**Study Number**
**TMC125-C177**

**Title**

Phase I, open-label trial to investigate the pharmacokinetic interaction between tenofovir (TDF) and TMC125 at steady-state in healthy subjects.

**Objectives**

The primary objectives of the trial were to determine the effect of steady state concentrations of TMC125 on the steady state pharmacokinetics and urinary excretion of TDF and to determine the effect of steady state concentrations of TDF on the steady state pharmacokinetics of TMC125 in healthy subjects.

**Study Design**

Phase 1, open label, randomized, 2-way, 1-sequence crossover trial in 24 healthy subjects divided equally into two groups, **group 1** and **group 2**.

<u>Session 1:</u>

Subjects received TMC125 200 mg b.i.d. from **day 1** to **day 7** (with a single dose on day 8). This was followed by a washout period of at least 14 days.

<u>Session 2:</u>

TDF 300 mg q.d. was administered from **day 1** to **day 16**. TMC125 200 mg b.i.d. was co-administered from **day 9** to **day 16** (inclusive) in 12 subjects randomized to **group 1** and from **day 1** to **day 8** (inclusive) in 12 subjects randomized to **group 2**.

Full pharmacokinetic profiles during the 12-hour dosing interval for TMC125 were determined on **day 8** of **session 1** in both the groups, and on **day 16** of **session 2** in **group 1** and on **day 8** of **session 2** in **group 2**. The pharmacokinetics during the 24-hour dosing interval for tenofovir was determined on **day 8** and **day 16** of **session 2** in both the groups. The urine was collected for 24 hours after the morning dose on **day 8** and **day 16** in **session 2** to investigate if TMC125 had an effect on the urinary excretion of tenofovir.

**Investigational Product(s)**

TMC125 was provided as a tablet containing 100 mg of TMC125 — spray-dried in combination with hydroxypropylmethylcellulose (HPMC) and microcrystalline cellulose, croscarmellose sodium, magnesium stearate, and lactose monohydrate (formulation **F060**). The batch # was 05A05 and the expiration date was January 2006.

TDF (Viread®) was formulated as a tablet containing 300 mg TDF, equivalent to 245 mg tenofovir disoproxil (or 136 grams of the tenofovir base). The batch # was T340651D and the expiration date was October 2006.

## Assay Methods

The plasma concentrations of TMC125 were determined using validated LC-MS/MS techniques. The plasma and urine concentrations of tenofovir were determined using HPLC with fluorescence detection. The lower limit of quantification (LLOQ) in plasma was 2 ng/mL for TMC125 and 20 ng/mL for tenofovir. The LLOQ in urine was 1 $\mu$g/mL for tenofovir.

## Pharmacokinetic, Pharmacodynamic, and Statistical Data Analysis

### Pharmacokinetic Analysis

Pharmacokinetic and statistical analysis was performed using Winonlin Professional (version 4.1, Pharsight Corporation). A non-compartmental model with extravascular input was used for the pharmacokinetic analysis. Based on the individual plasma concentration-time data and using the scheduled sampling times, the standard pharmacokinetic parameters were calculated.

### Statistical Analysis

The statistical analyses were performed for TMC125 in plasma using treatment with TDF as test treatment and treatment without TDF as reference treatment. The statistical analyses were performed for TDF in plasma using treatment with TMC125 as test treatment and treatment without TMC125 as reference treatment. The primary plasma pharmacokinetic parameters were $C_{0h}$, $C_{min}$, $C_{max}$ and $AUC_{12h}$ for TMC125, and $C_{0h}$, $C_{min}$, $C_{max}$ and $AUC_{24h}$ for tenofovir on the logarithmic scale. The primary urine pharmacokinetic parameter was $D_{urine,24hours}$ (% dose excreted in the urine in 24 hours), for tenofovir.

## RESULTS

## Subject Disposition and Demographics

Out of the 32 subjects screened, 24 subjects were randomized to 2 treatment groups and started treatment. Out of the 12 subjects randomized to **group 1**, 2 subjects dropped out in session 1 (due to adverse event and withdrawal of consent) and no subjects dropped out in **session 2**, therefore 10 subjects randomized to **group 1** completed all assessments. Out of the 12 subjects randomized to **group 2**, 3 subjects dropped out in session 1 (all due to adverse events) and no subjects dropped out in **session 2**, therefore 9 subjects randomized to **group 2** completed all assessments.

Table 1 shows the demographics in the trial.

## Table 1: Demographics in Trial TMC125-C177

| Parameter | Group 1 N = 12 | Group 2 N = 12 | All Subjects N = 24 |
|---|---|---|---|
| Age, years Median (range) | 27.5 (19 - 54) | 23.0 (20 - 40) | 25.5 (19 - 54) |
| Height, cm Median (range) | 181.5 (177 - 187) | 180.5 (174 - 188) | 181.5 (174 - 188) |
| Weight, kg Median (range) | 77.0 (58 - 100) | 79.5 (61 - 102) | 77.0 (58 - 102) |
| BMI, kg/m² Median (range) | 23.2 (18 - 30) | 24.2 (19 - 29) | 23.5 (18 - 30) |
| Sex, n (%) Male | 12 (100%) | 12 (100%) | 24 (100%) |
| Ethnic Origin, n (%) White Hispanic | 12 (100%) | 11 (91.7%) 1 (8.3%) | 23 (95.8%) |
| Type of Smoker, n (%) Light/Nonsmoker | 6 (50%) / 6 (50%) | 3 (25%) / 9 (75%) | 9 (37.5%) / 15 (62.5%) |

## Pharmacokinetics

All blood samples taken to determine TMC125 and tenofovir plasma concentrations were available for analysis. There were 3 deviations noted (deviation > 10 %) from the scheduled sampling time; in case of these deviations, the actual sampling time was used for the pharmacokinetic analysis. The use of actual sampling time (instead of scheduled sampling time) is not expected to alter the conclusions of the trial.

One subject did not take the TMC125 evening intake on **day 12** of **session 2**. Therefore, the pre-dose plasma concentration of tenofovir and TMC125 on **day 13** was excluded from the descriptive statistics.

Five subjects discontinued the trial prematurely; 4 subjects discontinued the trial because of AEs after **session 1**. Therefore, the treatment in **session 2** could not be administered to these subjects. One subject withdrew consent after the pharmacokinetic sample on **day 5** of **session 1**. Therefore, a plasma concentration-time profile of TMC125 could not be determined on **day 8** of **session 1** and the treatment in **session 2** could not be administered to this subject. Thus, full pharmacokinetic profiles of TMC125 were available for 23 subjects for **day 8** of **session 1** and for 19 subjects from **day 8** and **day 16** of **session 2**.

## TMC125

Fig 1 shows the mean plasma concentrations time curves of TMC125 200 mg b.i.d. (**F060**), with and without co-administration of TDF (300 mg q.d.)

292

**Fig 1: Mean plasma concentrations time curves of TMC125 200 mg b.i.d. (F060), with and without co-administration of TDF (300 mg q.d.)**
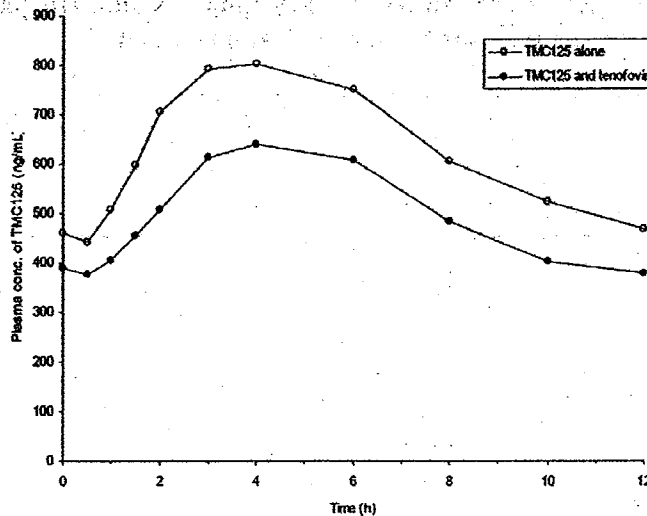


The mean plasma concentrations of TMC125 after co-administration with TDF were lower as compared to the mean plasma concentrations of TMC125 when administered alone.

Table 2 shows the pharmacokinetic parameters of TMC125, with and without co-administration of TDF.

**Table 2: Pharmacokinetic parameters of TMC125, with and without co-administration of TDF**

| Pharmacokinetics of TMC125 (mean ± SD, $t_{max}$ median [range]) | TMC125 alone (reference) | TMC125 and TDF (test) |
|---|---|---|
| n | 23 | 19 |
| $C_{0h}$, ng/mL | 461.3 ± 170.5 | 388.8 ± 126.3 |
| $C_{min}$, ng/mL | 426.1 ± 154.6 | 338.4 ± 113.5 |
| $t_{max}$, h | 4.0 (2.0 - 6.0) | 4.0 (2.0 - 6.0) |
| $C_{max}$, ng/mL | 875.7 ± 232.8 | 695.2 ± 144.3 |
| $AUC_{12h}$, ng.h/mL | 7638 ± 2254 | 6040 ± 1557 |
| $C_{ss,av}$, ng/mL | 636.5 ± 187.9 | 503.3 ± 129.7 |
| FI, % | 72.55 ± 20.30 | 74.02 ± 23.51 |

The mean estimates of all the pharmacokinetic parameters (except median $t_{max}$) were lower when TMC125 was co-administered with TDF, as compared to when TMC125 was administered alone. The inter-individual variability in $C_{0h}$, $C_{min}$, $C_{max}$, and $AUC_{12h}$ estimates of TMC125 (combined for both groups) was 37 % and 32 %, 36 % and 34 %, 27 % and 21 %, and 30 % and 26 %, respectively, when TMC125 was administered alone and when co-administered with TDF.

Table 3 shows the summary of the statistical analysis of the pharmacokinetic parameters of TMC125 with and without co-administration of TDF.

**Table 3: Summary of the statistical analysis of the pharmacokinetic parameters of TMC125 with and without co-administration of TDF**

| Parameter | LSmeans [a] | | | 90% CI,% [b] | p-value |
| | TMC125 alone (reference) | TMC125 and TDF (test) | LSmeans ratio, % | | Treatment |
|---|---|---|---|---|---|
| $C_{0h}$, ng/mL | 437.0 | 383.2 | 87.70 | 77.51 - 99.23 | 0.0818 |
| $C_{min}$, ng/mL | 405.2 | 330.6 | 81.59 | 72.76 - 91.49 | 0.0064[c] |
| $C_{max}$, ng/mL | 849.6 | 690.9 | 81.32 | 75.39 - 87.72 | 0.0002[c] |
| $AUC_{12h}$, ng.h/mL | 7384 | 6004 | 81.30 | 74.81 - 88.35 | 0.0004[c] |

[a] n= 23 for TMC125 alone (reference) and n=19 for TMC125 and TDF (test)

[b] 90% confidence intervals.

[c] Statistically significant difference

The $LS_{means}$ ratio of $C_{0hr}$, $C_{min}$, $C_{max}$ and $AUC_{12hr}$ of TMC125 were decreased by 12 %, 18 %, 17 %, and 17 % respectively, when TMC125 was co-administered with TDF as compared to when TMC125 was administered alone.

## Tenofovir

### Plasma

Fig 2 shows the mean plasma concentration time profiles of tenofovir (300 mg q.d.) alone or when co-administered with TMC125 200 mg b.i.d. (**F060**).

**Fig 2: Mean plasma concentration time profiles of tenofovir (300 mg q.d.) alone or when co-administered with TMC125 200 mg b.i.d. (F060)**
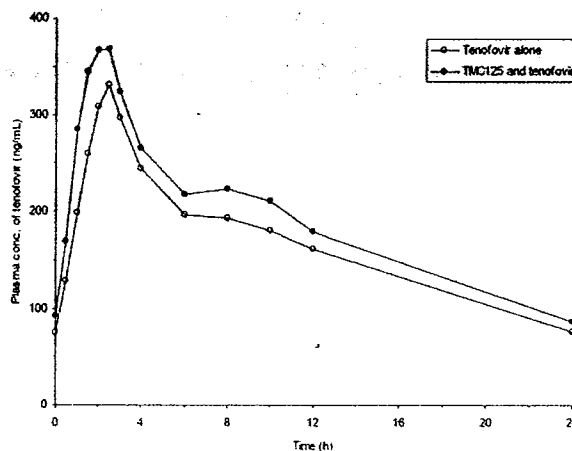
The mean plasma concentrations of tenofovir, when tenofovir was co-administered with TMC125 (session 2: day 16 for group 1 and day 8 for group 2) were higher, as compared to when tenofovir was administered alone (session 2: day 8 for group 1 and day 16 for group 2).

Table 4 shows the pharmacokinetic parameters of tenofovir, with and without co-administration of TMC125.

