-dependent mild increase in airway resistance, macular edema, a mild increase in blood pressure, and asymptomatic elevation in serum levels of hepatic transaminases.

Results of Sponsor's Analysis

PK analyses

Due to the sparse sampling, long-half life of FTY720-p (6-9 days), the sponsor used linear mixed effect modeling with a random effect on the intercept parameter, a residual variance with an auto-regressive 1st order (AR1) structure and three independent covariates affecting the intercept: gender, weight and race. The final model is shown in below;

$$CWGT = WGT - 70$$

$$\mu = (\beta_0 + b_0) + (\beta_1 \times CWGT) + (\beta_2 \times MALE) + (\beta_3 \times RACE) + (\beta_4 \times TGP)$$

$$Y \sim MVN\left(\begin{bmatrix}\mu_1\\ \mu_2\\ \dots\\ \mu_9\end{bmatrix}, \sigma^2\begin{bmatrix}1 & \rho & \rho^2 & \dots\\ & 1 & \rho & \dots\\ & & \dots & \dots\\ & & & 1\end{bmatrix}\right), \quad b_0 \sim N(0, \sigma_0^2)$$

where μ_1, μ_2, ..., μ_9 correspond to the estimated mean FTY720-P concentration at month 2, 3, 6, 9, 12, 15, 18, 21 and 24 in study CFTY720D2301; month 6 and month 12 in study CFTY720D2302. CWGT : bodyweight centered at 70kg, TGP=1 if dose=0.5mg, 0 if dose=1.25mg.

Source: the sponsor's report : Modeling of the exposure-response relationship in patients

with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 27.

Although, the male and weight covariates were associated with significant p-values, the magnitude of the effects associated with these covariates was deemed to be not clinically relevant; a male would have on average a 10.4% lower concentration than a female, and a gain in weight of 14 kg (from 70 to 84 kg) would be on average associated with a 6.2% decrease in concentration. (b) (4) -For 70kg female who were assigned to 0.5mg, estimated mean concentrations at SS in Asian and Black population are 62% higher and 14% lower than Caucasian (b) (4) . However, it should be cautious that there was not sufficient observed data in Asian (N=14).

Due to their potential risk of interaction with FTY720-P concentration, the effects of the following concomitant medication on the FTY720-P average concentration were investigated: fluoxetine and paroxetine, itraconazole and ketoconazole, carbamazepine, clarithromycin, corticosteroids and oral contraceptive. Besides, due to their high frequency of use in the studied population, the effect of the most frequent concomitant medication on FTY720-P average concentration was also checked. The most frequent concomitant medication include: baclofen, gabapentin, oxybutin, amantadine, amitriptyline, pregabalin and modafinil. For all these concomitant treatments, no unexpected effect was observed on FTY720-P concentrations (see Table 1).

Table 1. Change in FTY720-p geometric mean concentration with concomitant treatment relative to without concomitant treatment. Nsamples* refers to the number of samples with concomitant treatment and FTY720 0.5 mg / FTY720 1.25 mg. The number of samples without concomitant treatment was approximately ranging between 3000 and 4000, in all cases.

Concomitant treatment	Effect	$N_{samples}$*	Relative change (0.5 mg)	Relative change (1.25 mg)
Fluoxetine and Paroxetine	Minor	141 / 105	-11.6%	-4.3%
Itra- and keta-conazole	? (N small)	16 / 3	-13.7%	+20.0%
Carbamazepine	As expected	59 / 49	-27.4%	-28.2%
Clarithromycin	? (N small)	5 / 4	+41.0%	+16.3%
Oral corticosteroids	? (N small)	11 / 2	-8.8%	-47.5%
IV corticosteroids	Minor	28 / 19	-15.4%	-10.0%
Oral contraceptive	Minor	666 / 594	+15.9%	+15.8%
Baclofen	Null	103 / 164	-9.7%	+9.8%
Gabapentin	Null	67 / 85	+4.5%	-16.0%
Oxybutin	Null	72 / 91	+2.9%	-4.8%
Amantadine	Null	91 / 118	-17.8%	+6.9%
Amitriptyline	Null	52 / 90	-6.4%	-13.4%
Pregabalin	Minor	26 / 44	-20.2%	-30.0%
Modafinil	Minor	82 / 22	-20.2%	-35.5%

Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 30.

Exposure-Response analyses

The sponsor conducted exposure-response analyses to characterize the relationship between FTY720-p concentrations at steady state and key efficacy (MRI lesion count, relapses) and safety endpoints (lymphocyte reduction, liver enzyme, FEV1 and HR).
Two phase III studies (CFTY720D2301, CFTY720D2302) were included in the sponsor's exposure-response analyses; CFTY720D2301 was a 24-month double blind, multicenter, parallel-group study comparing the efficacy and safety of 0.5 mg and 1.25mg fingolimod QD versus placebo; CFTY720D2302 was a 12-month double-blind, randomized, multicenter, parallel-group study comparing the efficacy and safety of 0.5 mg and 1.25 mg fingolimod QD versus interferon β-1a i.m. (Avonex®) administered i.m. once weekly in patients with relapsing-remitting multiple sclerosis.

Primary endpoint was the aggregate annualized relapse rate (ARR) which was computed as total number of confirmed relapses for all the patients divided by the total number of days on the study for all patients, multiplied by 365.25. Fingolimod at both dose groups (0.5mg and 1.25mg) showed superior effectiveness compared to placebo and active control (interferon β-1a) in the efficacy analysis with little difference between two dose groups (see **Table 2**, **Table 3**). The similar results were shown in other secondary endpoints such as disability-related endpoint (EDSS score) or MRI measures of inflammation (MRI lesion count).

Table 2. Aggregate annualized relapse rate up to month 24 : study D2301

	FTY720D 1.25mg N=429	FTY720D 0.5mg N=425	Placebo N=418
Aggregate ARR estimate (95% CI)	0.16 (0.13, 0.19)	0.18 (0.15, 0.22)	0.40 (0.34, 0.47)
ARR ratio (95% CI) vs. placebo	0.40 (0.32, 0.50)	0.46 (0.37, 0.57)	--
P-value vs. placebo	<0.001*	<0.001*	--
P-value fingolimod 1.25mg vs. 0.5mg		0 226	--

Aggregate ARR estimate (95% CI), ARR ratio, and p-value are calculated using negative binomial regression adjusted by treatment, pooled country, number of relapses in the previous 2 years, and baseline EDSS.

Source: the sponsor's report : clinical overview, page27.

Table 3. Aggregate annualized relapse rate up to month 12 : study D2302

	FTY720D 1.25 mg N=420	FTY720D 0.5 mg N=429	IFN-β-1a N=431
Adjusted ARR estimate (95% CI)	0.20 (0.16, 0.26)	0.16 (0.12, 0.21)	0.33 (0.26, 0.42)
ARR ratio vs. IFN beta-1a	0.62	0.48	--
P-value vs. IFN beta-1a	<0.001*	<0.001*	--
P-value FTY720D 1.25mg vs. 0.5mg		0.159	

* Indicates two-sided statistical significance at the 0.05 level.

Source: the sponsor's report : clinical overview, page 32.

Data from 2552 patients from studies CFTY720D2302 and CFTY720D2301 were pooled in an exposure-response analysis for FTY720 in RRMS patients. The data of all patients with evaluable PK measurements in these two studies was proposed to be used for an exposure-response analysis, focusing on the relationship between FTY720-P average concentrations at steady state and the following key endpoints: relapse rate, MRI lesion counts, lymphocytes and infections.

Summary of baseline and demographic characteristics in two studies are given in **Table 4**.

Table 4. Mean (SD) of the baseline and demographic characteristics in study D2301 (top) and D2302 (bottom).

Study '2301'	Placebo	FTY720 0.5 mg	FTY720 1.25 mg
Treatment duration (days)	621 (209)	655 (179)	595 (244)
Age (years)	37.2 (8.6)	36.6 (8.8)	37.4 (8.9)
Weight (kg)	70.7 (14.6)	71.6 (15.2)	70.8 (16.3)
BMI (kg/m^2)	24.7 (4.4)	24.9 (4.8)	24.4 (4.8)
EDSS	2.5 (1.3)	2.3 (1.3)	2.4 (1.4)
Volume of T2 lesions	6162 (7085)	6128 (7623)	6829 (8491)
Number of Gd-enhanced T1 lesions	1.26 (2.93)	1.65 (5.57)	1.80 (4.66)
Relapses in the year prior study	1.44 (0.73)	1.46 (0.76)	1.46 (0.81)
Relapses in the 2 years prior study	2.16 (1.19)	2.12 (1.13)	2.15 (1.25)
Lymphocytes (10E9/L)	1.82 (0.57)	1.86 (0.62)	1.84 (0.55)
Duration of MS since 1st symptoms	8.14 (6.35)	8.01 (6.60)	8.40 (6.86)
Ethnicity (Caucasian/ Black/ Asian/ Native American/ Others)	95.5/ 0.5/ 0.7/ 0/ 3.3	95.5/ 0.3/ 0.7/ 0/ 3.5	95.1/ 0/ 0/ 0/ 4.9
Gender (M:F)	0.29 : 0.71	0.30 : 0.70	0.31 : 0.69

Study '2302'	FTY720 0.5 mg	FTY720 1.25 mg	IFN-1β 30 µg
Treatment duration (days)	347 (72)	336 (89)	342 (81)
Age (years)	36.8 (8.8)	35.8 (8.4)	35.9 (8.3)
Weight (kg)	71.5 (16.2)	71.2 (16.9)	71.6 (17.4)
BMI (kg/m^2)	24.9 (4.7)	25 (5.1)	25.2 (5.4)
EDSS	2.2 (1.3)	2.2 (1.3)	2.2 (1.3)
Volume of T2 lesions	5170 (6642)	5085 (5962)	4924 (5711)
Number of Gd-enhanced T1 lesions	0.98 (2.81)	1.49 (4.77)	1.06 (2.8)
Relapses in the year prior study	1.52 (1.20)	1.52 (0.86)	1.45 (0.79)
Relapses in the 2 years prior study	2.29 (2.20)	2.17 (1.18)	2.25 (1.22)
Lymphocytes (10E9/L)	1.77 (0.53)	1.78 (0.53)	1.77 (0.53)
Duration of MS since 1st symptoms	7.46 (6.20)	7.3 (5.96)	7.38 (6.33)
Ethnicity (Caucasian/ Black/ Asian/ Native American/ Others)	93.7/ 0.9/ 1.6/ 0.3/ 3.5	94.8/ 1.4/ 1.6/ 0.3/ 1.9	93.7/ 1.4/ 0.9/ 0.3/ 3.7
Gender (M:F)	0.35 : 0.65	0.31 : 0.69	0.32 : 0.68