**Table 4: Pharmacokinetic parameters of tenofovir, with and without co-administration of TMC125**

| Pharmacokinetics of tenofovir (mean ± SD, $t_{max}$ median [range]) | TDF alone (reference) | TDF and TMC125 (test) |
|---|---|---|
| n | 19 | 19[a] |
| $C_{0h}$, ng/mL | 74.52 ± 19.38 | 92.69 ± 22.09 |
| $C_{min}$, ng/mL | 69.61 ± 16.37 | 82.15 ± 16.71 |
| $t_{max}$, h | 2.0 (0.5 – 4.0) | 1.5 (0.5 – 3.0) |
| $C_{max}$, ng/mL | 388.7 ± 97.28 | 443.1 ± 98.54 |
| $AUC_{24h}$, ng.h/mL | 3946 ± 778.2 | 4511 ± 827.6 |
| $C_{ss,av}$, ng/mL | 164.4 ± 32.43 | 187.9 ± 34.48 |
| FI, % | 194.8 ± 35.72 | 192.6 ± 39.74 |
| $D_{urine,0-24h}$, % | 46.36 ± 6.123 | 53.86 ± 10.96 |

[a] n = 10 for $D_{urine,0-24h}$

The mean estimates of all the pharmacokinetic parameters of tenofovir were higher when TDF was co-administered with TMC125 as compared to when TDF was administered alone. The individual ratios TDF + TMC125/TDF ratios for $C_{0hr}$, $C_{min}$, $C_{max}$, and $AUC_{24hr}$ ranged (combined for both groups) from 80 % to 186 %, 94 % to 166 %, 50 % to 166 %, and 86 % to 147 %, respectively, when TDF was combined with TMC125, as compared to when TDF was administered alone. The inter individual variability in $C_{0hr}$, $C_{min}$, $C_{max}$, and $AUC_{24hr}$ estimates of TDF (combined from both groups) was 26 % and 24 %, 24 % and 20 %, 25 % and 22 %, 20 % and 18 %, respectively, for TDF alone and TDF co-administered with TMC125.

Table 5 shows the summary of the statistical analysis of the pharmacokinetic parameters of tenofovir with and without co-administration of TMC125.

## Table 5: Summary of the statistical analysis of the pharmacokinetic parameters of tenofovir with and without co-administration of TMC125

| Parameter | LSmeans [a] | | LSmeans ratio, % | 90% CI,% [b] | p-value | | |
|---|---|---|---|---|---|---|---|
| | TDF alone (reference) | TDF + TMC125 (test) | | | Treatment | Period | Sequence |
| $C_{0h}$, ng/mL | 72.14 | 90.43 | 125.4 | 116.3 - 135.1 | 0.0001[c] | 0.2474 | 0.8186 |
| $C_{min}$, ng/mL | 67.65 | 80.64 | 119.2 | 112.9 - 125.9 | <0.0001[c] | 0.0517 | 0.8890 |
| $C_{max}$, ng/mL | 377.3 | 432.7 | 114.7 | 103.7 - 126.8 | 0.0304[c] | 0.2486 | 0.6738 |
| $AUC_{24h}$, ng.h/mL | 3858 | 4432 | 114.9 | 109.3 - 120.6 | 0.0001[c] | 0.1378 | 0.3111 |
| $D_{urine, 0-24h}$, % | 47.94 | 52.80 | 110.1 | 97.49 - 124.4 | 0.1808 | - | - |

[a] For $C_{0h}$, $C_{min}$, $C_{max}$ and $AUC_{24h}$ n= 19 for TDF alone (reference) and for TMC125 and TDF (test), for $D_{urine, 0-24h}$, n=10 for TDF alone (reference) and for TMC125 and TDF (test)

[b] 90% confidence intervals.

[c] Statistically significant difference

The $LS_{mean}$ ratios of $C_{0hr}$, $C_{min}$, $C_{max}$, and $AUC_{24hr}$ estimates of TDF were increased by 25 %, 19 %, 15 %, and 15 %, respectively, when TDF was co-administered with TMC125 as compared to when TDF was administered alone.

## URINE

For subjects in group 2, the urine samples collected between 12 and 24 hours post-dose on day 8 of session 2 were not sent to the bioanalytical laboratory. Therefore, the concentration of tenofovir could not be measured in these samples. For these subjects $A_{etotal}$ and $D_{urine,total}$ could not be determined. The urinary pharmacokinetic parameters $A_{e,0-24h}$ (amount excreted in the urine in 24 hours) and $D_{urine,0-24h}$ (% of dose excreted in 24 hours) of tenofovir was calculated for 10 subjects after co-administration of TMC125 **(group 1, day 16 of session 2)** and tenofovir and for 19 subjects when tenofovir was administered alone **(group 1, day 8 of session 2 and group 2, day 16 of session 2)**. A statistical analysis was performed for the 10 subjects for which $A_{e,0-24h}$ and $D_{urine,0-24h}$ were available for both treatments, i.e., the subjects of **group 1**.

The results of the statistical analysis showed that the $LS_{mean}$ ratios of TDF $D_{urine,0-24}$ % was 10 % higher when TDF was co-administered with TMC125, as compared to when TDF was administered alone.

## Pharmacokinetic Results Summary

- The $LS_{means}$ ratio of $C_{0hr}$, $C_{min}$, $C_{max}$ and $AUC_{12hr}$ of TMC125 were decreased by 12 %, 18 %, 17 %, and 17 % respecfively, when TMC125 was co-administered with TDF as compared to when TMC125 was administered alone. The decrease in TMC125 exposures is not expected to be clinically relevant since the

magnitude of decrease in TMC125 exposures in the presence of TDF in lower than the magnitude of decrease in TMC125 exposures when TMC125 was co-administered with darunavir/ritonavir (for which efficacy data is available).

- The $LS_{mean}$ ratios of $C_{0hr}$, $C_{min}$, $C_{max}$, and $AUC_{24hr}$ estimates of TDF were increased by 25 %, 19 %, 15 %, and 15 %, respectively, when TDF was co-administered with TMC125 as compared to when TDF was administered alone. The increase in TDF exposures is not expected to be clinically relevant since a greater magnitude of increase in the concentrations of TDF has been observed when TDF and darunavir/ritonavir is co-administered and for which no dose adjustment is recommended for tenofovir.

### Conclusion

*TMC125 and tenofovir can be co-administered without any dose adjustments.*

The Clinical Pharmacology Review of the drug-drug interaction study (Study 026) between etravirine and raltegravir (MK-0518) was conducted by ———

****************

Protocol 026

TITLE: An Open-Label, 3-Period, Fixed-Sequence Study to Evaluate the 2-Way Interaction of MK-0518 and TMC125 in Healthy Adult Subjects

OBJECTIVES: To evaluate the effect of coadministration of TMC125 and MK-0518 on the plasma pharmacokinetic profiles of MK-0518 (e.g. $AUC_{0-12hr}$, $C_{12\,hr}$, $C_{max}$) and to evaluate the safety and tolerability of multiple doses of TMC125 alone, MK-0518 alone, and TMC125 coadministered with multiple doses of MK-0518, and to assess the effect of MK-0518 on pharmacokinetics of TMC-125

SUBJECTS AND STUDY DESIGN: This was an open label, 3-period, fixed-sequence study in healthy adult subjects to assess the effects of co-administration of MK-0518 and TMC125. Twenty subjects each received MK-0518 and TMC125 in an open label fashion. In Period 1, all subjects were administered oral doses of 400 mg MK-0518 every 12 hours for 4 days. However, in Period 1, the Day 4 MK-0518 PM dose was not given. Period 1 was followed by a wash-out of at least 4 days. In Period 2, the same 20 subjects were administered 200-mg TMC125 q12 hours for 8 days. There was no wash-out between Periods 2 and 3. In Period 3, all 20 subjects received a combination of TMC125 (200 mg q12 hours) and MK-0518 (400 mg q12 hours) for 4 days. In Period 3, the Day 4 PM doses were not administered. All dosing was in an open label fashion.

All doses of TMC125 and MK-0518 were administered with food including days when pharmacokinetic samples were collected.

Subject Baseline Demographics

APPEARS THIS WAY
ON ORIGINAL

| AN | Gender | Race | Age (Years) | Height (cm) | Weight (kg) |
|---|---|---|---|---|---|
| 0441 | Male | White | 41 | 175.0 | 78.2 |
| 0442 | Male | White | 37 | 181.0 | 79.1 |
| 0443 | Male | White | 20 | 179.1 | 88.2 |
| 0444 | Male | Black | 33 | 182.0 | 100.0 |
| 0445 | Male | White | 22 | 173.0 | 58.2 |
| 0446 | Male | White | 38 | 183.7 | 99.6 |
| 0447 | Male | White | 22 | 175.2 | 67.7 |
| 0448 | Male | White | 34 | 177.4 | 87.7 |
| 0449 | Male | White | 37 | 176.7 | 88.6 |
| 0450 | Male | White | 24 | 187.0 | 103.2 |
| 0451 | Male | Black | 32 | 179.0 | 72.7 |
| 0452 | Male | White | 24 | 177.1 | 76.8 |
| 0453 | Male | White | 19 | 177.0 | 71.8 |
| 0454 | Female | White | 31 | 165.5 | 70.9 |
| 0455 | Female | White | 45 | 167.0 | 78.6 |
| 0456 | Female | Black | 29 | 164.0 | 83.6 |
| 0457 | Female | White | 29 | 176.5 | 63.6 |
| 0458 | Female | White | 30 | 166.5 | 60.9 |
| 0459 | Female | White | 23 | 174.5 | 80.0 |
| 0460 | Female | Black | 26 | 145.0 | 59.6 |

**INVESTIGATORS AND STUDY LOCATIONS**

**FORMULATION**: MK-0518 poloxamer formulation tablets (FMI) 400 mg, TMC125 100 mg tablets

**SAMPLE COLLECTION**: Serial blood samples were obtained for plasma concentrations of MK-0518 at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours postdose. Serial blood samples were obtained for plasma concentrations of TMC-125 at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours postdose.

**ASSAYS**: Validated HPLC-MS/MS assays were used for plasma MK-0518 concentrations. The lower limit of quantitation (LLOQ) for the plasma assay was 2 ng/mL (4.5 nM) and the linear calibration range was 2 to 1000 ng/mL. The accuracy and precision were < 6% and $\leq 9\%$ respectively.

The bioanalysis of TMC125 was performed by Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium. Plasma concentrations of TMC125 were determined using a validated LC-MS/MS method. The lower limit of quantification was 2 ng/mL in human heparin plasma.

**PHARMACOKINETIC DATA ANALYSIS**: Plasma concentrations of MK-0518 were used to calculate pharmacokinetic parameters including $AUC_{0-12hr}$, $C_{max}$, $C_{12 hr}$, $T_{max}$, and apparent $t_{1/2}$ for each subject in the presence or absence of multiple doses of TMC125. Geometric mean ratios (MK-0518 + TMC125/MK-0518) and associated 90% confidence intervals (CIs) of primary plasma MK-0518 PK parameters ($C_{12h}$, $C_{max}$, and $AUC_{0-12hr}$) were calculated for treatment comparisons.

The plasma pharmacokinetic profile (e.g., $C_{12 hr}$, $AUC_{0-12 hr}$, $C_{max}$, $T_{max}$) of TMC125 in the presence and absence of MK-0518 was calculated for each subject. Geometric mean ratios (TMC125 + MK0518/ TMC125) and associated 90% confidence intervals (CIs) of primary plasma TMC125 PK parameters ($C_{12h}$, $C_{max}$, and $AUC_{0-12hr}$) were calculated for treatment comparisons.

## PHARMACOKINETIC RESULTS:

### MK-0518 Pharmacokinetics:

Figure 1. Arithmetic Mean MK-0518 Plasma Concentration Profiles Following Multiple Doses of 400-mg MK-0518 Twice-Daily With or Without Coadministration of Multiple Doses of 200 mg TMC125 Twice-Daily to Healthy Adult Subjects (Inset = Semilog Scale)



APPEARS THIS WAY
ON ORIGINAL

300
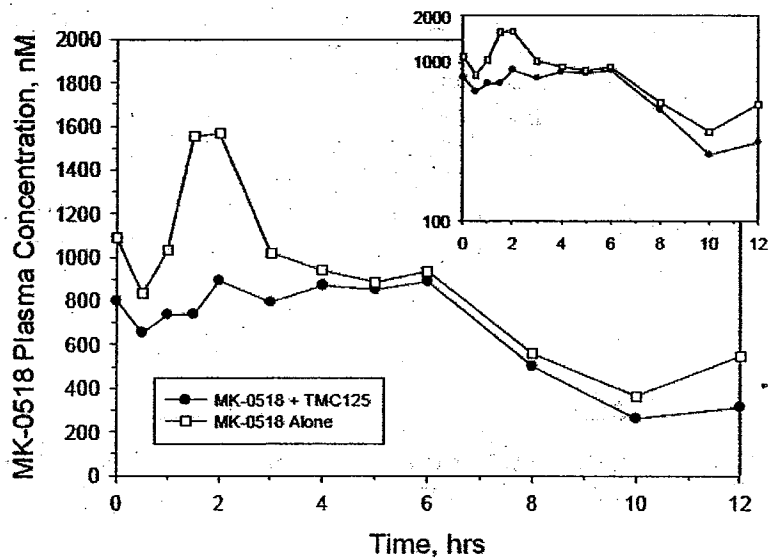
Table 1. Comparison of MK-0518 Plasma Pharmacokinetics Following Administration of Multiple Doses of 400 mg MK-0518 Twice-Daily With or Without Coadministration of Multiple Doses of 200 mg TMC125 Daily to Healthy Adult Subjects

| Pharmacokinetic Parameter | MK-0518 + TMC125 | | | MK-0518 | | | (MK-0518 + TMC125 / MK-0518) | | | MSE [†] |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Geometric Mean | 95% Confidence Interval for Geometric Mean | N | Geometric Mean | 95% Confidence Interval for Geometric Mean | N | Geometric Mean Ratio | 90% Confidence Interval for Geometric Mean Ratio | |
| $C_{12hr}$ (nM) [‡] | 19 | 141.6 | (77.2, 259.6) | 19 | 216.0 | (117.8, 396.0) | 19 | 0.66 | (0.34, 1.26) | 1.347 |
| $AUC_{0-12hr}$ (µM·hr) [‡] | 19 | 6.28 | (4.49, 8.78) | 19 | 7.01 | (5.03, 9.81) | 19 | 0.90 | (0.68, 1.18) | 0.240 |
| $C_{max}$ (µM) [‡] | 19 | 1.54 | (1.09, 2.18) | 19 | 1.74 | (1.23, 2.46) | 19 | 0.89 | (0.68, 1.15) | 0.213 |
| $T_{max}$ (hr) | 19 | 3.0 [§] | | 19 | 1.5 [§] | | 19 | 1.8 [‖] | (0.3, 3.0) [‖] | |

[†] Mean square error on log-scale.
[‡] Geometric mean computed from least squares estimate from an ANOVA performed on the natural-log transformed values.
[§] Median reported for $T_{max}$.
[‖] Hodges-Lehman estimate of median treatment difference with corresponding 90% CI for true median treatment difference.

Table 2. Individual MK-0518 Plasma Pharmacokinetics and Summary Statistics Following Administration of Multiple Doses of 400 mg MK-0518 Twice-Daily With or Without Coadministration of Multiple Doses of 200 mg TMC125 Twice-Daily to Healthy Adult Subjects
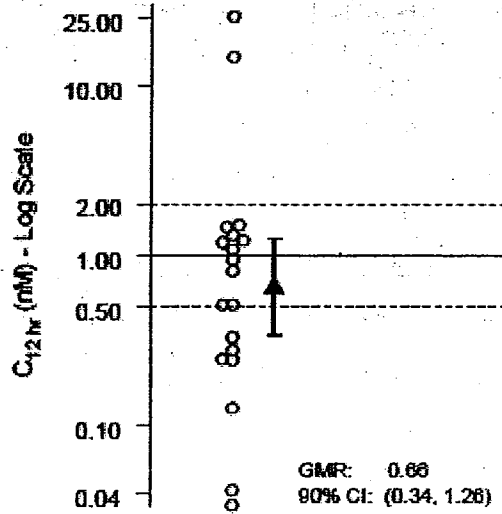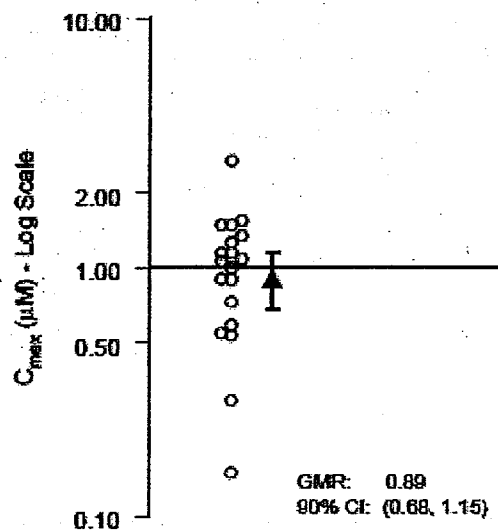
| AN | $C_{12hr}$ nM A | C | C/A | $AUC_{0-12hr}$ µM·hr A | C | C/A | $C_{max}$ µM A | C | C/A | $T_{max}$ hr A | C | C-A |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0441 | | | 14.76 | 4.06 | 9.51 | 2.34 | | | 0.99 | | | 0.0 |
| 0442 | | | 0.13 | 9.31 | 11.41 | 1.23 | | | 1.14 | | | 4.0 |
| 0443 | | | 0.51 | 1.69 | 2.33 | 1.38 | | | 1.08 | | | 4.0 |
| 0444 | | | 1.50 | 3.46 | 6.88 | 1.99 | | | 2.69 | | | -0.5 |
| 0445 | | | 1.31 | 7.98 | 4.71 | 0.59 | | | 0.89 | | | -4.0 |
| 0446 | | | 0.95 | 6.56 | 6.94 | 1.06 | | | 1.15 | | | -0.5 |
| 0447 | | | 1.08 | 5.40 | 4.63 | 0.86 | | | 0.90 | | | 3.0 |
| 0448 | | | 0.81 | 4.17 | 5.18 | 1.24 | | | 1.48 | | | 1.0 |
| 0449 | | | 1.19 | 2.75 | 3.04 | 1.11 | | | 1.06 | | | 5.0 |
| 0450 | | | 1.47 | 2.56 | 5.47 | 2.14 | | | 1.34 | | | 3.0 |
| 0451 | | | 0.04 | 5.70 | 2.59 | 0.4? | | | 0.73 | | | 0.0 |
| 0452 | | | 0.24 | 15.35 | 6.63 | 0.4? | | | 0.54 | | | 0.0 |
| 0453 | | | 0.51 | 3.00 | 3.82 | 1.27 | | | 1.55 | | | 2.5 |
| 0454 | | | 0.03 | 16.31 | 10.34 | 0.63 | | | 0.53 | | | -12.0 |
| 0455 | | | 0.33 | 30.87 | 5.92 | 0.19 | | | 0.15 | | | 0.5 |
| 0456 | | | 25.42 | 12.26 | 8.09 | 0.66 | | | 0.59 | | | 7.0 |
| 0457 | | | 0.28 | 18.77 | 25.15 | 1.3 | | | 1.48 | | | 4.0 |
| 0458 | | | 0.25 | 23.56 | 6.01 | 0.2 | | | 0.29 | | | 2.5 |
| 0460 | | | 1.23 | 9.01 | 13.00 | 1.4+ | | | 1.26 | | | 3.0 |
| AM | 547.5 | 315.3 | — | 9.62 | 7.46 | — | 2.43 | 1.92 | — | 2.0 | 3.2 | — |
| SD | 957.4 | 554.0 | — | 8.04 | 5.19 | — | 2.26 | 1.62 | — | 2.9 | 2.9 | — |
| Med | 133.4 | 130.3 | — | 6.56 | 6.01 | — | 1.40 | 1.31 | — | 1.5 | 3.0 | 1.8 [1] |
| GM [2] | 216.0 | 141.6 | 0.66 | 7.01 | 6.28 | 0.90 | 1.74 | 1.54 | 0.89 | — | — | — |

Treatment A: 400-mg MK-0518 q12h x 3.5 days

Treatment C: 400-mg MK-0518 + 200-mg TMC125 q12h x 3.5 days

AN = Allocation Number; AM = Arithmetic Mean; SD = Standard Deviation; Med = Median; GM = Geometric Mean

[1] For $T_{max}$, represents Hodges-Lehman estimate of median treatment difference

[2] Geometric mean computed from least squares estimate from an ANOVA performed on the natural-log transformed values

Figure 2. Individual MK-0518 $C_{12 hr}$ Ratios (MK-0518 Coadministered With TMC125/MK-0518 Administered Alone) With Geometric Mean Ratio and 90% Confidence Interval Following Multiple Doses of 400 mg MK-0518 Twice-Daily With or Without Coadministration of Multiple Doses of 200 mg TMC125 Twice-Daily to Healthy Adult Subjects



Figure 3. Individual MK-0518 $AUC_{0-12 hr}$ Ratios (MK-0518 Coadministered With TMC125/MK-0518 Administered Alone) With Geometric Mean Ratio and 90% Confidence Interval Following Multiple Doses of 400 mg MK-0518 Twice-Daily With or Without Coadministration of Multiple Doses of 200 mg TMC125 Twice-Daily to Healthy Adult Subjects
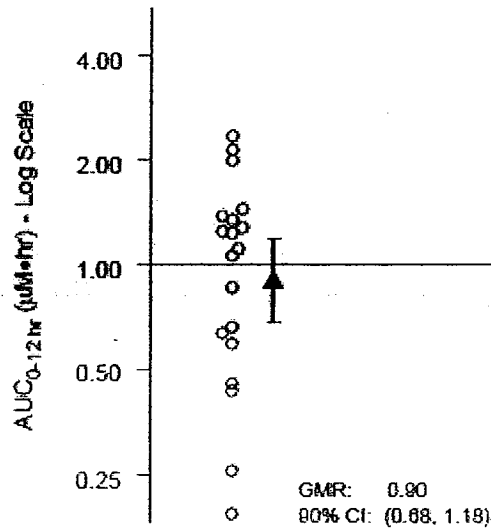
Figure 4. Individual MK-0518 $C_{max}$ Ratios (MK-0518 Coadministered With TMC125/MK-0518
Administered Alone) With Geometric Mean Ratio and 90% Confidence Interval Following Multiple Doses
of 400 mg MK-0518 Twice-Daily With or Without Coadministration of Multiple Doses of 200 mg TMC125
Twice-Daily to Healthy Adult Subjectscts

Figure 5. Arithmetic Mean TMC125 Plasma Concentration Profiles Following Multiple Doses of 200-mg TMC125 Twice-Daily With or Without Coadministration of Multiple Doses of 200 mg MK-0518 Twice-Daily to Healthy Adult Subjects (Inset = Semilog Scale)
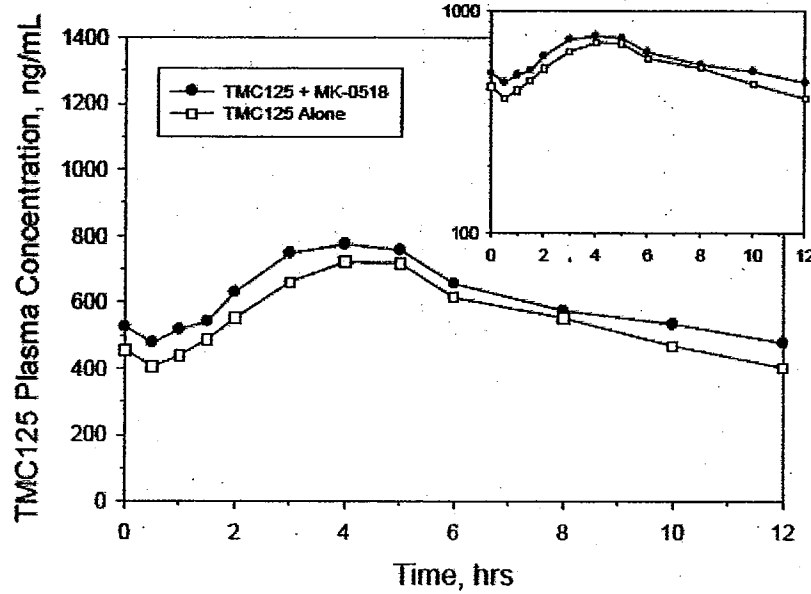


Table 3. Comparison of TMC125 Plasma Pharmacokinetics Following Administration of Multiple Doses of 200 mg TMC125 Twice-Daily With or Without Coadministration of Multiple Doses of 400 mg MK-0518 Twice Daily to Healthy Adult Subjects

| Pharmacokinetic Parameter | MK-0518 + TMC125 | | | TMC125 | | | (MK-0518 + TMC125/ TMC125) | | | MSE [†] |
|---|---|---|---|---|---|---|---|---|---|---|
| | N | Geometric Mean | 95% Confidence Interval for Geometric Mean | N | Geometric Mean | 95% Confidence Interval for Geometric Mean | N | Geometric Mean Ratio | 90% Confidence Interval for Geometric Mean Ratio | |
| $C_{12 hr}$ (ng/mL) [‡] | 19 | 439 | (359, 536) | 19 | 373 | (306, 457) | 19 | 1.17 | (1.10, 1.26) | 0.015 |
| $AUC_{0-12hr}$ (ng·hr/mL) [‡] | 19 | 6813 | (5633, 8240) | 19 | 6216 | (5139, 7518) | 19 | 1.10 | (1.03, 1.16) | 0.011 |
| $C_{max}$ (ng/mL) [‡] | 19 | 766 | (633, 926) | 19 | 734 | (607, 888) | 19 | 1.04 | (0.97, 1.13) | 0.017 |
| $T_{max}$ (hr) | 19 | 4.0 [§] | | 19 | 4.0 [§] | | 19 | -0.5 [‖] | (-1.0, 0.0) [‖] | |

[†] Mean square error on log-scale.

[‡] Geometric mean computed from least squares estimate from an ANOVA performed on the natural-log transformed values.

[§] Median reported for $T_{max}$.

[‖] Hodges-Lehman estimate of median treatment difference with corresponding 90% CI for true median treatment difference.