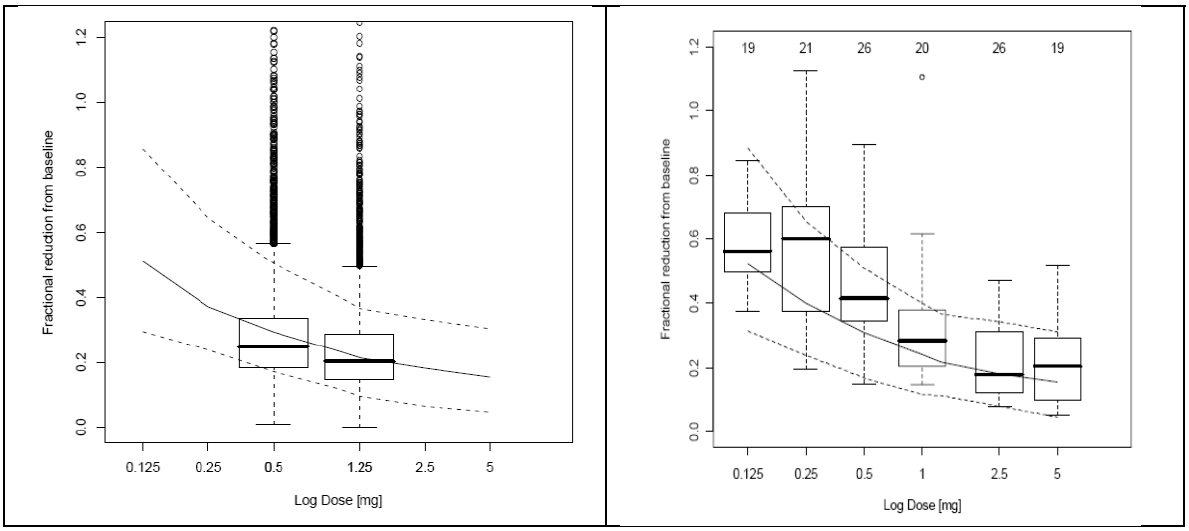
Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 25.

For each variable (Lymphocyte counts, MRI lesion and Relapse), an inhibitory E_{max} model was used to describe the exposure-response relationship, focused on estimating IC50 parameter which indicates a relative potency of the respective doses; the non-linear mixed effect model with two correlated random effects was the final model for the relationship between lymphocyte counts and FTY720-p concentration; the negative binomial regression model was used to model new T2 lesion count related to FTY720-p concentration; the continuous-time Markov model, used to analyze the relapse data, described both the time spent in a relapse or remitting state and the transition rates between states as a function of predictors.

Sex was one significant covariate in the relationship for lymphocyte counts-FTY720-p concentration but the difference is not clinically meaningful (the estimated maximum reduction was 85% in female and 80% in male).

Also the predicted dose response in lymphocytes was found to be similar between RRMS patients, stable renal transplant patients (study CFTY720A B102), and healthy volunteers (pooled phase 1 data). See **Figure 8**.

Figure 8. Predicted dose-response (lymphocyte counts). Left panel :The boxplots characterize the distribution of the lymphocyte responses at each dose level of the phase 3 studies. The solid line is the predicted median dose-response based on a simulation of 500 healthy volunteers at each dose level following 90 days treatment and the dashed lines are the 5th and 95th percentiles; Right panel: The box plots characterize the distribution of the lymphocyte responses in each cohort in study FTY270A-B102. The numbers at the top are the sizes of the respective cohorts. The solid line is the predicted median dose-response based on a simulation of 500 healthy volunteers at each dose level; the dashed lines are the 5th and 95th percentiles



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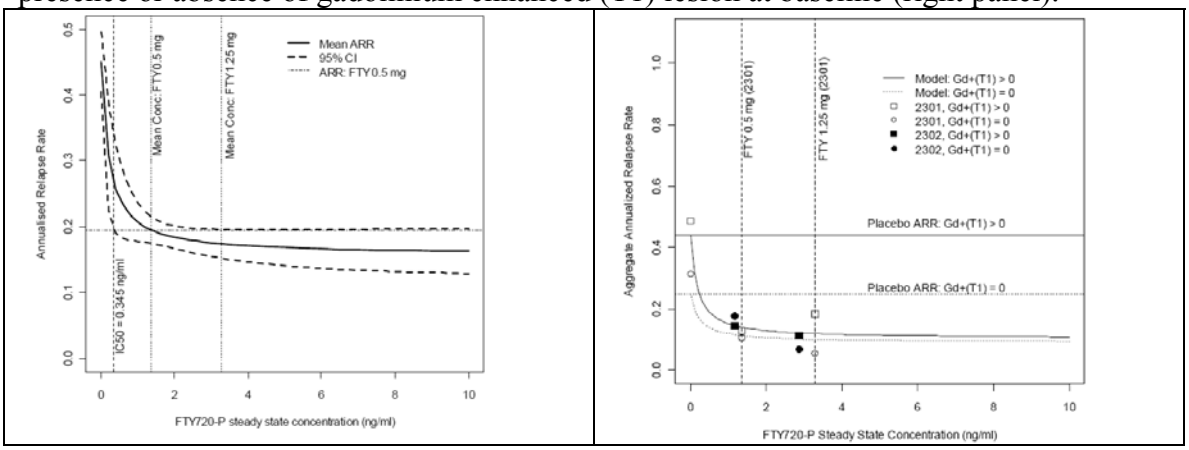
Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 35-36.

Figure 9 displays the model-predicted relationship with FTY720-p concentration for ARR from the sponsor's models. The sponsor's analyses showed that 0.5mg is on the shoulder and 1.25mg on plateau. The most important covariates in the relapse model are the disease severity (as expressed by EDSS at baseline (<3 or ≥3)) and the disease activity at baseline (as expressed by the number of gadoliniumenhanced(T1) lesions at baseline (none or >0) and number of relapses prior to study start).

Figure 9. The model predicted relationship for ARR and FTY720-p concentration with no covariate (left panel) and with EDSS at baseline lower than 3 and R2Y=1, stratified by presence or absence of gadolinium enhanced (T1) lesion at baseline (right panel).



Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 50 and 53.

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Table 5 lists the estimated IC50 values and relative potency at each dose for each variable. The three IC50s were similar, indicating a comparable potency of the drug for the respective endpoints. Furthermore, for all three pharmacodynamic variables the typical response of the lower dose (0.5 mg) was 6-10 percentage points further from the maximum response than the typical response of the higher dose (1.25 mg).

Table 5. Summary of IC50 and relative potencies for lymphocyte counts, new T2 lesions and relapse rates, in patients treated with FTY720.

Variable	Structural feature	IC50: Concentration of half-maximal response (ng/mL)	Relative potency for FTY720 0.5 mg (at 1.326 ng/mL)	Relative potency for FTY720 1.25 mg (at 3.224 ng/mL)
Lymphocytes	Mean count at steady state	0.18	88%	95%
New T2 lesions	Mean count at month 12	0.17	88%	95%
Relapses	Transition rate from remission to relapse state	0.34	80%	90%

Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 60.

Due to the presence of S1P receptors in multiple tissues, fingolimod manifests a number of other biological effects in addition to the reduction in circulating lymphocytes. These include a transient reduction in heart rate and atrio-ventricular conduction on treatment initiation, a dose-

dependent mild increase in airway resistance, macular edema, a mild increase in blood pressure, and asymptomatic elevation in serum levels of hepatic transaminases.

In terms of safety analyses, no formal modeling method was employed. Most safety endpoints including FEV1, heart rate (HR) and infection rate were not related to FTY720-p concentration but liver enzyme level such as AST and ALT slightly increases with increasing concentration especially in male population (**Table 6**, **Figure 10**). Also within the observed lymphocyte count range, the lymphocyte count was not identified as a predictor of infection count either.

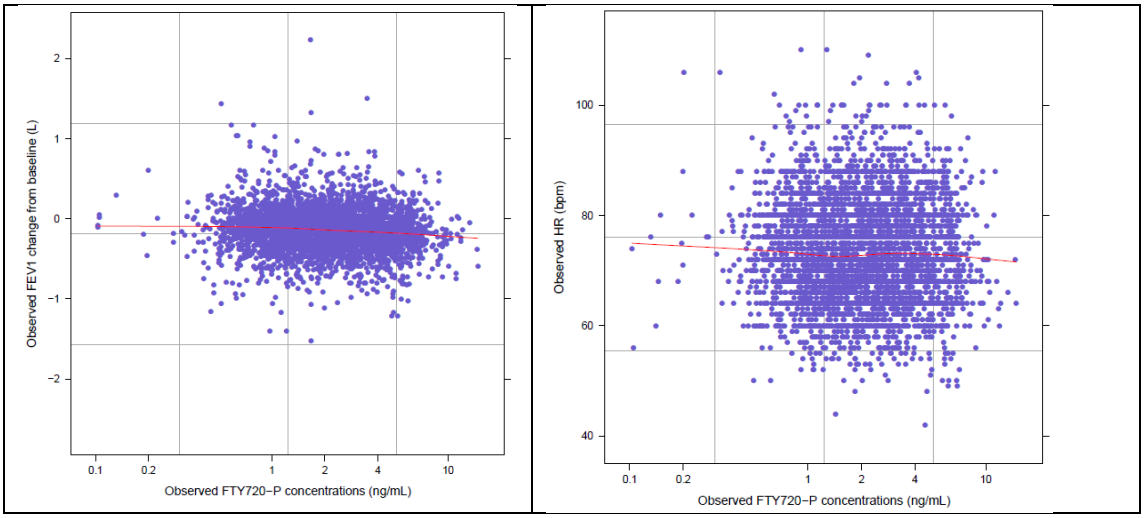
Table 6. The number of infections normalized to treatment duration at each dose group by the study.

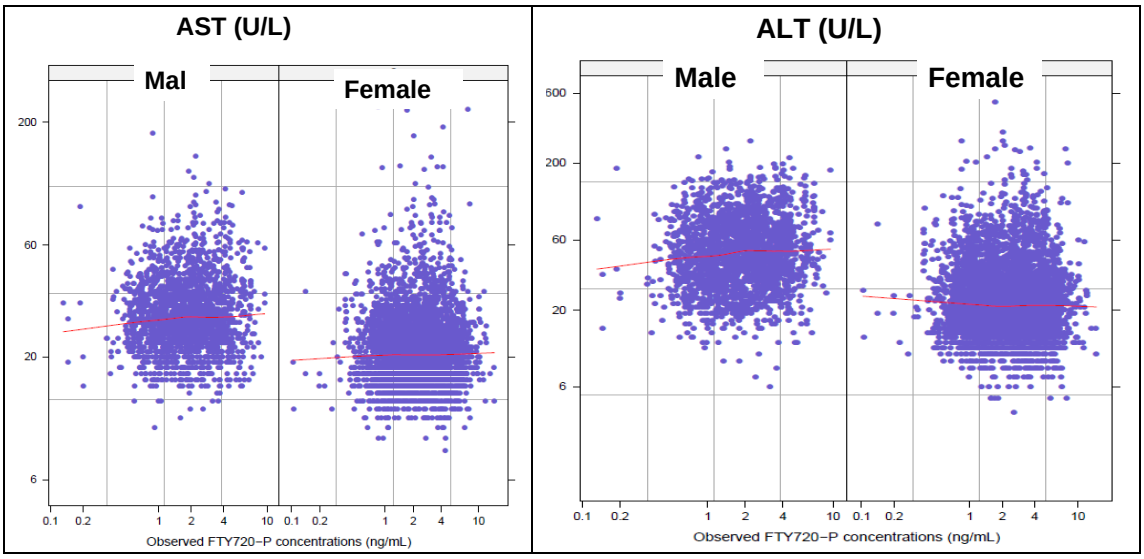
Analysis Variable : NINF								
Study Number	Planned Dose	N Obs	N	Mean	Std Dev	Minimum	Maximum	Sum
2301	0	411	411	1.5021549	1.8473442	0	14.0667331	617.3856499
	0.5	421	421	1.3964009	1.7392772	0	13.0446429	587.8847959
	1.25	421	421	1.4123612	2.4016693	0	26.0892857	594.6040463
2302	0.5	425	425	1.2368766	2.6807649	0	42.9705882	525.6725497
	1.25	414	414	1.4057922	2.3505375	0	27.5660377	581.9979510
	30	417	417	1.3347521	5.4133856	0	104.3571429	556.5916206

NINF = number of infections normalized to treatment duration

Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 114..

Figure 10. The relationship between safety endpoints (at day >=60) and FTY720-p concentration; change in FEV1 (L) from the baseline, Heart Rate(bpm), AST (U/L) and ALT (U/L), clockwise.





Source: the sponsor's report : Modeling of the exposure-response relationship in patients with relapsing remitting multiple sclerosis, based on data collected in two phase 3 studies (CFTY720D2301 and CFTY720D2302), page 172-175.

The sponsor's final models with parameter estimates for each endpoint are summarized in appendix.

Reviewer's comments:

- *The sponsor' modeling methods are acceptable*
- *However, without placebo, E-R relationship appears to be flat, and therefore lower dose than 0.5mg could provide similar effect size.*

Reviewer's Analyses

Introduction

The sponsor proposes the dose of 0.5 mg QD for the treatment of RRMS. In the efficacy analyses results as well as the sponsor's exposure-response analyses across all endpoints, there was little difference shown between low (0.5mg) and high (1.25mg) doses although both doses showed superior effectiveness compared to placebo and active control.
There has been safety concern with fingolimod due to the presence of S1P receptors in multiple tissues, including a transient reduction in heart rate and atrio-ventricular conduction on treatment initiation, a dose-dependent mild increase in airway resistance, macular edema, a mild increase in blood pressure, and asymptomatic elevation in serum levels of hepatic transaminases. Hence, given these safety concerns, the reviewer performed the independent analyses to see if lower dose than 0.5mg would produce sufficient effectiveness based on primary endpoint (ARR).

Objectives

- To predict ARR at lower dose than the proposed dose of 0.5mg based on modeling approach
  - To characterize the relationship between clinical endpoint (ARR) and PD marker (lymphocyte counts at steady state)
  - To use of PD biomarker as a bridge to the relationship between clinical endpoint (ARR) and concentration

Methods

The data from two studies were pooled. Negative binomial model was used to model the relationship between ARR and FTY720-p concentration as follows;

$$Y_i \sim NB(\mu_i, k)$$

$$\log(\mu_i) = \beta_0 \times (1 - f_i(conc)) + \log(t_i)$$

where

$$f_i(conc) = 0 \text{ if placebo}$$

$$= \frac{\text{Imax} \times conc^{\wedge h}}{IC50^{\wedge h} + conc^{\wedge h}} \text{ if fingolimod}$$

$$= \beta_1 \text{ if interferon}\beta$$

Model (1)

Y : the number of confirmed relapse for each patient

$\log(t_i)$ =on study day/365.25, offset variable to account for a patient's different study period in negative binomial model.
k is dispersion parameter.
Because of estimation problem due to the lack of data around declining part on the model, hill-coefficient (h) was fixed to the range over (0.5, 1, 1.5, 2, 2.5, 3).

For the lymphocyte counts, the sponsor's base model was utilized (see appendix). For the model for the relationship between ARR and lymphocyte counts, the similar model to the model (1) was employed as follows;

$$Y_i \sim NB(\mu_i, k)$$

$$\log(\mu_i) = \beta_0 \times (1 - f_i(lymphocyte)) + \log(t_i)$$

where

$$f_i(lymphocyte) = \frac{\text{Ilmax} \times lymphocyte^{\wedge h}}{Ilym50^{\wedge h} + lymphocyte^{\wedge h}}$$

Model (2)

Data Sets

Data sets used are summarized in Table 7.

Table 7. Analysis Data Sets

Study Number	Name	Link to EDR
D2301, D2302	nm.xpt	

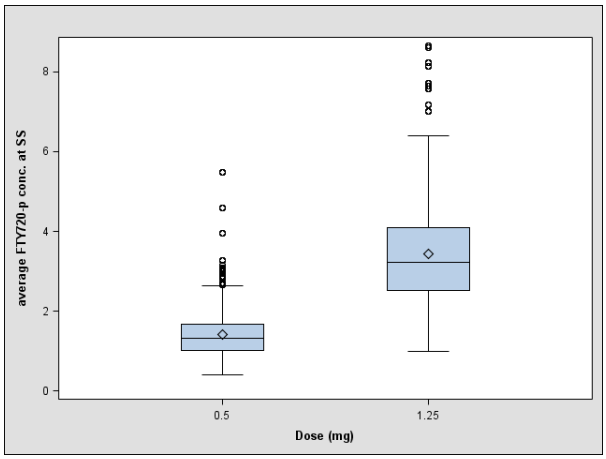
Software

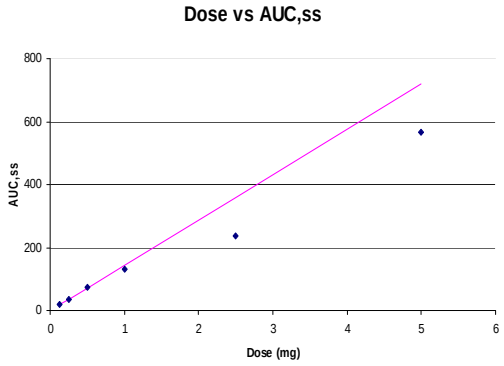
SAS 9.2 and R 2.5 were used for the analysis.

Model Results

First, the dose-proportionality was examined. Figure 1 displays the distribution of predicted FTY720-p concentration from two phase III studies(left panel) and the relationship between AUC at SS and dose (right panel) with dose proportionality at steady state at doses lower than 1 mg. The predicted median concentration at 0.5mg and 1.25mg are 1.25ng/mL and 3.13ng/mL, respectively. Based on this observation, the concentration at the dose of 0.25mg would be approximately a half of concentration at the dose of 0.5mg if 0.25mg would have studied in the clinical trial.

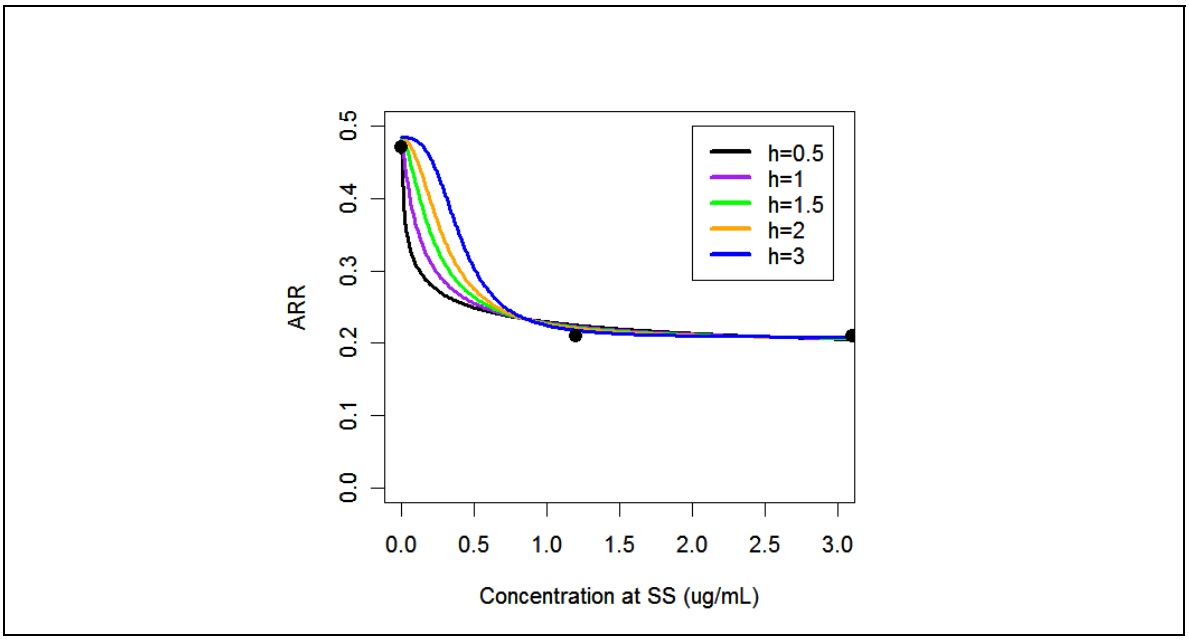
Figure 11. The distribution of predicted FTY720-p concentration at each dose (left panel) and dose-proportionality (right panel)