304

## Table 4. Individual TMC125 Plasma Pharmacokinetics and Summary Statistics Following Administration of Multiple Doses of 200 mg TMC125 Twice-Daily With or Without Coadministration of Multiple Doses of 400 mg MK-0518 Twice-Daily to Healthy Adult Subjects

| | $C_{t\text{rough}}$, ng/mL | | | $AUC_{0-12h}$, ng·hr/mL | | | $C_{max}$, ng/mL | | | $T_{max}$, hr | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AN | B | C | C/B | B | C | C/B | B | C | C/B | B | C | C-B |
| 0441 | | | 1.24 | 6710 | 7394 | 1.10 | | | 0.96 | | | 1.0 |
| 0442 | | | 0.91 | 7901 | 7378 | 0.93 | | | 1.06 | | | -1.0 |
| 0443 | | | 1.14 | 4193 | 4173 | 1.00 | | | 0.99 | | | 0.0 |
| 0444 | | | 1.05 | 5420 | 6173 | 1.14 | | | 1.10 | | | 0.0 |
| 0445 | | | 0.80 | 7054 | 5514 | 0.78 | | | 0.76 | | | 1.0 |
| 0446 | | | 1.27 | 5233 | 6024 | 1.15 | | | 1.00 | | | 0.0 |
| 0447 | | | 1.20 | 6907 | 8228 | 1.19 | | | 1.10 | | | -1.0 |
| 0448 | | | 1.18 | 9769 | 11450 | 1.17 | | | 1.36 | | | -1.0 |
| 0449 | | | 1.49 | 8786 | 10770 | 1.23 | | | 1.06 | | | 0.0 |
| 0450 | | | 1.23 | 3584 | 4863 | 1.36 | | | 1.40 | | | -2.0 |
| 0451 | | | 1.27 | 12870 | 14400 | 1.12 | | | 1.13 | | | 0.0 |
| 0452 | | | 1.04 | 6510 | 7102 | 1.09 | | | 1.08 | | | 0.0 |
| 0453 | | | 0.99 | 8013 | 7546 | 0.94 | | | 0.85 | | | -1.0 |
| 0454 | | | 1.60 | 6291 | 7584 | 1.21 | | | 1.21 | | | 0.0 |
| 0455 | | | 1.24 | 5310 | 5541 | 1.04 | | | 0.81 | | | 0.0 |
| 0456 | | | 1.53 | 3770 | 5462 | 1.45 | | | 1.37 | | | 0.0 |
| 0457 | | | 1.06 | 2550 | 2682 | 1.05 | | | 0.92 | | | -1.0 |
| 0459 | | | 1.12 | 6012 | 5248 | 0.87 | | | 0.76 | | | -3.0 |
| 0460 | | | 1.26 | 9732 | 11860 | 1.22 | | | 1.19 | | | -1.0 |
| AM | 404 | 478 | — | 6664 | 7336 | — | 783 | 829 | — | 4.4 | 3.9 | — |
| SD | 162 | 206 | — | 2500 | 2937 | — | 279 | 345 | — | 1.3 | 0.9 | — |
| Med | 369 | 438 | — | 6510 | 7102 | — | 800 | 782 | — | 4.0 | 4.0 | -0.5[†] |
| GM[‡] | 373 | 439 | 1.17 | 6216 | 6813 | 1.10 | 734 | 766 | 1.04 | -- | -- | -- |

Treatment B: 200-mg TMC125 q12h x 8 days

Treatment C: 400-mg MK-0518 + 200-mg TMC125 q12h x 3.5 days

AN = Allocation Number; AM = Arithmetic Mean; SD = Standard Deviation; Med = Median; GM = Geometric Mean

[†] For $T_{max}$, represents Hodges-Lehman estimate of median treatment difference

[‡] Geometric mean computed from least squares estimate from an ANOVA performed on the natural-log transformed values

305

Figure 6. Individual TMC125 $C_{12\,hr}$ Ratios (TMC125 Coadministered With MK-0518/TMC125 Administered Alone) With Geometric Mean Ratio and 90% Confidence Interval Following Multiple Doses of 200 mg TMC125 Twice-Daily With or Without Coadministration of Multiple Doses of 400 mg MK-0518 Twice-Daily to Healthy Adult Subjects



GMR: 1.17
90% CI: (1.10, 1.26)
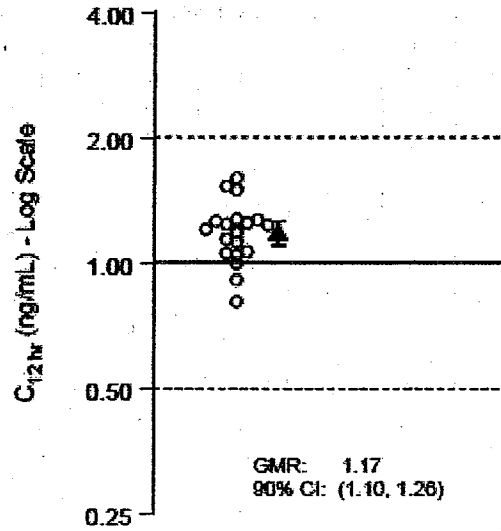
Figure 7. Individual TMC125 $AUC_{0-12\,hr}$ Ratios (TMC125 Coadministered With MK-0518/TMC125 Administered Alone) With Geometric Mean Ratio and 90% Confidence Interval Following Multiple Doses of 200 mg TMC125 Twice-Daily With or Without Coadministration of Multiple Doses of 400 mg MK-0518 Twice-Daily to Healthy Adult Subjects



GMR: 1.10
90% CI: (1.03, 1.16)

306
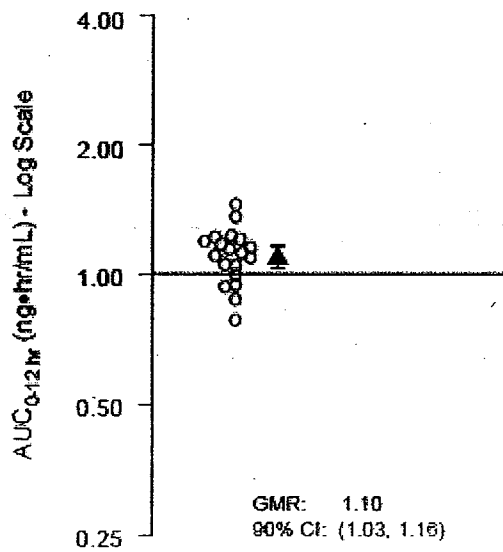
Figure 8. Individual TMC125 Cmax Ratios (TMC125 Coadministered With MK-0518/TMC125 Administered Alone) With Geometric Mean Ratio and 90% Confidence Interval Following Multiple Doses of 200 mg TMC125 Twice-Daily With or Without Coadministration of Multiple Doses of 400 mg MK-0518 Twice-Daily to Healthy Adult Subjects
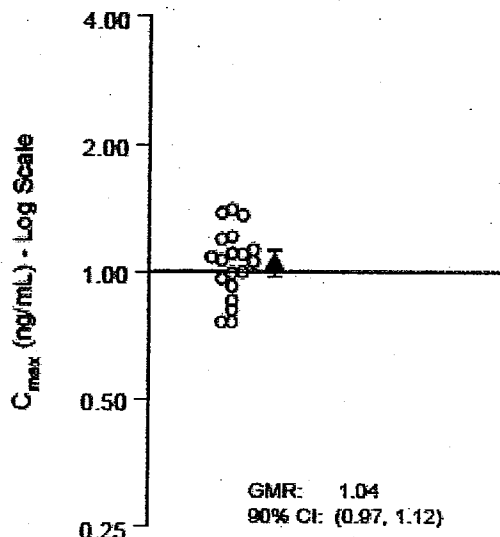


**SAFETY RESULTS**: Administration of MK-0518 with concurrent administration of TMC125 was generally well-tolerated. No serious clinical adverse experiences were reported and no subject discontinued due to an adverse experience. A total of sixteen subjects reported a total of forty-four non-serious clinical adverse experiences, twenty-one of which were deemed by the investigator to be possibly related to either drug. The most common drug related adverse event was headache. There were no laboratory adverse experiences reported in this study. All adverse experiences reported were transient and rated mild to moderate in intensity.

**DISCUSSION AND CONCLUSIONS**: With co-administration of 200 mg TMC125 twice daily for 12 days, the MK-0518 $C_{12hr}$ geometric mean ratio for (MK-0518 + TMC125/MK-0518) was 0.66 with a 90% CI of (0.34, 1.26). The $AUC_{0-12hr}$ geometric mean ratio (MK-0518 + TMC125/MK-0518) was 0.90 with a corresponding 90% confidence interval of (0.68, 1.18), while the $C_{max}$ geometric mean ratio was 0.89 with a corresponding 90% confidence interval of (0.68, 1.15).

The 90% confidence interval is quite wide implying a large degree of uncertainty in the effect of TMC125 on MK-0518 $C_{12\,hr}$. This makes a definitive conclusion about the magnitude of the effect difficult.

The applicant concluded that effects up to a 2-fold increase in exposure (AUC) and a 60% decrease (equivalent to geometric mean ratio of 0.4) in trough concentration ($C_{12hr}$) were considered to be not clinically relevant based on available clinical experience from Phase I and Phase II studies with regard to safety and efficacy.

The lower bound of 90% CI of MK-0518 $C_{12hr}$ ratios was 0.34 (<0.4) and a few individual MK-0518 $C_{12hr}$ ratios were much lower than 0.4.

The effect of MK-0518 on the pharmacokinetics of TMC125 is negligible. TMC125 $C_{12\,hr}$ is unaffected when dosed in the presence of 400 mg MK-0518 with a geometric mean ratio of 1.17 and a 90% confidence interval of (1.10, 1.26). $AUC_{0-12\,hr}$ with a GMR of 1.10 and 90% CI of (1.03, 1.16), $C_{max}$ with a GMR of 1.04 and a 90% CI of (0.97, 1.12).

307

**MASS BALANCE STUDY**

**Study Number
TMC125-C130**

**Title**

A Phase I, open-label, single dose, mass balance trial with $^{14}$C-labeled TMC125.

**Objectives**

The primary objectives of the trial were to characterize the excretion pathways and the overall metabolic profile after a single dose of $^{14}$C-TMC125 in humans.

**Study Design**

Phase I, open-label, single dose, mass-balance trial in 6 healthy male subjects. $^{14}$C-TMC125 was administered as a single 800 mg dose as PEG4000 capsules. The plasma samples were collected at several pre-defined points up to 168 hours after dosing, and thereafter in 24 hour intervals, if less than 7 stools had been delivered or if the radioactivity in 1 of the latest 2 urine collections (120-144 hours or 144-168 hours) accounted for > 2 % of the administered dose. At 6 time points during the first 48 hours, additional plasma samples were collected for structural characterization of the metabolite profile in plasma. The urine was collected at pre-defined intervals and the feces was collected per stool up to at least 168 hours after dosing. The concentration of unchanged TMC125 in plasma, and the total radioactivity in whole blood, plasma, urine, and feces was determined.

**Discussion of Trial Design and Selection of Dose**

PEG4000 was used as solvent as TMC125 is practically insoluble in aqueous solvents. In this trial, a single oral dose of 800 mg TMC125 was administered as PEG4000 capsules. A dose-corrected comparison (and assuming dose proportional pharmacokinetics) of the exposures demonstrated in trials in healthy subjects with the formulations **TF002** and **TF035**, have shown that the AUC$_{12h}$ with 900 mg b.i.d. TMC125 of formulation **TF002** (capsule containing PEG4000) is comparable with that of 656 mg b.i.d. of TMC125 of formulation **TF035**, assuming dose-proportional pharmacokinetics. As a single dose of 800 mg TMC125 administered as **TF035** results in plasma concentrations well above the limit of quantification, administration of 800 mg TMC125 as PEG4000 capsules was considered to be high enough to draw relevant conclusions.

**Investigational Product(s)**

Unlabeled TMC125 was manufactured by ⎯⎯⎯ the lot number was ZR165335PUA131 (expiry date 22 december 2006).

[Pyrimidine-5-$^{14}$C]-TMC125 was synthesized by the ⎯⎯⎯⎯⎯ $^{14}$C-labeled

TMC125 (batch No. 1819) was purified by J&J PRD, Beerse, Belgium and supplied with a specific radioactivity of 2.04 GBq/mmol (55 mCi/mmol) as a solution in ethanol. The radiochemical purity was $\geq$ — (determined by HPLC). TMC125 was formulated by J&J PRD as capsules of TMC125 ($^{14}$C-labeled and unlabeled) in PEG4000 at a dose of 50 mg per capsule (**TF002**).

**Pharmacokinetic and Statistical Data Analysis**

*Pharmacokinetic Analysis*

The pharmacokinetic and statistical analysis was done by ⎯⎯⎯⎯⎯⎯.
⎯⎯⎯ Non-parametric pharmacokinetic analyses were performed using WinNonlin® Professional (version 3.3; Pharsight Corporation, California, U.S.A.), and Microsoft Excel® (version 2000; Microsoft, Redmond, Washington, U.S.A) for the calculation of the ratios. Non-compartmental analysis model 200 (extravascular input, plasma data) was used for the pharmacokinetic analysis.

Based on the individual plasma concentration-time data, using the scheduled sampling times, the standard pharmacokinetic parameters were computed. The individual concentrations of total radioactivity (in terms of dpm/mL) were converted into ng (equivalent)/mL, using the specific radioactivity of 2.31 KBq/mg TMC125.

*Statistical Analysis*

Descriptive statistics were calculated for total radioactivity and unchanged TMC125 in plasma at each time point and for the derived pharmacokinetic parameters.

**RESULTS**

**Subject Disposition and Demographics**

Out of the 13 subjects screened, 6 subjects received the trial medication. All 6 subjects who received the trial medication completed the trial. All subjects fulfilled the criteria for leaving the investigational site on day 8 (= 168 hours), i.e., the radioactivity excreted in 1 of the last two urine collections (120 hr-144 hr or 144 hr-168 hr) did not exceed 2 % of the administered dose, all subjects delivered at least 7 fecal stools prior to 168 hours after dosing. The collection of feces and urine was therefore discontinued in all 6 subjects on day 8.

Table 1 shows the demographic data collected in the trial.