As the first attempt to quantify the relationship between ARR and FTY720-p concentration, the negative binomial model (1) for the number of confirmed relapse was fitted to pooled dataset. The reviewer tried to estimate hill-coefficient parameter in the model. However, due to the lack of data around declining part of model, hill-coefficient could not be estimated. Hence the reviewer fixed it at the range over 0 to 3 rather than estimating it. Figure **2** shows the model-predicted relationship between ARR and FTY720-p concentration based on the reviewer's model by different hill-coefficient. The black dots represent the observed mean ARR at each dose group which were marked at the median of exposure range at each dose. As shown in Figure **2**, the model describes the observed data well regardless of different values of hill-coefficient, implying that there was uncertainty on the shape of relationship, making it impossible to predict ARR at unstudied lower dose such as 0.25 mg precisely.
Hence, given the availability of lymphocyte counts data and the presumed relationship between lymphocyte counts and ARR, the reviewer used lymphocyte counts (PD biomarker) as a bridge to link ARR and FTY720-p concentration as it was expected that lymphocyte counts-concentration relationship and the ARR- lymphocyte counts relationship would be quantified more precisely.

Figure 12. The model predicted ARR and FTY720-p concentration relationship (solid black line) by different hill-coefficient values. The black dots are observed ARR at each dose groups which are marked at the median exposure at each dose.



Before fitting the model for lymphocyte counts, the reviewer examined the time-profile of lymphocyte counts, which shows that the number of lymphocyte counts appears to be at steady state from month 2 (Figure 3). Therefore, the lymphocyte counts from month 2 were averaged for each patient to match to exposure and ARR. Hereafter, lymphocyte counts at SS refers to average lymphocyte counts at SS.
It is well known that Avonex has different mechanism of action from fingolimod. However, as shown in Figure 4, placebo and active control (Avonex) groups show very similar distribution with median of about 1.7E9/L so the patients who were assigned to Avonex were also included in the analyses.

Figure 13. The absolute number of lymphocyte counts at each month.

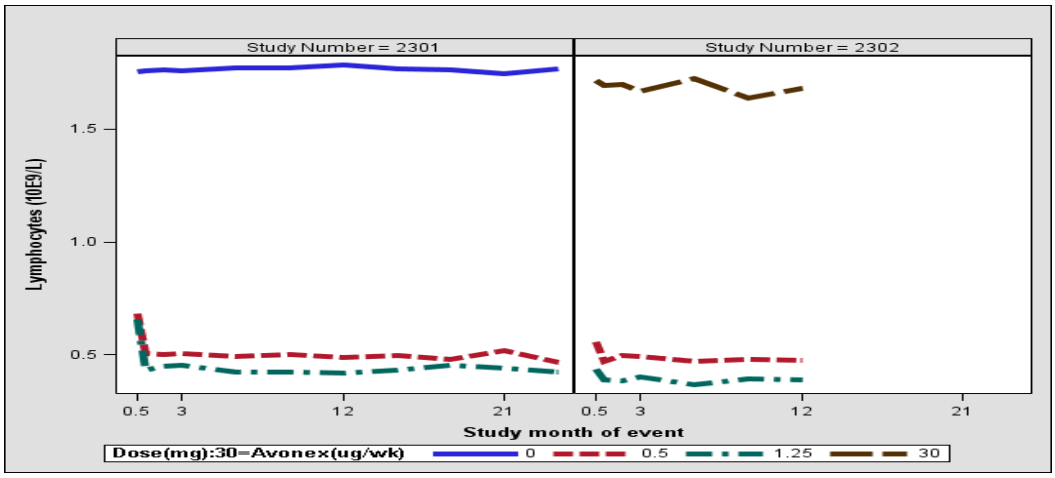
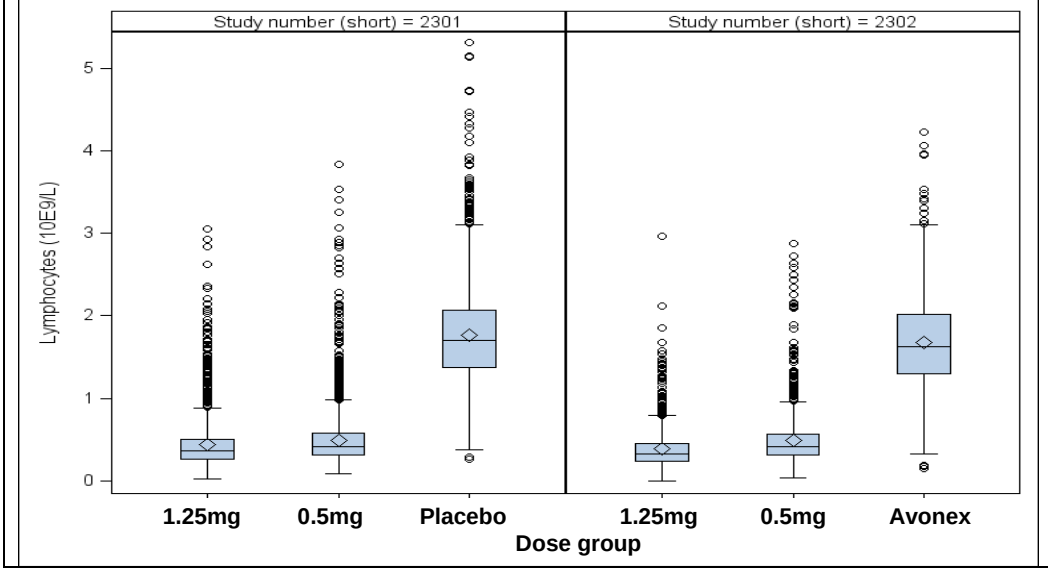
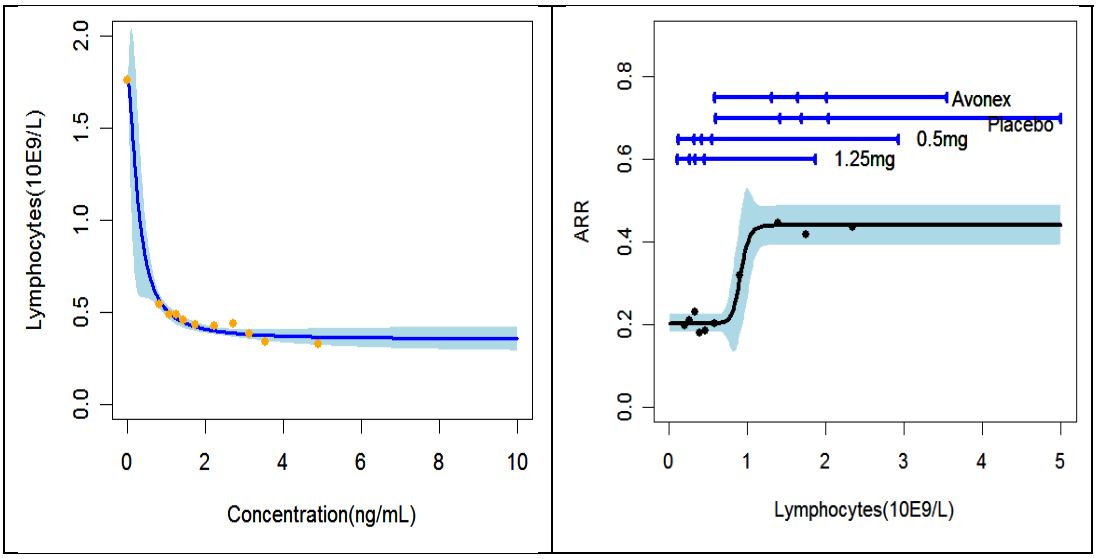


Figure 14. The distribution of absolute number of lymphocyte counts at SS at each treatment group by study.



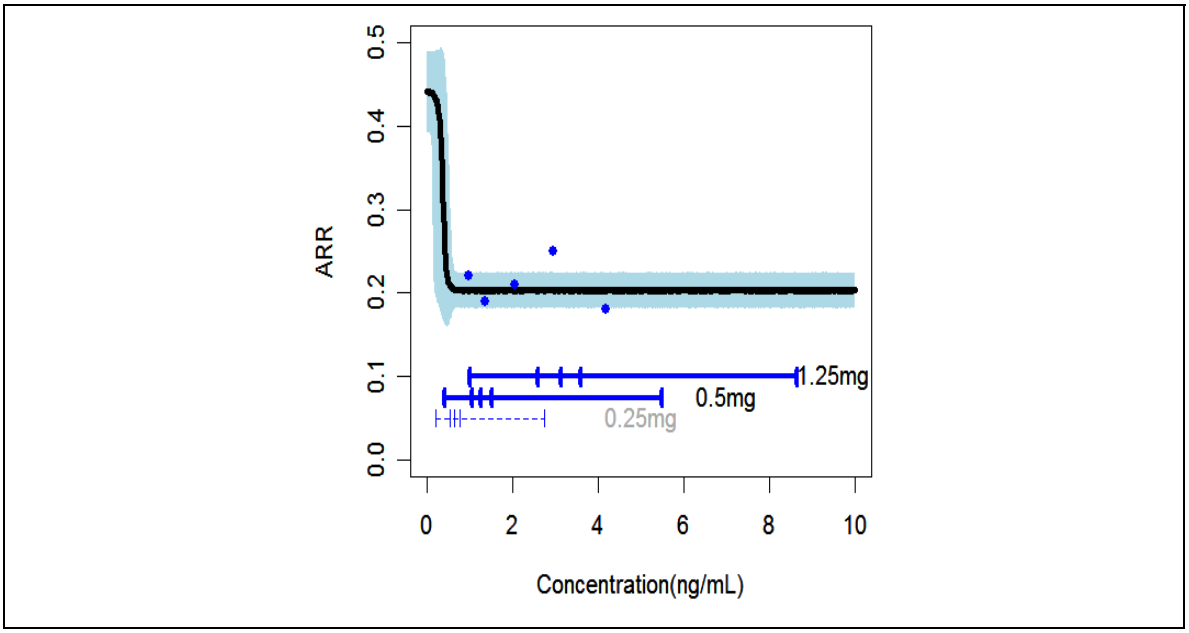
The left panel in Figure 5 displays the relationship between lymphocyte counts at steady state (SS) and FTY720-p concentration, and the right panel represents the relationship between ARR and lymphocyte counts at SS. Both models describe the observed data reasonably well. ARR-lymphocyte counts relationship suggest that mean lymphocyte reduction below 1E9/L would be necessary to get mean ARR of about 0.2 and further lower lymphocyte counts does not provide additional ARR benefit.

Figure 15. The model predicted relationship for lymphocyte counts at SS and FTY720-p concentration relationship (left) and the relationship for ARR and lymphocyte counts at SS (right) with 95% prediction interval (blue shaded area). The orange dots on the left are observed absolute number of lymphocyte at SS at decile of exposure range. The black dots on the right panel indicate the observed ARR at decile of lymphocyte counts at SS. Also blue vertical bars on the right panel shows the distribution of lymphocyte counts for each treatment group.



To link concentration with ARR, the lymphocyte counts were predicted over the observed concentration range (lymphocyte-concentration model) and then the predicted lymphocyte counts were used to predict ARR (ARR- lymphocyte model). Figure **16** shows the results for ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge. The shape of ARR-concentration relationship was quantified with improved precision. Based on this relationship, the model predicted average ARR of 0.26 (95%CI: 0.22-0.30) at 0.25 mg, which could be almost as effective as 0.5mg.

Figure 16. The predicted ARR-concentration relationship using lymphocyte counts (PD biomarker) as a bridge with 95% confidence interval. Four dots represent observed ARR at quintile of exposure range.



In conclusion, the reviewer's analyses showed that exposure-response relationship is flat within the observed exposure range and 0.25mg would be almost as effective as 0.5mg.

Listing of Analyses Codes and Output Files

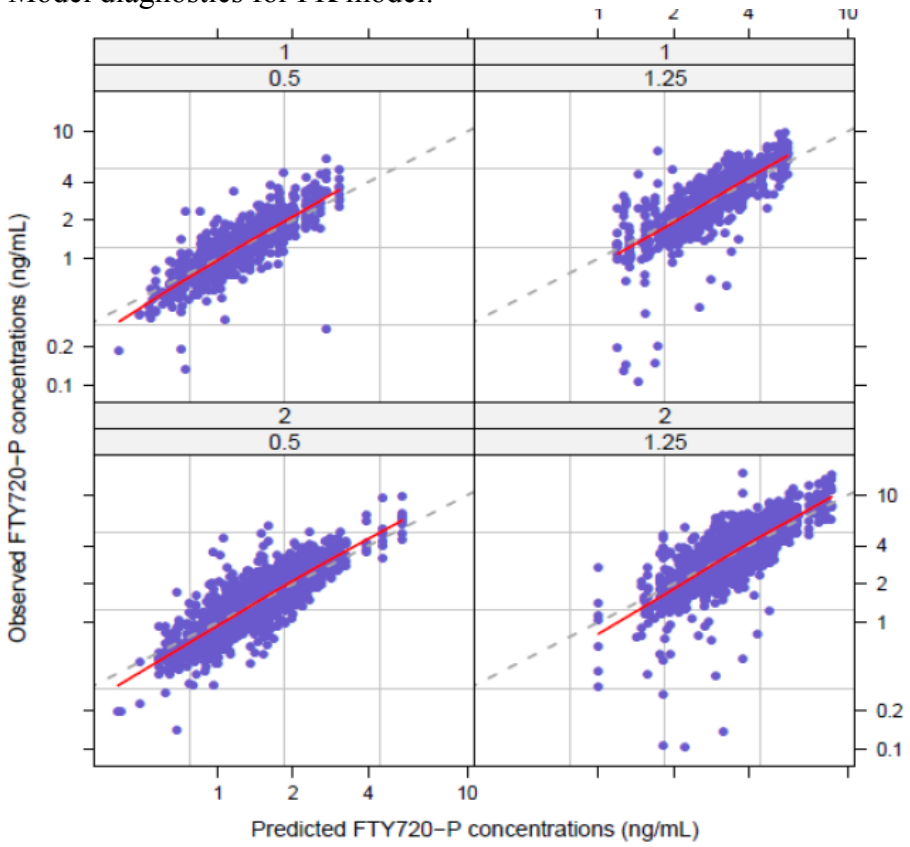
File Name	**Description**	**Location in \\cdsnas\pharmacometrics**
Relapse_reviewer.sas	The reviewer's ARR-FTY720-p concentration analyses	
Lym relapse reviewer.sas	The reviewer's ARR-lymphocyte counts at SS analyses	

Appendix 1

1. The parameter estimates from final population PK.

Parameter	Estimate (SE)	Pr>|t|
β_0 Intercept*	1.1698 (0.01966)	-
β_1 Weight	-0.00459 (0.000837)	<0.0001
β_2 Male	-0.1093 (0.02961)	0.0002
β_3 Race=Black	-0.1479 (0.1702)	0.39
Race=Asian	0.4846 (0.1197)	<0.0001
Race=Others	-0.07661 (0.06496)	0.24
β_4 Dose=0.5 mg	-0.8885 (0.02444)	<0.0001
σ_0^2 Intercept random effect	0.1484	
σ^2 Residual variance	0.1042	
ρ AR(1) correlation	0.1497	

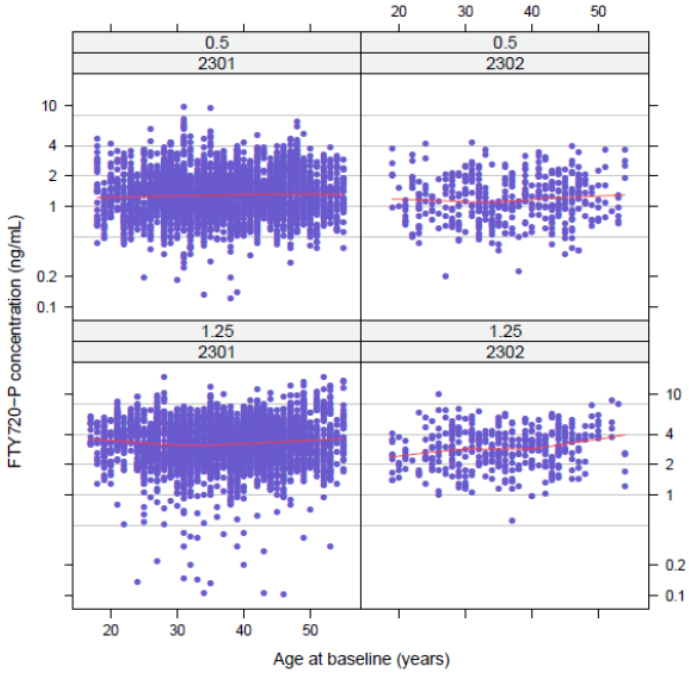
2. Model diagnostics for PK model.



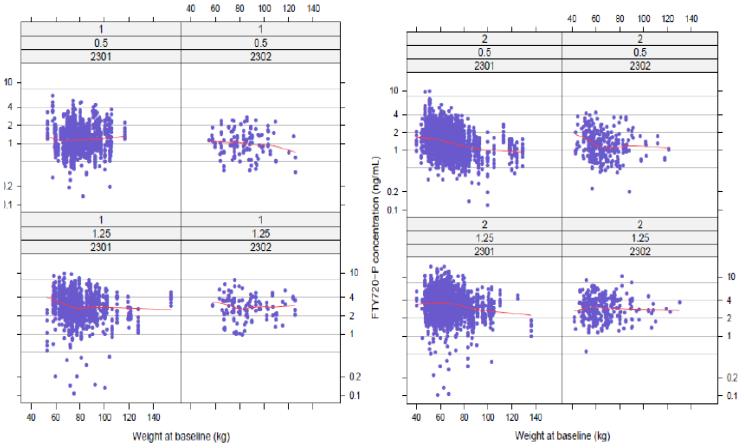
Note: 1=Male, 2=Female

** Grey dotted line represents 45 degree line and red solid line represents actual fitted regression line using observed and predicted concentrations.*

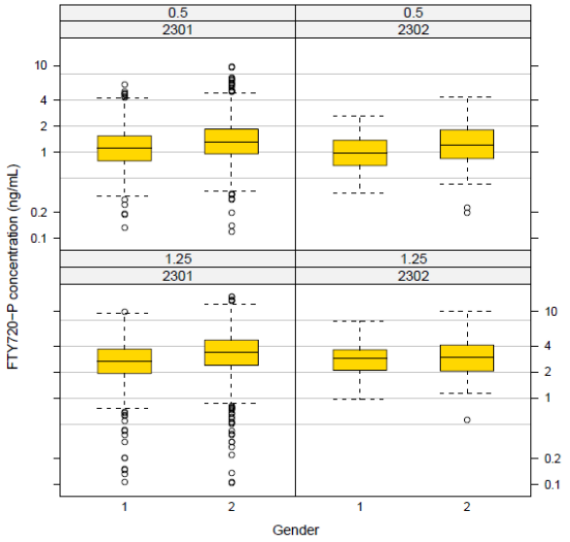
3. FTY720-p concentration .vs. covariates.
- Age