**Table 1: Demographic data collected in trial TMC125-C130**

| Parameter | All Subjects N = 6 |
|---|---|
| Age (years), median (range) | 49.5 (40, 60) |
| Height (cm), median (range) | 176.0 (170, 192) |
| Weight (kg), median (range) | 88.0 (64, 100) |
| Body Mass Index (kg/m$^2$), median (range) | 26.7 (21, 29) |

**Pharmacokinetics**

Unchanged TMC125

Fig 1 shows the mean plasma concentration-time profile of TMC125 (unchanged) and total $^{14}$C radioactivity after oral administration of 800 mg $^{14}$C-labeled TMC125.

**Fig 1: Mean plasma concentration-time profile of TMC125 (unchanged) and total $^{14}$C radioactivity after oral administration of 800 mg $^{14}$C-labeled TMC125**
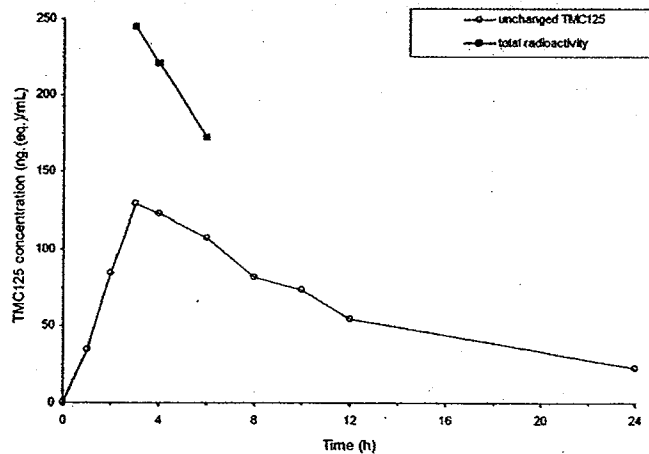


The plasma concentration of TMC125 was quantifiable up to 72 hours in all the subjects. In 3 subjects, the plasma concentrations were quantifiable at the last measured time point of 168 hours post dose.

Table 2 shows the mean pharmacokinetic parameters of TMC125 in plasma.

311

## Table 2: Mean pharmacokinetic parameters of TMC125 in plasma

| Pharmacokinetics of TMC125 | TMC125 in plasma | Total $^{14}$C-radioactivity in plasma |
|---|---|---|
| | N = 6 | N = 6 |
| $t_{max}$, h, median (range) | 3.5 (2.0 - 10.0) | 3.5 (2.0 - 10.0) |
| $C_{max}$, ng.(eq.)/mL, mean ± SD | 164 ± 50.3 | 319 ± 88.2 |
| $AUC_{last}$, ng.h/mL, mean ± SD | 2350 ± 935 | NA |
| $AUC_\infty$, ng.h/mL, mean ± SD | 2515 ± 1001 | NA |
| $t_{1/2term}$, h, mean ± SD | 41.1 ± 19.6 | NA |

NA: not assessable

### Total Radioactivity

The total $^{14}$C-radioactivity in plasma exceeded the LLOQ of 142 ng.eq/mL at limited time points. In some subjects, only 2-3 quantifiable concentrations could be determined. The highest concentrations determined was 478 ng*eq/mL. Table 3 shows the ratio of unchanged drug to total radioactivity.

### Table 3: Ratio of Unchanged Drug to Total Radioactivity

| Parameter/ CRF ID | 1300001 | 1300002 | 1300004 | 1300007 | 1300010 | 1300013 |
|---|---|---|---|---|---|---|
| Ratio $C_{2h}$, % | | | | | | |
| Ratio $C_{3h}$, % | | | | | | |
| Ratio $C_{4h}$, % | | | | | | |
| Ratio $C_{6h}$, % | | | | | | |
| Ratio $C_{8h}$, % | | | | | | |
| Ratio $C_{10h}$, % | | | | | | |
| Ratio $C_{12h}$, % | | | | | | |
| Ratio $C_{max}$, % | | | | | | |

The descriptive statistics for total radioactivity in plasma could only be calculated for the concentrations at 3, 4, and 6 hours after administration, with the highest concentrations at 3 hours post dose. The individual ratios for plasma concentrations of TMC125 versus total $^{14}$C radioactivity ranged from ⎯⎯ % over all time points and subjects.

### Radioactivity Recovery in Urine and Feces

Table 4 shows the levels of total radioactivity as a percentage of the administered radioactive dose in urine and feces from healthy male subjects after a single oral dose of 800 mg $^{14}$C-labelled TMC125 from the time of administration up to 168 hours after dosing.

**Table 4:** Cumulative percentage of the recovered radioactivity in Urine and Feces after a single dose of 800 mg $^{14}$C-labelled TMC125 up to 168 hours after dosing

| Subject # | 1 | 2 | 3 | 4 | 5 | 6 | Mean ± SD |
|---|---|---|---|---|---|---|---|
| Urine | | | | | | | 1.2 ± 0.3 |
| Feces | | | | | | | 93.7 ± 0.7 |
| Total | | | | | | | 94.9 ± 0.9 |

At 168 hours after the administration of a single dose of $^{14}$C-labelled TMC125, ⟶ to ⟶ of the administered radioactivity was recovered. The majority of the radioactivity was recovered in the feces ( ⟶ %). The percentage of the radioactivity recovered in the urine was ' ⟶ % of the administered dose.

*Reviewer's Note:*

*Due to limitations of the assay, the descriptive statistics of the parent (unchanged) drug and/or metabolites could not be computed beyond 6 hours after TMC125 dosing. Therefore, no conclusions can be drawn regarding the nature of the circulating moiety in the plasma (i.e., whether it is parent drug and/or metabolite(s)) and what proportion of the radioactivity in the plasma (beyond 6 hrs) is composed of the parent drug. However, the majority of the administered radioactivity was recovered in the feces and urine (~95 %) Further, the majority of the radioactivity in feces was composed of the parent drug. This indicates that the major proportion of the radioactivity in plasma beyond 6 hours may have been composed primarily of the parent drug. The metabolites formed in the plasma beyond 6 hours (if any) were either detected in the feces/urine or the concentrations of the metabolites was too low (and therefore inconsequential) to have any efficacious/toxic effect.*

<u>Metabolite Profiling and Identification</u>

Table 5 shows the mass balance and metabolic profile of TMC125 in the methanolic extracts of human feces as percentage of total radioactivity accounted for unchanged drug and metabolite.

313

**Table 5: Mass balance and metabolic profile of TMC125 in the methanolic extracts of human feces as percentage of total radioactivity accounted for unchanged drug and metabolite**

| Subject # | Collection Day | Metabolite 8 | Metabolite 12 | Metabolite 13 | Unchanged drug |
|-----------|----------------|--------------|---------------|---------------|----------------|
| 1300001 | 2 | | | | |
| | 3 | | | | |
| | 4 | | | | |
| | Sum 0-96h | 7.3 | 0.2 | NA | 82.0 |
| 1300002 | 2 | | | | |
| | 3 | | | | |
| | 4 | | | | |
| | Sum 0-96h | 3.8 | NA | NA | 86.4 |
| 1300004 | 1 | | | | |
| | 2 | | | | |
| | 3 | | | | |
| | 4 | | | | |
| | Sum 0-96h | 5.6 | 0.6 | NA | 82.1 |
| 1300010 | 2 | | | | |
| | 3 | | | | |
| | 3 | | | | |
| | 4 | | | | |
| | Sum 0-96h | 5.9 | 0.7 | 0.2 | 81.2 |
| 1300013 | 2 | | | | |
| | 3 | | | | |
| | 4 | | | | |
| | Sum 0-96h | 9.0 | 0.5 | NA | 81.2 |
| 1300007 | 2 | | | | |
| | 2 | | | | |
| | 3 | | | | |
| | Sum 0-96h | 5.1 | 0.4 | NA | 83.0 |

NA = not assessable

In methanolic fecal extracts up to 96 hours, the relative proportion of unchanged drug (as a function of the administered dose) decreased with time of collection for each subject. The unchanged drug accounted for the majority of the radioactivity in the methanolic feces extracts. Metabolite 8 was the major metabolite in the feces accounting for to ⸺ of the dose. Metabolite 12 was also present in low quantities (up to of the dose).

Table 6 shows the mass balance and metabolite profile of TMC125 in the human urine as the percentage of the radioactive dose accounted for by the TMC125 metabolites.

**Table 6: Mass balance and metabolite profile of TMC125 in the human urine as the percentage of the radioactive dose accounted for by the TMC125 metabolites**

| Subject # | Metabolite 1 | Metabolite 6 | Metabolite 8 |
|-----------|--------------|--------------|--------------|
| 1300001 [a] | | | |
| 1300002 [a] | | | |
| 1300004 [a] | | | |
| 1300010 [a] | | | |
| 1300013 [a] | | | |
| 1300007 [a] | | | |
| 1300007 [b] | | | |

b In pooled urine samples for the post-dose interval 0-8 hours
c Urine sample for the post-dose interval 8-24 hours

314

Unchanged drug was not detected in urine samples. Metabolite 1 was the only metabolite present in quantifiable amounts, representing ——— of the dose. In the pooled urine sample of subject 1300007 for the sampling interval 8 to 24 hours post-dose, Metabolites 6 and 8 were also present. Treatment with ß-glucuronidase/arylsulphatase and LC-MS/MS structural characterization confirmed that metabolite 1 and metabolite 6 were glucuronidated metabolites.

Fig 2 shows the metabolic pathways of TMC125 in humans. The most important pathway of TMC125 metabolism in humans is the hydroxylation of the methyl carbons of the dimethylbenzonitrile moiety to form **metabolite 12** and **metabolite 8**. The glucoronidation of these metabolites yielded **metabolite 6** and **metabolite 1** respectively. Overall, methyl hydroxylation accounts for 3.8-9.5 % of the dose. The aromatic hydroxylation at the dimethylbenzonitrile moiety (**metabolite 13**) was a very minor metabolic pathway.

In plasma (based on the concentrations above the limit of quantification at 2, 4, and 8 hours), TMC125 was represented the major fraction of the absorbed radioactivity. Metabolites 1, 8, and 12 were detected in the plasma.

**Fig 2: Metabolic pathways of TMC125 in humans.**



315
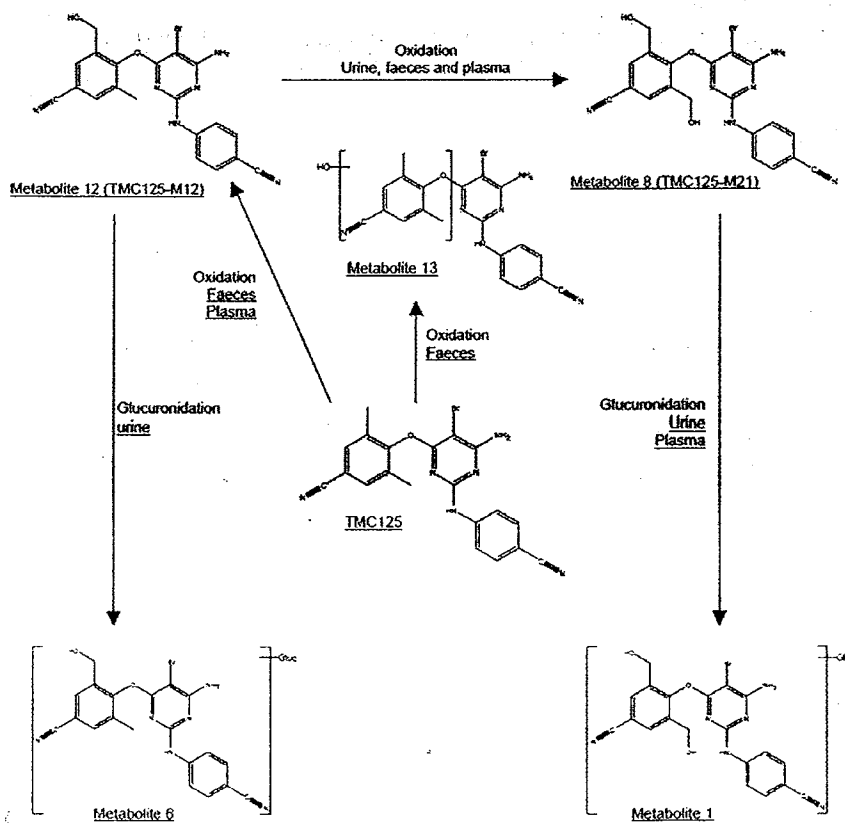
**Metabolite 12** and **metabolite 8** (TMC125-M12 and TMC125-M21) were tested on a panel of wild-type and mutant HIV-1 virus strains to determine the in vitro antiviral activity (TMC125-C130). When compared to TMC125, the $EC_{50}$ values for wild-type HIV-1 were similar for **metabolite 12** but about 200-fold higher (i.e. less active) for **metabolite 21**. The antiviral activity on mutant virus strains was lower for **metabolite 12** as compared to TMC125; **metabolite 21** did not show any activity on the mutant virus strains tested.

**Conclusion**

- At 168 hours after the administration of a single oral dose of TMC125, 94.9 % ± 0.9 % of the dose was recovered based on radioactivity.
- The total radioactivity recovered in the feces after 168 hours was 93.7 % ± 0.7 % of the administered dose. Unchanged drug accounted for the majority of the radioactivity in the feces ‘ ⸺ , of the dose).
- The total radioactivity recovered in the urine was 1.2 % ± 0.3 % of the administered dose. No unchanged drug was present in the urine.
- The most important Phase-1 metabolic pathway of TMC125 in humans was hydroxylation of the methyl carbons of the dimethylbenzonitrile moiety to form metabolite 12 and metabolite 8, and the glucuronidation of these metabolites to metabolite 6 and metabolite 1, respectively.
- For time points with both a quantifiable total ^{14}C-radioactivity and TMC125 concentration in plasma, the TMC125 cocentration was approximately half of the corresponding total ^{14}C-radioactivity.