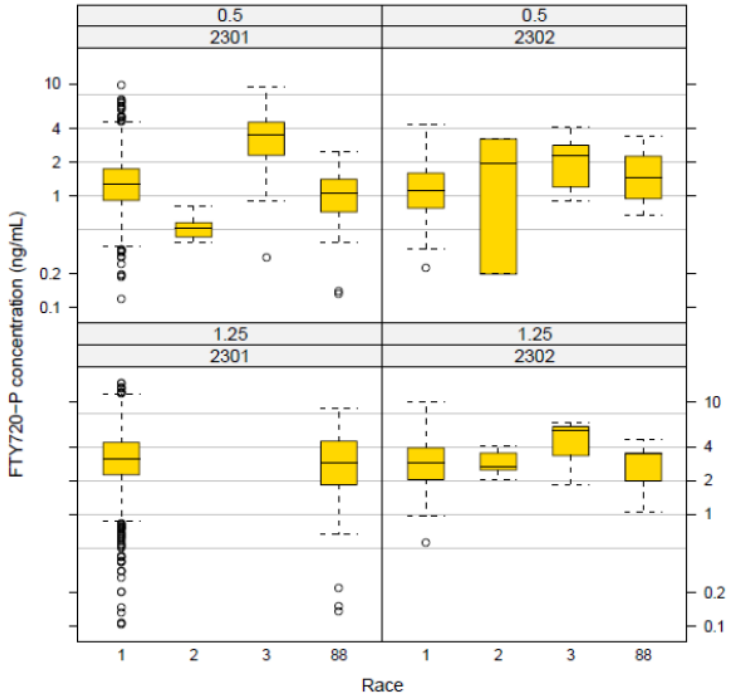
- Weight by gender (left : Male, right : Female)



- Gender



- Race



Note: 1=Caucasian, 2=Black, 3=Asian, 88=Other

3. The model and parameter estimates for the sponsor's exposure-response model.
   - Lymphocyte counts analyses

$$CLYMB = LYMB - 1.8$$

$$fI_{max} = ltI_{max} \times (1 + \beta_3 \times CLYMB) + (\beta_4 \times FEMALE)$$

$$I_{max}{}^{*} = \frac{\exp(fI_{max})}{1 + \exp(fI_{max})}$$

$$fCp = \begin{cases} 0, if\ group = Placebo \\ \dfrac{I_{max}{}^{*} \times Cp}{\exp(LIC50 + b_{LIC50}) + Cp}, if\ group = FTY720\ 0.5\ or\ 1.25\ mg \\ \beta_1 + (\beta_5 \times CLYMB), if\ group = IFN - 1\beta \end{cases}$$

$$\mu = (\beta_0 + b_0) \times (1 - fCp)$$

$$Y \sim N(\mu, \sigma_{res}^2) \quad and \begin{bmatrix} b_0 \\ b_{LIC50} \end{bmatrix} \sim MVN\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_0^2 & \text{cov} \\ \text{cov} & \sigma_{LIC50}^2 \end{bmatrix}\right)$$

Parameter	Estimate (SE)	Pr>|t|
β_0 Intercept	1.8204 (0.01147)	-
β_1	0.08330 (0.01290)	<0.0001
β_3	0.2232 (0.05292)	<0.0001
β_4	0.3257 (0.04146)	<0.0001
β_5	-0.3710 (0.02230)	<0.0001
ltI_{max}	1.2920 (0.05051)	<0.0001
LIC_{50}	-1.7251 (0.09673)	<0.0001
σ_0	0.4634 (0.008351)	-
σ_{LIC50}	1.2560 (0.1067)	-
cov	0.07444 (0.04342)	-
σ_{res}	0.2581 (0.001755)	-

- New T2 lesion count analyses

$$LT1B = \log(T1B + 1)$$

$$CCV2B = \sqrt[3]{V2B} - 15$$

$$IR1Y = 1, if\ R1Y > 1, 0\ else,$$

$$\mu^{*} = \mu_0 + (\beta_1 \times LT1B) + (\beta_4 \times CCV2B) + (\beta_5 \times IR1Y)$$

$$I_{max}{}^{*} = \frac{\exp(ltI_{max} + (\beta_3 \times LT1B))}{1 + \exp(ltI_{max} + (\beta_3 \times LT1B))}$$

$$fCp = \begin{cases} 0, if\ group = Placebo \\ \dfrac{I_{max}{}^{*} \times Cp}{\exp(LIC50) + Cp}, if\ group = FTY720\ 0.5\ or\ 1.25\ mg \\ \dfrac{\exp(\beta_2)}{1 + \exp(\beta_2)}, if\ group = IFN - 1\beta \end{cases}$$

$$\mu = \exp(\mu^{*}) \times (1 - fCp)$$

$$Y \sim NB(\mu, \theta)$$

Parameter	Estimate (SE)	Pr>|t|
μ_0 Intercept	0.9885 (0.09139)	-
β_1	0.7783 (0.08022)	<0.0001
β_2	0.2805 (0.2048)	0.1709
β_3	0.3972 (0.1695)	0.0192
β_4	0.0296 (0.00641)	<0.0001
β_5	0.1825 (0.07287)	0.0123
Itl_{max}	1.0526 (0.2152)	<0.0001
LIC_{50}	-1.7599 (0.4369)	<0.0001
k(1/θ)	1.9332 (0.0990)	-

- Relapse analyses

Assuming that the variable *S(t)* represents the state ('1=relapsing' or '0=remitting') of a patient at time *t*, the next state to which the patient moves, and the time of move, are governed by the transition probabilities:

- from state 0 ('remitting') to state 1 ('relapsing'): $p_{01}(t', t) = P(S(t)=1 \mid S(t')=0)$,
- and vice versa: $p_{10}(t', t) = P(S(t)=0 \mid S(t')=1)$,

for $t' \leq t$,

or by the transition intensities:

- $\lambda_{01}(t) = \lim_{\delta t \to 0} P(S(t+\delta t)=1 \mid S(t)=0)/\delta t$; $\lambda_{00}(t) = 1 - \lambda_{01}(t)$;
- and $\lambda_{10}(t) = \lim_{\delta t \to 0} P(S(t+\delta t)=0 \mid S(t)=1)/\delta t$; $\lambda_{11}(t) = 1 - \lambda_{10}(t)$.

Base model:

$$\lambda_{01} = \exp[\alpha_0 + \left(\frac{I_{max} \times C_p}{\exp(LIC50) + C_p}\right) + \beta_1 \times IFN - 1\beta]$$

$$\lambda_{10} = \exp[\alpha_1].$$

Parameter	Estimate	Standard Error	95% CI
α_0	-6.642	0.055	(-6.749, -6.535)
α_1	-3.770	0.032	(-3.833 -3.708)
I_{max}	-1.090	0.145	(-1.375, -0.804)
LIC50	-1.064	0.739	(-2.513 0.384)
β_1	-0.175	0.094	(-0.359, 0.0093)

Final model :

$$\alpha_0 * = \alpha_0 + \beta_1 \times LT1B + \beta_3 \times DSB + \beta_5 \times R2Y_2 + \beta_6 \times R2Y_3 + \beta_7 \times R2Y_4$$

$$I_{max} * = I_{max} + \beta_2 \times LT1B + \beta_4 \times DSB$$

$$\lambda_{01} = \exp\left[\alpha_0 * + \left(\frac{I_{max} * \times Cp}{\exp(LIC50) + Cp}\right) + \beta_8 \times IINF\right]$$

Parameter	Estimate	Standard Error	95% Confidence Interval
α_0	-6.471	0.109	(-6.684 -6.258)
α_1	-3.770	0.032	(-3.832, -3.708)
Imax	-1.154	0.194	(-1.535, -0.773)
log(IC50)	-1.083	0.753	(-2.559, 0.394)
β_1	-0.593	0.089	(-0.766, -0.419)
β_2	0.464	0.162	(0.147 0.782)
β_3	-0.197	0.0907	(-0.375 -0.019)
β_4	-0.322	0.159	(-0.634 -0.011)
β_5	0.259	0.086	(0.090, 0.427)
β_6	0.410	0.101	(0.212, 0.608)
β_7	0.795	0.103	(0.594, 0.997)
β_8	-0.203	0.094	(-0.388, -0.018)

4. Parameter estimates for the reviewer's models

- ARR-FTY720-p concentration model (h=2.5)

Parameter	Estimate	SE	P-value
Beta0	-0.7260	(0.069)	<0.0001
Beta1	-0.1576	0.16	0.3322
Imax	-1.1690	0.23	<0.0001
Log(IC50)	-0.8769	0.49	0.0728
K	0.7884	0.11	<0.0001
IC50	0.4161	0.20	0.0408

- Lymphocyte-ARR model

Parameter	Estimate	SE	P-value
Beta0	-1.6568	0.023	<0.0001
IC50	0.8916	0.026	<0.0001
Imax	0.4747	0.016	<0.0001
h	17.4536	7.43	0.0188
K	0.7472	0.04741	<0.0001

4.5 APPENDIX V

DRUG SAFETY REVIEW

Fingolimod

Submission: NDA 22527

Indication: Relapsing Remitting Multiple Sclerosis

Executive summary

Several severe adverse events associated with fingolimod treatment show dose-dependence. Considering the pharmacological actions of fingolimod and its phosphate form as well as the metabolic pathways of fingolimod, a hypothesis is proposed. We hypothesize that CYP4F2 can affect the systemic exposure of fingolimod, its phosphate form, and metabolites, resulting in dose-dependent occurrence of treatment related adverse events. Such hypothesis should be conveyed to the sponsor for conducting relevant pharmacogenetic analyses.

Drug Safety, Office of Clinical Pharmacology

Clinical Pharmacology reviewer: PeiFan Bai, Ph.D.

Secondary reviewer

Darrell Abernethy, M.D., Ph.D.
Associate director for drug safety

Date: April 30, 2010

Activation of Fingolimod: FTY-720 is activated to its phosphate form by sphingosine kinase 2 (SphK2) and a kinetic equilibrium exists between FTY-720 and its phosphate form. In mice, FTY720 was efficiently phosphorylated by sphingosine kinase 2 (SphK2) in lymphoid tissues including Peyer's patches, spleen, and lymph nodes, but not in heart, liver, and kidney.(1) There are two types of sphingosine kinases, and only sphingosine kinase 2 efficiently phosphorylates FTY720.(2)

Receptors of Phosphorylated - FTY720: Phosphorylated FTY720, not FTY720, was an efficient agonist of human S1P receptors of $S1P_1$, $S1P_3$, $S1P_4$, and $S1P_5$. According to an e-mail from the pharm/tox reviewer, Dr. Richard Siarey), both fingolimod and its phosphate form binds to these S1P receptors. FTY720-phosphate was found to downregulate S1P1. FTY720-induced inactivation of lymphocyte S1P1 explained the egress blocking effects of FTY720.(3)

Physiological functions and distribution of S1P receptors to which FTY-720 and FTY-720-phosphate bind:

Tissue distributions of 4 Sphingosine 1-phosphate receptors with which fingolimod-phosphoate interacts:

Sphingosine 1-phosphate receptor 1: Endothelial cells, and to a lesser extent, in vascular smooth muscle cells, fibroblasts, melanocytes, and cells of epithelioid origin.

Sphingosine 1-phosphate receptor 3: Expressed in all tissues, but most abundantly in heart, placenta, kidney, and liver

Sphingosine 1-phosphate receptor 4; peripheral leukocytes and lung

Sphingosine 1-phosphate receptor 5: Widely expressed in the brain, most prominently in the corpus callosum, which is predominantly white matter. Detected in spleen, peripheral blood leukocytes, placenta, lung, aorta and fetal spleen.

Possible pharmacological actions of FTY-720 could be derived a publication by Spiegel et al.(4)

Receptor	G Protein Coupling	Signaling Pathways	Tissue Distribution	Physiological Actions
$S1P_1$/EDG1	$G_{i/o}$	↓ AC, ↑ ERK, ↑ PLC, ↑ PI3K/Akt, ↑ eNOS, ↑ Rac, ↑ Rho	Wide	Cell motility, lymphocyte trafficking, angiogenesis, vascular maturation and tone, neurogenesis
$S1P_2$/EDG5	$G_{i/o}$, G_q, $G_{12/13}$	↕ AC, ↑ PLC, ↑ JNK, ↑ p38, ↓ Rho, ↓ Rac	Wide, vascular smooth muscle cells	Inhibits motility and proliferation, neuronal excitation, hearing
$S1P_3$/EDG3	$G_{i/o}$, G_q, $G_{12/13}$	↓ AC, ↑ ERK, ↑ PLC, ↑ Rac, ↑ Rho	Heart, lung, spleen, kidney, intestine, diaphragm, cartilage	Bradycardia, pulmonary epithelial barrier, hearing
$S1P_4$/EDG6	$G_{i/o}$, G_q, $G_{12/13}$	↑ ERK, ↑ PLC	Immune cells, leukocytes	Cytokines?
$S1P_5$/EDG8	$G_{i/o}$, $G_{12/}$	↓ AC, ↓ ERK, ↑ JNK, ↑ p64JNK	Central nervous system, NK cells	Cell motility

Best Available Copy

The information in the table above could be useful for understanding the adverse events caused by FTY-720 treatment.

Are adverse events observed in clinical trials dose dependent?

The summary below is based on Dr. Lourdes Villalba's review

Pulmonary functions: Pulmonary function tests including FEV1 (Forced expiratory volume in one second), and Diffusion lung capacity for carbon monoxide (DLCO) showed a dose-dependent increase in the mean % changes from baseline for a 24 month period of monitoring. The dose-dependent percent changes in FVC (Forced vital capacity) seemed to diminish after 6 months of chronic treatment.

Eye disorders: Macular edema and retinal detachment showed a dose-dependent increase in occurrence rate.

Cardiovascular disorders: Based on a pooled analysis by medical officer (Lourdes Villalba, M.D), occurrence of cardiac ischemia in patients younger than 40 years showed dose-dependence. Hypertension also showed dose-dependent occurrence. Mean changes in Holter hourly heart rate showed a dose dependent decrease with the lowest heart rate occurring approximately at 6 hr post dose.

Liver: In terms of hepatic enzyme abnormalities, increase in alanine aminotransferase (ALT) (U/L) from normal baseline greater than 3x ULN showed a dose-dependent occurrence rate.

Clin pharm comments: In a publication, Fingolimod reportedly caused dose-dependent occurrence of nasopharyngitis, headache, dyspnea, diarrhea, nausea, increase in alanine aminotransferase, dizziness, gastroenteritis, and hypertension, and bradycardia.(5). A most recent report of a clinical trial also revealed a dose-dependent occurrence of FTY-720-induced serious adverse events.(6) These published results are consistent with what is submitted in the NDA.

What is the major suspect for causing dose-dependent manifestation of FTY-720 treatment related adverse events?

CYP4F2 is the major enzyme metabolizing FTY-720. *CYP4F2* is genetically polymorphic. Reportedly, the variant allele (*V433M* ; rs2108622) of CYP4F2 resulted in a lower CYP4F2 activity and was associated with warfarin dose.(8, 9) Frequencies of this variant allele reported are available on the web site of Internal HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). With Dr. Mike Pacanowski's assistance, we found a study in 112 Utah residents of northern and western European ancestry, and the results of that study showed that homozygous carriers of this mutation constituted 7% of the Caucasian subjects studied (see attached). The homozygous carriers of this variant allele in African American in south United States, Chinese, and Japanese consisted 0%, 3% and 10%, respectively. These frequencies of 3%, 7% and 10% can not be ignored.

What is the hypothesis generated based on our review?

CYP4F2 polymorphism can cause variability in the systemic exposures of FTY-720, it phosphate form, and metabolites, contributing to differential clinical manifestation of adverse drug reactions. It is hypothesized that *CYP4F2* polymorphism can play a role in patients' differential experiences of FTY-720-induced adverse events. This hypothesis is proposed and the importance of testing this hypothesis is elaborated below.

1. The organs involved in the dose-dependent occurrence of treatment-related adverse events seem to correspond to the distributions of S1P receptors, to which fingolimod and its active phosphate form bind.
2. Treatment-related adverse events seem to exhibit dose dependence. Therefore the factors that affect the systemic exposure of FTY-720, its phosphate form and metabolites are possible suspects.

3. Multiple sclerosis appears to be more prevalent in White women. Since there is a 7% of *V433M* homozygotes reported in a Utah Caucasian group, the prevalence of adverse events, if partially attributable to *CYP4F2* polymorphism, can not be ignored.
4. According to the population PK analysis, there is no clear trend whether race impacts the plasma concentration of FTY-720 and its active form. However, there are substantial percentages of *V433M* homozygotes in Asians well.

Appendices

Appendix I. CYP4F2 V433M genotype frequencies from Internal HapMap Project

Population	Genotype frequencies							
	genotype	freq	count	genotype	freq	count	genotype	freq
ASW (A)	C/C	0.811	43	C/T	0.189	10	T/T	0
CEU (C)	C/C	0.607	68	C/T	0.321	36	T/T	0.071
CHB (H)	C/C	0.573	45	C/T	0.396	32	T/T	0.035
CHD (D)	C/C	0.6	51	C/T	0.365	31	T/T	0.035
GIH (G)	C/C	0.33	29	C/T	0.477	42	T/T	0.193
JPT (J)	C/C	0.635	54	C/T	0.259	22	T/T	0.106
LWK (L)	C/C	0.8	72	C/T	0.189	17	T/T	0.011
MEX (M)	C/C	0.551	27	C/T	0.449	22	T/T	0
MKK (K)	C/C	0.615	88	C/T	0.357	51	T/T	0.028
TSI (T)	C/C	0.455	40	C/T	0.398	35	T/T	0.148
YRI (Y)	C/C	0.894	101	C/T	0.097	11	T/T	0.009

Note: the 'reference'-allele is the base observed in the reference genome sequence at this location

Population descriptors:
ASW (A): African ancestry in Southwest USA
CEU (C): Utah residents with Northern and Western European ancestry from the CEPH collection
CHB (H): Han Chinese in Beijing, China
CHD (D): Chinese in Metropolitan Denver, Colorado
GIH (G): Gujarati Indians in Houston, Texas
JPT (J): Japanese in Tokyo, Japan
LWK (L): Luhya in Webuye, Kenya
MEX (M): Mexican ancestry in Los Angeles, California
MKK (K): Maasai in Kinyawa, Kenya
TSI (T): Toscans in Italy
YRI (Y): Yoruba in Ibadan, Nigeria

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Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22527	ORIG-1	NOVARTIS PHARMACEUTICALS CORP	FINGOLIMOD HCL ORAL CAPSULES

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/s/

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07/21/2010

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08/02/2010

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA/BLA Number	N 22-527	**Brand Name**	Gilenia®
OCP Division (I, II, III, IV, V)	DCP-I	**Generic Name**	Fingolimod (FTY720)
Medical Division	HFD-120	**Drug Class**	sphingosine 1-phosphate (S1P) receptor modulator
OCP Reviewer	Ju-Ping Lai Jagan Parepally	**Indication(s)**	Relapsing Remitting Multiple Sclerosis (RRMS)
OCP Team Leader	Angela Men	**Dosage Form**	Capsule (0.5 mg)
Pharmacometrics Reviewer	Joo-Yeon Lee	**Dosing Regimen**	0.5 mg once daily
Date of Submission	12/21/2009	**Route of Administration**	Oral
Estimated Due Date of OCP Review	4/19/2010	**Sponsor**	Novartis
Medical Division Due Date	4/26/2010	**Priority Classification**	Priority
PDUFA Due Date	6/21/2010		

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Clin. Pharm. and Biopharm. Information

The sponsor submitted this original NDA 22527 (NME) on December 21st, 2009 seeking for approval of Gilenia® (Fingolimod, FTY720) for the treatment of Relapsing Remitting Multiple Sclerosis (RRMS). This NDA is under the priority review classification.