APPEARS THIS WAY
ON ORIGINAL

316

## SPECIAL POPULATIONS (HEPATIC IMPAIRMENT STUDY)

317

**Study Number**
**TMC125-C125**

**Title**

Pharmacokinetic and safety assessment of multiple dose TMC125 in subjects with impaired hepatic function.

**Objectives**

The primary objective of the trial was to assess the steady state pharmacokinetics of TMC125 200 mg b.i.d. in subjects with mild or moderate hepatic impairment and compare it to the pharmacokinetics of TMC125 200 mg b.i.d. in matched, healthy subjects.

**Study Design**

Phase 1, open label trial. The trial population (n = 32) was divided into the following two panels, **panel A** and **panel B**.

**Panel A:** 8 subjects with mild hepatic impairment (**A1; Child Pugh Score 5-6**) and 8 healthy matched controls (**A2**).

**Panel B:** 8 subjects with moderate hepatic impairment (**B1; Child Pugh Score 7-9**) and 8 healthy matched controls (**B2**).

The control group was derived from a comparable, but healthy matched subject population. The control group was similar to subjects with hepatic impairment with respect to age (± 5 years), BMI (± 15 %), gender, race, and smoking status. The mild and moderate hepatic impairment was defined by using the Child-Pugh classification score.

The subjects in **panel A** received TMC125 200 mg b.i.d. (**F060**) in the fed state (standard breakfast) for 7 days and an additional dose in the morning of day 8. Full pharmacokinetic profiles of TMC125 were determined on **day 1** up to 12 hours post dose and on **day 8** up to 96 hours post dose. The dosing of subjects in **panel B** started if no major safety, tolerability, or pharmacokinetic concerns were raised in subjects of **panel A**. The dose of TMC125 administered to subjects in **panel B** was either the same dose as for **panel A** (200 mg b.i.d.) with food or a lower dose (100 mg b.i.d.) with food depending on the results of **panel A**. **Based on the results from panel A, 200 mg b.i.d. with food was administered to subjects in panel B.**

**Investigational Product(s)**

TMC125 was provided as a tablet containing 100 mg (formulation **F060**) of TMC125 — spray dried in a fixed ratio with hydroxypropylmethylcellulose and microcrystalline cellulose, _____ croscarmellose sodium, magnesium stearate, and lactose monohydrate. The batch # was 05E12 and 05G19 and the expiration date was November 2006 and July 2007 respectively.

**Assay Methods**

The plasma concentrations of TMC125 were determined using a validated LC-MS/MS method. The lower limit of quantification (LLOQ) of TMC125 was 2 ng/mL.

**Pharmacokinetics and Statistical Data Analysis**

*Pharmacokinetic Analysis*

Pharmacokinetic and statistical analysis was performed using Winonlin Professional™ (version 4.1, Pharsight Corporation). A non-compartmental model with extravascular input was used for the pharmacokinetic analysis. Based on the individual plasma concentration-time data and using the scheduled sampling times, the standard pharmacokinetic parameters were calculated.

*Statistical Analysis*

The statistical analysis was performed by comparing the subjects with mild hepatic impairment (**panel A**) or moderate hepatic impairment (**panel B**) versus healthy matched subjects within the same panel. The primary pharmacokinetic parameters of TMC125 were $C_{max}$ and $AUC_{12h}$ on the logarithmic scale on day 1 and $C_{min}$, $C_{max}$, and $AUC_{12hr}$ on the logarithmic scale on day 8.

**RESULTS**

**Subject Disposition and Demographics**

Out of the 34 subjects screened, 32 subjects were assigned to receive treatment. 2 subjects were not assigned to treatment as they did not fulfill all the inclusion and exclusion criteria. All 32 subjects completed the trial.

Table 1 shows the demographics of the trial.

## Table 1: Demographics in Trial TMC125-C125

| | Panel A | | Panel B | |
|---|---|---|---|---|
| | Healthy controls | Subjects with mild hepatic impairment[a] | Healthy controls | Subjects with moderate hepatic impairment[a] |
| Parameter | N = 8 | N = 8 | N = 8 | N = 8 |
| Age, years | 56.0 | 57.0 | 51.0 | 54.0 |
| Median (range) | (44-66) | (41-65) | (42-63) | (44-64) |
| Height, cm | 171.5 | 170.5 | 175.0 | 173.5 |
| Median (range) | (157-181) | (160-183) | (155-190) | (158-198) |
| Weight, kg | 72.4 | 74.3 | 82.1 | 78.5 |
| Median (range) | (57-89) | (58-101) | (55-96) | (60-125) |
| BMI, kg/m² | 26.1 | 25.8 | 27.1 | 25.7 |
| Median (range) | (23-29) | (20-32) | (23-31) | (22-32) |
| Gender, n (%) Male/female | 5/3 | 5/3 | 6/2 | 6/2 |
| Ethnic Origin, n (%) Caucasian/White | 8 (100.0) | 8 (100.0) | 8 (100.0) | 8 (100.0) |
| Smoker: Yes/No[b] | 4/4 | 4/4 | 4/4 | 4/4 |
| HCV cirrhosis: Yes/No | 0/8 | 0/8 | 0/8 | 2[c]/6 |
| Alcoholic cirrhosis: Yes/No | 0/8 | 8/0 | 0/8 | 7[c]/1 |

[a] All subjects in Panel A belonged to child-Pugh class A, and all subjects in Panel B belonged to child-Pugh class B, as per protocol.
[b] All smokers were light smokers, i.e., no more than 10 cigarettes, or 1 cigar, or 1 pipe per day.
[c] Subject 125-0020 had cirrhosis secondary to both alcoholism and hepatitis C infection.
N = total number of subjects

## Pharmacokinetics

Fig 1 shows the mean plasma concentration-time profiles after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with mild hepatic impairment.

**Fig 1: Mean plasma concentration-time profiles after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with mild hepatic impairment**
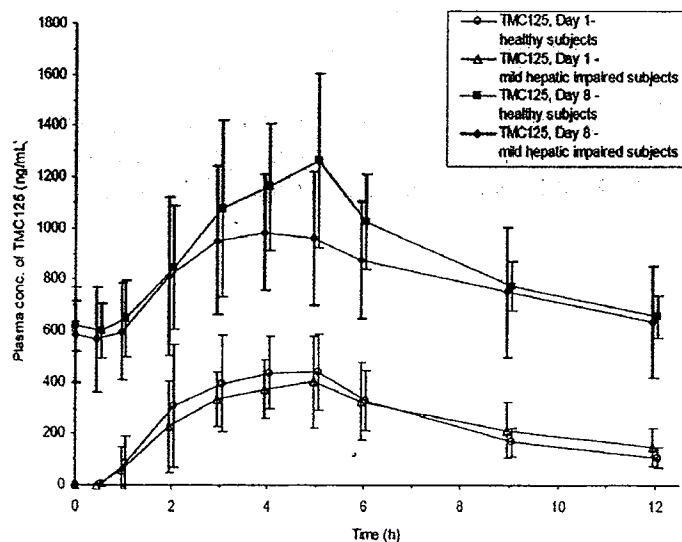
The mean plasma concentrations of TMC125 on day 1 were comparable for healthy subjects and subjects with mild hepatic impairment. On day 8, from 2-6 hours post dose, the plasma concentrations of TMC125 were lower for subjects with mild hepatic impairment, however, the trough concentrations were comparable.

Fig 2 shows the mean plasma concentration-time profiles after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with moderate hepatic impairment.

**Fig 2: Mean plasma concentration-time profiles after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with moderate hepatic impairment**
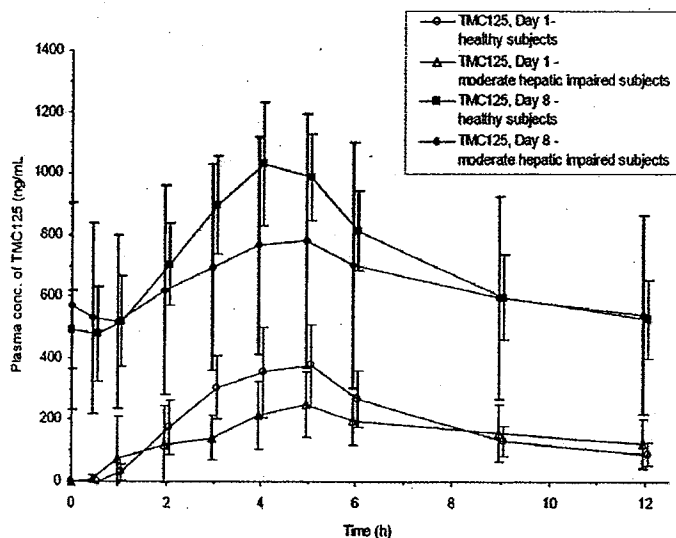


Fig 3 shows the pharmacokinetic parameter plots ($C_{max}$ and AUC) on day 8 after administration of TMC125 200 mg b.i.d. to subjects with different degrees of hepatic impairment.

**Fig 3:  Pharmacokinetic parameter plots ($C_{max}$ and AUC) on day 8 after administration of TMC125 200 mg b.i.d. to subjects with different degrees of hepatic impairment**



$C_{max}$ - Day 8

$AUC_{12h}$ - Day 8
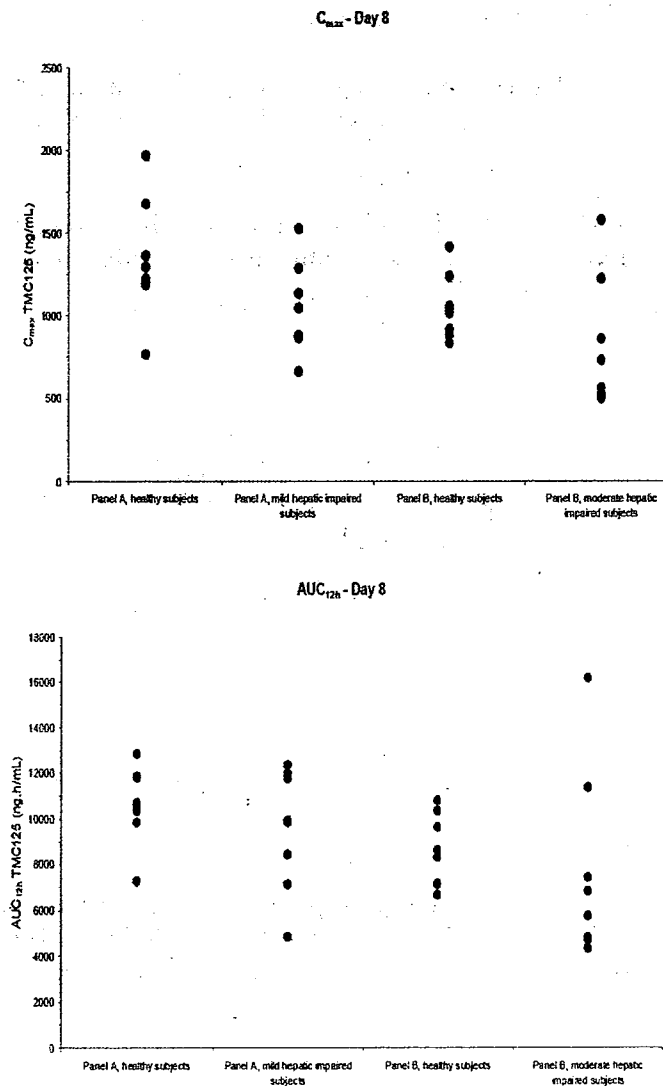
There was overlap in the pharmacokinetic parameters of subjects with mild and moderate hepatic impairment and the pharmacokinetic parameters of subjects with normal hepatic function.

Table 2 shows the pharmacokinetic parameters of TMC125 after administration of TMC125 200 mg b.i.d. to healthy subjects, subjects with mild hepatic impairment, and subjects with moderate hepatic impairment.

Table 2: Pharmacokinetic parameters of TMC125 after administration of TMC125 200 mg b.i.d. to healthy subjects, subjects with mild hepatic impairment, and subjects with moderate hepatic impairment

| Pharmacokinetic parameter (Mean ± SD, $t_{max}$: median [range]) | Panel A | | Panel B | |
|---|---|---|---|---|
| | Healthy controls N=8 | Subjects with mild hepatic impairment N=8 | Healthy controls N=8 | Subjects with moderate hepatic impairment N=8 |
| **Day 1** | | | | |
| $C_{min}$, ng/mL | 498.8 ± 149.2 | 466.5 ± 157.8 | 413.9 ± 123.3 | 267.5 ± 100.6 |
| $t_{max}$, h | 4.0 (2.0 - 5.0) | 4.5 (2.0 - 5.0) | 5.0 (2.0 - 5.0) | 5.0 (4.0 - 9.0) |
| $AUC_{12h}$, ng.h/mL | 2972 ± 1105 | 2903 ± 816.1 | 2293 ± 663.9 | 1846 ± 808.0 |
| **Day 5** | | | | |
| $C_{0h}$, ng/mL | 451.3 ± 117.4 | 437.5 ± 212.3 | 338.3 ± 84.51 | 406.5 ± 170.8 |
| **Day 6** | | | | |
| $C_{0h}$, ng/mL | 525.5 ± 107.8 | 514.8 ± 191.2 | 408.1 ± 91.53 | 475.8 ± 274.9 |
| **Day 7** | | | | |
| $C_{0h}$, ng/mL | 578.0 ± 90.62 | 541.4 ± 195.9 | 453.9 ± 105.8 | 536.9 ± 303.7 |
| **Day 8** | | | | |
| $C_{0h}$, ng/mL | 618.6 ± 100.7 | 585.4 ± 186.4 | 491.6 ± 127.5 | 568.5 ± 336.5 |
| $C_{min}$, ng/mL | 593.8 ± 99.64 | 549.9 ± 192.1 | 461.6 ± 128.4 | 499.0 ± 293.4 |
| $C_{max}$, ng/mL | 1339 ± 356.9 | 1060 ± 267.8 | 1054 ± 193.5 | 817.6 ± 393.7 |
| $t_{max}$, h | 4.5 (3.0 - 5.0) | 4.0 (3.0 - 6.0) | 4.0 (4.0 -5.0) | 5.0 (4.0 - 6.0) |
| $AUC_{12h}$, ng.h/mL | 10650 ± 1688 | 9546 ± 2630 | 8584 ± 1560 | 7665 ± 4122 |
| $t_{1/2term}$, h* | 85.64 ± 27.44 | 86.73 ± 38.22 | 71.16 ± 27.50 | 189.9 ± 58.80 |
| $C_{av}$, ng/mL | 887.5 ± 140.7 | 795.5 ± 219.2 | 715.3 ± 130.0 | 638.8 ± 343.5 |
| FI, % | 81.78 ± 23.49 | 67.94 ± 26.98 | 84.19 ± 17.36 | 54.15 ± 13.41 |

* Accurate determination not possible

For subjects in panel A and B with normal hepatic function, the inter subject variability in $C_{max}$ and $AUC_{12h}$ of TMC125 on **day 1** ranged from 30 % to 37 % and 29 % to 30 %, respectively. On **day 8**, the inter subject variability in $C_{max}$ and $AUC_{12h}$ ranged from 16 % to 27 % and 18 % to 28 % respectively. In subjects with mild hepatic impairment, the inter subject variability in $C_{min}$, $C_{max}$, and $AUC_{12h}$ ranged from 28 % to 34 % on **day 1** and 25 % to 35 % on **day 8**. In subjects with moderate hepatic impairment, the inter subject variability in $C_{min}$, $C_{max}$, and $AUC_{12h}$ ranged from 38 % to 44 % on **day 1** and 48 % to 59 % on **day 8**.

Table 3 shows the statistical evaluation of the pharmacokinetic parameters of TMC125 after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with mild hepatic impairment.

**Table 3:** **Statistical evaluation of the pharmacokinetic parameters of TMC125 after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with mild hepatic impairment**

| Pharmacokinetic parameter | LSmeans [a] | | LSmeans ratio | 90% CI [b] |
| --- | --- | --- | --- | --- |
| | Panel A, healthy subjects (reference) | Panel A, subjects with mild hepatic impairment (test) | | |
| *Day 1* | | | | |
| $C_{max}$, ng/mL | 482.5 | 441.7 | 0.92 | 0.69 – 1.21 |
| $AUC_{12h}$, ng.h/mL | 2834 | 2795 | 0.99 | 0.75 – 1.29 |
| *Day 8* | | | | |
| $C_{min}$, ng/mL | 586.0 | 512.0 | 0.87 | 0.65 – 1.17 |
| $C_{max}$, ng/mL | 1297 | 1030 | 0.79 | 0.63 – 1.00 |
| $AUC_{12h}$, ng.h/mL | 10520 | 9168 | 0.87 | 0.69 – 1.09 |
| Pharmacokinetic parameter | Median | | Treatment difference median | 90% CI [b] |
| | Panel A, healthy subjects (reference) | Panel A, subjects with mild hepatic impairment (test) | | |
| $t_{max}$, h on Day 1 | 4.0 | 4.5 | 0.0 | (-1.0) - (1.0) |
| $t_{max}$, h on Day 8 | 4.5 | 4.0 | 0.0 | (-1.0) - (1.0) |

[a] n = 8 for Reference and n = 8 for Test
[b] 90% confidence intervals.

On **day 1**, the $LS_{means}$ ratios of $C_{max}$ and $AUC_{12h}$ of TMC125 were not significantly altered (change < 10 %) when TMC125 200 mg b.i.d. was administered to subjects with mild hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal hepatic function.

On **day 8**, the $LS_{means}$ ratios of $C_{min}$, $C_{max}$, and $AUC_{12h}$ of TMC125 were decreased by 13 %, 21 %, and 13 % respectively, when TMC125 200 mg b.i.d. was administered to subjects with mild hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal hepatic function.

Table 4 shows the statistical evaluation of the pharmacokinetic parameters of TMC125 after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with moderate hepatic impairment.

**Table 4:** **Statistical evaluation of the pharmacokinetic parameters of TMC125 after administration of TMC125 200 mg b.i.d. to healthy subjects and subjects with moderate hepatic impairment**

| Pharmacokinetic parameter | LSmeans[a] | | LSmeans ratio | 90% CI[b] |
|---|---|---|---|---|
| | Panel B, healthy subjects (reference) | Panel B, subjects with moderate hepatic impairment (test) | | |
| **Day 1** | | | | |
| $C_{max}$ ng/mL | 398.6 | 251.3 | 0.63 | 0.47-0.85 |
| $AUC_{12h}$ ng.h/mL | 2201 | 1698 | 0.77 | 0.55-1.08 |
| **Day 8** | | | | |
| $C_{max}$ ng/mL | 446.5 | 439.1 | 0.98 | 0.68-1.42 |
| $C_{min}$ ng/mL | 1040 | 749.4 | 0.72 | 0.54-0.96 |
| $AUC_{12h}$ ng.h/mL | 8460 | 6908 | 0.82 | 0.60-1.11 |

| Pharmacokinetic parameter | Median | | Treatment difference median | 90% CI[b] |
|---|---|---|---|---|
| | Panel B, healthy subjects (reference) | Panel B, subjects with moderate hepatic impairment (test) | | |
| $t_{max}$ h on Day 1 | 5.0 | 5.0 | -0.5 | (-2.0) - (0.0) |
| $t_{max}$ h on Day 8 | 4.0 | 5.0 | 0.0 | (-1.0) - (0.0) |

[a] n = 8 for Reference and n=8 for Test
[b] 90% confidence intervals.

On **day 1**, the $LS_{means}$ ratios of $C_{max}$ and $AUC_{12h}$ of TMC125 were decreased by 37 % and 23 % respectively, when TMC125 200 mg b.i.d. was administered to subjects with moderate hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal hepatic function.

On **day 8**, the $LS_{means}$ ratios of $C_{max}$ and $AUC_{12h}$ of TMC125 were decreased by 28 % and 18 % respectively, when TMC125 200 mg b.i.d. was administered to subjects with mild hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal hepatic function. The $LS_{means}$ ratio of $C_{min}$ was similar in subjects with moderate hepatic impairment and subjects with normal hepatic function.

## Pharmacokinetic Results Summary

- **Mild Hepatic Impairment:**
  - **Day 1:** The $LS_{means}$ ratios of $C_{max}$ and $AUC_{12h}$ of TMC125 were not significantly altered (change < 10 %) when TMC125 200 mg b.i.d. was administered to subjects with mild hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal hepatic function.
  - **Day 8:** The $LS_{means}$ ratios of $C_{min}$, $C_{max}$, and $AUC_{12h}$ of TMC125 were decreased by 13 %, 21 %, and 13 % respectively, when TMC125 200 mg b.i.d. was administered to subjects with mild hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal hepatic function.

- **Moderate Hepatic Impairment:**
  - **Day 1:** The $LS_{means}$ ratios of $C_{max}$ and $AUC_{12h}$ of TMC125 were decreased by 37 % and 23 % respectively, when TMC125 200 mg b.i.d. was administered to subjects with moderate hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal hepatic function.
  - **Day 8:** The $LS_{means}$ ratios of $C_{max}$ and $AUC_{12h}$ of TMC125 were decreased by 28 % and 18 % respectively, when TMC125 200 mg b.i.d. was administered to subjects with mild hepatic impairment as compared to when TMC125 200 mg b.i.d was administered to subjects with normal

hepatic function. The $LS_{means}$ ratio of $C_{min}$ was similar in subjects with moderate hepatic impairment and subjects with normal hepatic function.

- **Severe Hepatic Impairment-Not evaluated in the trial**

## Conclusion

*No dose adjustment of TMC125 is required in patients with mild or moderate hepatic impairment. There are no data available regarding the use of TMC125 in patients with severe hepatic impairment, therefore, TMC125 is not recommended for use in patients with severe hepatic impairment.*

## IN VITRO STUDIES

An *in vitro* study to determine the kinetics of TMC125 metabolism in human liver microsomes, and to identify the microsomal cytochrome P-450 iso-enzymes mediating TMC125 metabolism (reaction phenotyping) (TMC125-NC210)

Objectives: To determine the enzyme kinetics of TMC125 metabolism in human liver microsomes (HLMs), and to identify the cytochrome P-450 (CYP) enzymes mediating TMC125 metabolism (reaction phenotyping) in HLMs.

Methods:

The enzyme kinetics of TMC125 metabolism in a pooled batch of HLMs was determined. On the basis of enzyme kinetics, a substrate concentration (TMC125) was selected and used in the subsequent set of experiments (reaction phenotyping). To identify the CYP enzymes responsible for the formation of TMC125 Phase I metabolites, "reaction phenotyping" was performed by three different approaches,

1. Diagnostic CYP chemical inhibitors in a pooled batch of HLMs
2. Recombinant heterologously expressed human CYP isoforms
3. Correlation of CYP isoenzyme-specific activities of the microsomes using a panel of 10 different individual batches of HLMs

*Enzyme Kinetics*: To determine kinetics (Km and Vmax) of TMC125 metabolism, varying concentrations of $^{14}$C-TMC125 [0.5 µM (1 kBq/ml), 1 µM (2 kBq/ml), 3 µM (6 kBq/ml), 5 µM (10 kBq/ml), 10 µM (10 kBq/ml), 20 µM (10 kBq/ml), 30 (10 kBq/ml) and 50 µM (10 kBq/ml)] were incubated in HLMs. On the basis of preliminary optimization experiments, a protein concentration (HLMs) of 0.25 mg/ml with 15 min incubation time at 37°C was adopted for all incubations in this experiment. All incubations were carried out in triplicates and the total volume of reaction mixture per incubate was 1.5 ml. The final concentration of DMSO in all incubations was 0.5 % v/v. All samples were analyzed by radio-HPLC.
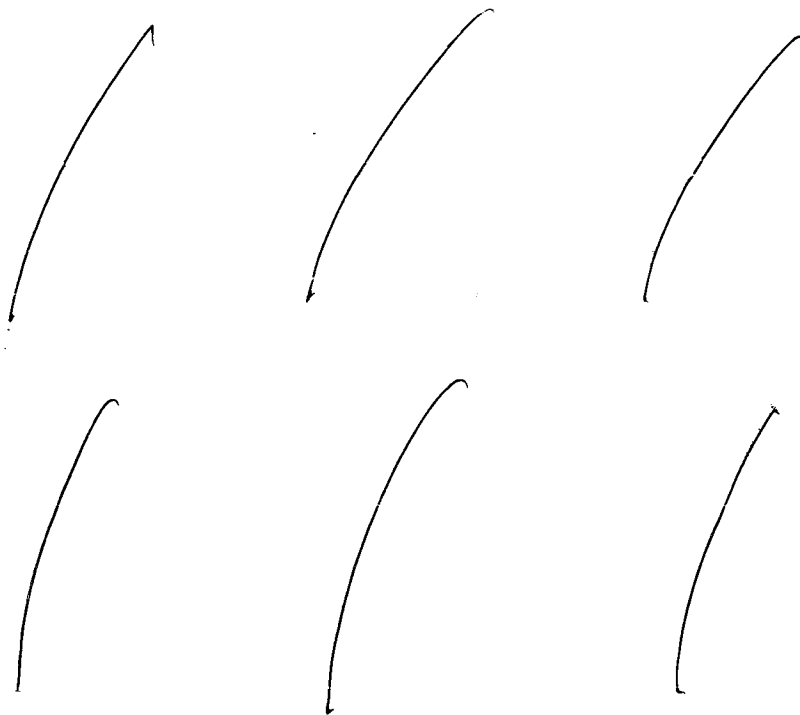
Results:

The apparent Michaelis constant Km and Vmax values for the overall metabolism of TMC125 amounted to 12.4 µM (Std. Error ± 5.4) and 360.8 (Std. Error ± 58.6) pmol/mg/min, respectively. The Km value obtained from this experiment was used in selecting the TMC125 concentration (5 µM) in the subsequent reaction phenotyping experiments in the same pooled batch of HLMs.