Fingolimod is a sphingosine-1-phosphate (S1P) receptor modulator. Fingolimod phosphorylated to the active moiety, *S*-enantiomer fingolimod-P. Fingolimod-P is a functional antagonist of sphingosine-1-phosphate (S1P) receptor which reduces the peripheral lymphocyte count mediated by down-modulation of the S1P1 receptor on lymphocytes. This results in a reduced egress of lymphocytes from the lymph nodes; in particular auto-aggressive T-cells that perform a central role in the MS inflammatory disease process are prevented from recirculating to the CNS. Fingolimod-P reversibly dephosphorylated back to the inactive form fingolimod. At steady state, fingolimod and fingolimod-P are in dynamic equilibrium.

The proposed product is hard capsule, with only one proposed strength of 0.5 mg. The recommended dosing regimen is 0.5 mg once-daily administered orally.

There are 56 human study reports, including 31 clinical pharmacology studies, which include 1 exposure-response report, 1 population PK report and 1thorough QT study, and for efficacy and safety, 4 controlled trials, 5 cross study reports and 16 other study reports. A clinical pharmacology study (D2109) will be submitted at 120 day safety update under the agreement with the Agency at the Pre-NDA meeting. The submission also contains 15 in vitro studies for information regarding protein binding (4 reports), hepatic metabolism and drug interactions (8 reports) and transporters (3 reports) and 7 bioanalytical validation and assay reports for fingolimod, its only active metabolite, fingolimod-P, and two inactive metabolites, M2 and M3. In addition, one model based report characterizing the effect of FTY720 on heart rate in healthy volunteers was provided as an analysis from more than one study.

All clinical studies were conducted with hard gelatin capsule formulations. Early clinical studies utilized formulations that included [redacted] (b) (4) (clinical service form, CSF). Subsequent clinical trials, including all phase II and phase III studies of the MS program, used the formulation intended for the market with yellow/white capsule shells (final market image, FMI). The CSF and the FMI formulations are bioequivalent. Absolute bioavailability of fingolimod was assessed for the FMI.

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This NDA consists of

- **Biopharmaceutics studies (5 studies):**
  1. BA: (3 studies)
     FTY720A0106: Food effect, SD 1 mg CSF, fasted vs high fat, n=14 (No effect on AUC and Cmax for FTY720, FTY720-P not measured)
     FTY720A0108: Absolute BA, 1 mg IV infusion 2h vs 1.25 PO FMI, n=11 (mean apparent absolute oral BA= 93%)
     FTY720A0107: Food effect, SD 1.25 mg FMI, fasted vs fed with high-fat, n=29 (No effect on AUC and Cmax for FTY720 and AUC for FTY720-P but Cmax↓34%, no clinical impact?)
  2. Comparative BA/BE: (2 study)
     FTY720A0116: Relative BA, SD 1.25 and 2.5 mg, CSF vs FMI, n=25 (BE)
     FTY720A2309: BE, SD 1.25x2 vs 2.5 mg FMI, n=34 (BE for FTY720, BE for FTY720-P Cmax but not AUC)
  3. Analytical methods: (7 Methods)

- **In vitro studies pertinent to PK using human biomaterials (15 studies):**
  1. Plasma protein binding: (4 studies) (Fingolimod and fingolimod-P are highly protein bound (>99.7%))
  2. Hepatic metabolism and drug interaction: (8 studies) (mainly via CYP4F2, not inducers or inhibitors of major cytochrome P450 isoenzymes)
  3. Studies using other human biomaterials: (3 studies)

- **Human pharmacokinetic studies (12 studies):**
  1. Healthy subject PK and tolerability: (2 studies)
     (dose-proportional SD 0.25-40 mg, MD QD 0.125-5 mg)
     FTY720A 2215: SAD high dose (5-40 mg), n=56
     FTY720A 2217: Mass balance, SD 4.47 mg, n=4 (Urinary and faecal excretion of radioactivity reached 81 % and 11 %)
  2. Patient PK and initial tolerability study reports: (3 studies)
     FTY720A B101: SAD (0.125 – 3.5 mg)in renal transplant patients with cyclosporine, n=20 (dose-proportional)
     FTY720A B102: MAD (0.125 – 5 mg)in renal transplant patients with cyclosporine, n=65
     FTY720A 0115: SD 0.07 mg/kg pediatric renal transplant patients with cyclosporine, n=7
  3. Intrinsic factors: (4 studies)
     FTY720A 0112: SD 5 mg, in stable cirrhotic liver disease patients with mild (n=8) or moderate (n=8) hepatic insufficiency (AUCb was increased by 12% and 44% in mild and moderate)
     FTY720A 2204: SD 1.25 mg in severe hepatic insufficient patient
     FTY720D 2108: SD 1.25 mg in severe renal insufficient patient
     FTY720A 2304: SD and MD 1.25 and 2.5 mg, Japanese vs Caucasien
  4. Extrinsic factors: (2 studies)
     FTY720A 0107: DDI, FTY720 1 mg SD vs steady state cyclosporine 200 mg BID
     FTY720A 2311: DDI, FTY720 5 mg SD vs ketoconazole 200 mg BID x 9 days.
  5. **Population PK (1 report)**

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- **Human pharmacodynamic studies (14 studies):**
  1. Healthy PD and PK/PD: (12 studies)
     FTY720A 0114: DDI, FTY720 SD vs atenolol or diltiazem.
     FTY720A 0118: DDI, FTY720 SD vs i.v. atropine.
     FTY720A 0119: DDI, FTY720 SD vs isoproterenol.
     FTY720A 2213: effects of FTY720 (1.25 and 5 mg) on heart rate and rhythm.
     FTY720A 2213E1: effects of a desensitizing dosing regimen of FTY720 on heart rate.
     FTY720A 2305: effect of various inter-treatment intervals on the heart rate.
     FTY720A 2306: MAD, safety and tolerability
     FTY720D 2101: QT
     FTY720D 2105: MD, pulmonary and cardiac pharmacodynamics of FTY720 (0.5 & 1.25 mg)
     FTY720D 2106: DDI, SD oral inhaled salmeterol on the negative chronotropic effect
     FTY720D 2110: small doses of FTY720 on the negative chronotropic effect of FTY720
     FTY720D 2113: effect of FTY720 on mean flow velocity in cerebral vessels, platelet function, and macular thickness
  2. Patient PD and PK/PD: (2 studies)
     FTY720D 2102: magnitude and time course of the effect of FTY720 on FEV1 and other pulmonary function tests
     FTY720D PKPD: Modeling of the exposure-response relationship

- **Efficacy and safety studies (25 studies):**
  1. Controlled trials: (4 studies)
  2. Cross study reports: (5 reports) Including one modeling report on heart rate in healthy subjects
  3. Other study reports: (16 reports)

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Clin. Pharm. and Biopharm. Information				
	"X" if included at filing	**Number of studies submitted**	**Number of studies reviewed**	**Critical Comments If any**
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x	7		
I. Clinical Pharmacology				
Mass balance:	x	1		
Isozyme characterization:	x	8		
Transporters:	x	3		
Blood/plasma ratio:	x	(1)		
Plasma protein binding:	x	4		
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	x	1		
multiple dose:				
Patients-				
single dose:	x	2		
multiple dose:	x	1		
Dose proportionality -				
fasting / non-fasting single dose:	x	2		
fasting / non-fasting multiple dose:	x	1		
Drug-drug interaction studies -				
In-vivo effects on primary drug:	x	2PK+(4PD)		
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:	x	1		
gender:				
pediatrics:				
geriatrics:				
renal impairment:	x	3		
hepatic impairment:	x	2		
Obese subject:				
PD -				
Phase 2:				
Phase 3:				
PK/PD -				
Phase 1 and/or 2, proof of concept:	x	14		12 in healthy/2 in patients
Phase 3 clinical trial:				
Population Analyses -		1		
Data rich:	x	(3)		
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability	x	1		
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:	x	1		
Bioequivalence studies -				
traditional design; single / multi dose:	x	1		
replicate design; single / multi dose:				
Food-drug interaction studies	x	2		

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Bio-waiver request based on BCS				
BCS class				
Dissolution study to evaluate alcohol induced dose-dumping				
III. Other CPB Studies				
Genotype/phenotype studies				
Chronopharmacokinetics				
Pediatric development plan				
Literature References	271			
Total Number of Studies				
	16 PK + 1 Pop PK+ 1 Exp.-Res.+ 12 PKPD+ 1 QTc+ 15 in vitro+ 7 Assay+ Literature			

Filability and QBR comments		
	"X" if yes	Comments
Application filable?	X	Reasons if the application <u>is not</u> filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?
Comments sent to firm?		
QBR questions (key issues to be considered)	• Is dose proportionality established in the therapeutic range? • Is there any food effect? • Is dose adjustment necessary for concomitant use of other drugs? • Is dose adjustment necessary for renal or hepatic dysfunction patients? • Are there exposure(dose)-response(efficacy and safety) relationships?	
Other comments or information not included above		
Primary reviewer Signature and Date	Ju-Ping Lai	
Secondary reviewer Signature and Date	Angela Men	

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

On **initial** review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	x			
2	Has the applicant provided metabolism and drug-drug interaction information?	x			
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?	x			
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
	Data				
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?	x			
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
	Studies and Analyses				
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?				Would be a review issue
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	x			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	x			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x			

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

General					
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x			
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?			x	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE?

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- Please provide all datasets and programs with outputs for population PK abnd exposure-response analyses as following direction below;
"All datasets used for model development and validation should be submitted as a SAS transport files (*.xpt). A description of each data item should be provided in a Define.pdf file. Any concentrations and/or subjects that have been excluded from the analysis should be flagged and maintained in the datasets.

Model codes or control streams and output listings should be provided for all major model building steps, e.g., base structural model, covariates models, final model, and validation model. These files should be submitted as ASCII text files with *.txt extension (e.g.: myfile ctl.txt, myfile out.txt)."

- If all datasets / programs already have been submitted with valid format, please provide an exact location.

Reviewing Clinical Pharmacologist Date

Team Leader/Supervisor Date

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22527	ORIG-1	NOVARTIS PHARMACEUTICALS CORP	FINGOLIMOD HCL ORAL CAPSULES

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Ju Ping LAI
01/21/2010

YUXIN MEN
01/22/2010