1. Diagnostic CYP chemical inhibitors and CYP inhibitory antibodies in a pooled batch of HLMs

Table 1. Effect of diagnostic CYP isoform specific chemical inhibitors on the overall metabolism of $^{14}$C-TMC125 and the formation of its metabolites 12 and 13 in human liver microsomes. Each value is mean ± S.D of 3 observations

| Diagnostic CYP inhibitor (CI) | CYP P450 Isoform selectivity | Final Conc. CI in the incubate (µM) | Substrate turnover/product formation rate (pmol/min.mg protein) | | |
|---|---|---|---|---|---|
| | | | TMC125 | M12 | M13 |
| | | | Mean ± S.D | Mean ± S.D | Mean ± S.D |
| Furafylline | CYP1A2 | 10 | 72.0 ± 11.9 | 21.3 ± 8.1 | 45.8 ± 6.6 |
| Coumarin | CYP2A6 | 100 | 97.3 ± 10.7 | 24.9 ± 6.0 | 63.1 ± 6.2 |
| Sulphaphenazole | CYP2C9 | 10 | 72.9 ± 7.3 | 21.3 ± 4.8 | 50.2 ± 3.9 |
| Quinidine | CYP2D6 | 10 | 70.2 ± 6.8 | 22.2 ± 12.0 | 47.1 ± 6.7 |
| 4-methylpyrazole | CYP2E1 | 20 | 73.3 ± 11.6 | 15.6 ± 6.7 | 56.9 ± 5.4 |
| Ticlopidine HCl | CYP2C19/D6 | 5 | 97.8 ± 20.0 | 21.8 ± 5.6 | 61.3 ± 15.1 |
| Ketoconazole | CYP3A4 | 1 | 6.7 ± 7.4 | 3.1 ± 3.4 | 0.0 ± 0.0 |
| Troleandomycin | CYP3A4 | 200 | 12.4 ± 1.5 | 6.7 ± 1.3 | 0.0 ± 0.0 |
| Clarithromycin | CYP3A | 15 | 25.8 ± 0.8 | 8.4 ± 2.0 | 16.9 ± 2.8 |
| Ritonavir | CYP3A4 | 0.15 | 6.7 ± 1.3 | 6.7 ± 1.3 | 0.0 ± 0.0 |
| 1-aminobenzotriazole | CYP P450 | 1000 | 8.4 ± 2.0 | 8.4 ± 2.0 | 0.0 ± 0.0 |
| Control (+ methanol) | | - | 73.3 ± 2.3 | 16.4 ± 4.1 | 53.8 ± 3.4 |
| Control (+ water) | | - | 87.6 ± 20.1 | 25.8 ± 9.7 | 54.2 ± 2.8 |

328

Table 2. Effect of diagnostic CYP isoform specific chemical inhibitors on the overall metabolism of $^{14}$C-TMC125 and the formation of its metabolites 12 and 13 in human liver microsomes. The percentage inhibition of TMC125 metabolism and metabolite formation

| Diagnostic Inhibitor | CYP isoform | % Inhibition of Metabolism[1] | | |
| | | Overall[2] | M12 | M13 |
| --- | --- | --- | --- | --- |
| Furafylline (10 µM) | CYP1A2 | 1.8 | -29.7 | 14.9 |
| Coumarin (100 µM) | CYP2A6 | -32.7 | -51.4 | -17.4 |
| Sulphaphenazole (10 µM) | CYP2C8/9/10 | 0.6 | -29.7 | 6.6 |
| Quinidine (10 µM) | CYP2D6 | 4.2 | -35.1 | 12.4 |
| 4-methylpyrazole (20 µM) | CYP2E1 | 0.0 | 5.4 | -5.8 |
| Ticlopidine (5 µM) | CYP2C19/D6 | -11.7 | 15.5 | -13.1 |
| Ketoconazole (1 µM) | CYP3A4 | 90.9 | 81.1 | 100.0 |
| Troleandomycin (200 µM) | CYP3A4 | 83.0 | 59.5 | 100.0 |
| Clarithromycin (15 µM) | CYP3A | 64.8 | 48.6 | 68.6 |
| Ritonavir (0.15 µM) | CYP3A4 | 90.9 | 59.5 | 100.0 |
| 1-aminobenzotriazole (1000 µM) | CYP P450 | 88.5 | 48.6 | 100.0 |

Additional Information
1. Calculated from control incubation (without inhibitor).
2. Negative values indicates higher % product formation in test sample compared to the control. For all qualitative purposes, all negative values were considered as no inhibition.

APPEARS THIS WAY
ON ORIGINAL

Figure 1. Effect of diagnostic CYP isoform specific chemical inhibitors on the overall metabolism of $^{14}$C-TMC125 and the formation of its metabolites 12 and 13 in human liver microsomes. Each value is mean ± S.D of 3 observations
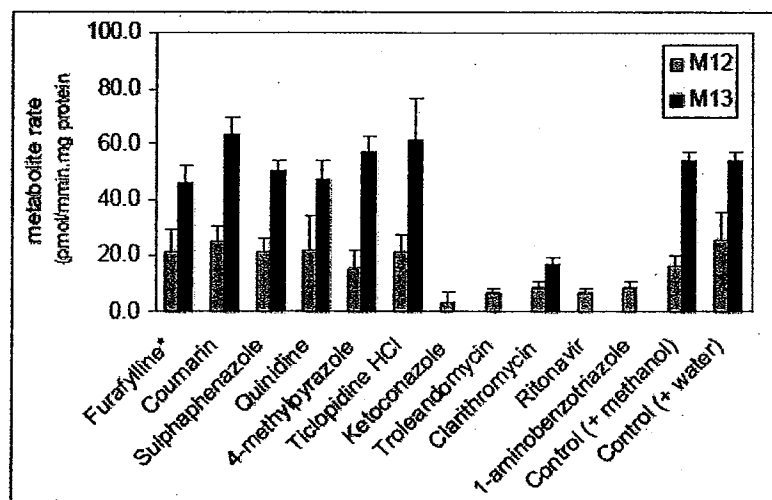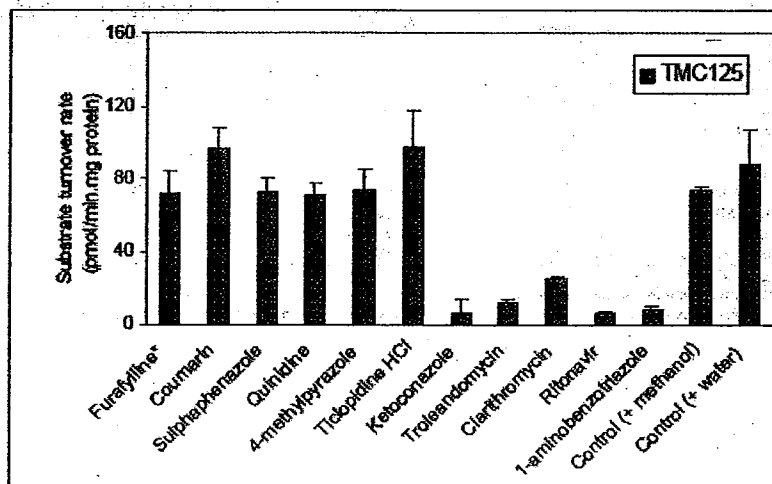
## 2. Recombinant heterologously expressed human CYP isoforms

**Table 3. CYP reaction phenotyping- Metabolism of ^{14}C-TMC125 in E. coli membranes containing heterologously expressed human CYP isoforms. Metabolites 12 and 13 were major metabolites of TMC125 observed in E. coli membranes, metabolite 8 was a minor metabolite**

| Cytochrome P-450 Form (100 pmol/ml) | Overall % Metabolism[1] | Product formation rate (pmol/min.100 pmol P450) | | |
|---|---|---|---|---|
| | | M8 | M12 | M13 |
| CYP1A2 | 0.15 ± 0.30 | -[2] | - | - |
| CYP2A6 | 0.43 ± 0.15 | - | 0.72 ± 0.06 | - |
| CYP2B6 | 0.48 ± 0.30 | - | 0.50 ± 0.10 | - |
| CYP2C8 | 0.26 ± 0.31 | - | 0.44 ± 0.12 | - |
| CYP2C9 | 13.54 ± 0.81 | - | 13.33 ± 0.62 | - |
| CYP2C19 | - | - | - | - |
| CYP2D6 | - | - | - | - |
| CYP2E1 | 0.04 ± 0.15 | - | - | - |
| CYP3A4 | 32.15 ± 0.85 | 0.28 ± 0.15 | 8.94 ± 0.15 | 14.50 ± 0.57 |
| CYP3A5 | 0.54 ± 0.31 | - | 0.28 ± 0.15 | 0.44 ± 0.25 |

Additional Information
1. Overall % metabolism of TMC125 calculated from % drug that remained in the sample at the end of the incubation
2. No measurable product observed in radio-HPLC profile (LLOQ= 200 dpm)

**Table 4. CYP reaction phenotyping- Metabolism of ^{14}C-TMC125 in Supersomes® (heterologously expressed human CYP isoforms). Metabolites 8, 12 and 13 were the major metabolites of TMC125 observed in these systems**

| Cytochrome P-450 Form (100 pmol/ml) | Overall % Metabolism[1] | Product formation rate (pmol/min.100 pmol P450) | | |
|---|---|---|---|---|
| | | M8 | M12 | M13 |
| CYP1A2 | - | -[2] | - | - |
| CYP2A6 | - | - | - | - |
| CYP2B6 | 3.50 ± 0.21 | - | 3.39 ± 0.25 | - |
| CYP2C8 | 0.61 ± 0.10 | - | 0.67 ± 0.0 | - |
| CYP2C9 | 3.44 ± 0.62 | - | 3.61 ± 0.59 | - |
| CYP2C19 | 94.44 ± 9.79 | 76.89 ± 7.13 | 7.50 ± 0.26 | - |
| CYP2D6 | 0.17 ± 0.06 | - | - | - |
| CYP3E1 | - | - | - | - |
| CYP3A4 | 13.61 ± 3.04 | - | 3.89 ± 0.85 | 8.17 ± 0.64 |
| CYP3A5 | 7.94 ± 0.40 | - | 2.83 ± 0.20 | 3.94 ± 0.65 |
| CYP3A7 | 2.50 ± 0.32 | - | 1.67 ± 0.20 | 0.67 ± 0.21 |

Additional Information
1. Overall % metabolism of TMC125 calculated from % drug remained in the sample at the end of the incubation
2. No measurable product observed in radio-HPLC profile (LLOQ= 200 dpm)

APPEARS THIS WAY
ON ORIGINAL

# 3. Correlation of CYP isoenzyme-specific activities of the microsomes using a panel of 10 different individual batches of HLMs

Table 5. CYP reaction phenotyping by correlation analysis - Pair-wise correlation analysis of $^{14}$C-TMC125 metabolism and formation of metabolites 12 and 13 with various CYP isoform dependent enzyme activities in 10 individual batches of human liver microsomes.

| Enzyme activities (CYP isoform) | Overall TMC125 metabolism Correlation (r²) | TMC125 Metabolite Correlation coefficient (r²) | |
|---|---|---|---|
| | | M12 | M13 |
| 7-ethoxyresorufine O-deethylase (1A2) | 0.232 | 0.394 | -0.127 |
| Phenacetin O-deethylase (1A2) | -0.086 | 0.145 | -0.194 |
| Coumarin 7-hydroxylase (2A6) | -0.059 | -0.348 | -0.216 |
| Taxol 6-α-hydroxylase (2C8) | -0.457 | 0.044 | -0.249 |
| Tolbutamide methyl hydroxylase (2C9,10) | -0.598 | -0.147 | -0.580 |
| S-mephenytoin 4-hydroxylase (2C19) | **0.578** | 0.221 | **0.735** |
| Dextromethorphan O-demethylase (2D6) | -0.301 | 0.108 | -0.130 |
| Bufuralol hydroxylase (2D6) | -0.356 | 0.061 | -0.140 |
| Chlorozoxazone 6-hydroxylase (2E1) | -0.458 | -0.059 | 0.106 |
| Lauric acid (ω-1)-hydroxylase (2E1) | -0.673 | -0.211 | -0.431 |
| Testosterone 6-β-hydroxylase (3A4) | **0.481** | **0.491** | **0.887** |
| Cyclosporine oxidase (3A) | 0.285 | 0.124 | **0.854** |
| Taxol 3'-hydroxylase (3A4) | **0.599** | 0.349 | **0.813** |
| Midazolam 4-hydroxylase (3A4/A5) | **0.538** | **0.458** | **0.939** |
| Midazolam 1'-hydroxylase (3A5/A4) | 0.274 | 0.193 | **0.820** |
| Lauric acid ω-hydroxylase (4A) | -0.269 | 0.198 | 0.108 |

**Additional Information**

1. Bolded numbers: Positive correlations higher than 0.400

## Conclusions:

Overall TMC125 metabolism as well as formation of its major metabolites 12 and 13 was mainly catalyzed by CYP3A enzymes. Involvement of CYP2C isoforms in TMC 125 metabolism was also suggested.

APPEARS THIS WAY
ON ORIGINAL

**Methods:**

Incubations were performed with CYP450 probe substrates, selective towards human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4, in the absence and presence of TMC125.

### CYP450 Probe Substrates and Their Metabolites

| Cytochrome P450 enzyme | Substrate | Metabolite |
|---|---|---|
| CYP1A2 | 7-ethoxyresorufin | resorufin |
| CYP2A6 | coumarin | 7-hydroxycoumarin |
| CYP2B6 | 7-ethoxy-4-trifluoro-methyl coumarin | 7-hydroxy-4-trifluoro-methyl coumarin |
| CYP2C9 | diclofenac | 4'-hydroxydiclofenac |
| CYP2C19 | S-mephenytoin | 4'-hydroxy-mephenytoin |
| CYP2D6 | bufuralol | 1'-hydroxybufuralol |
| CYP2E1 | chlorzoxazone | 6-hydroxy-chlorzoxazone |
| CYP3A | testosterone | 6β-hydroxy-testosterone |

The Km value for each CYP marker substrate was listed below.

| CYP1A2 | CYP2A6 | CYP2B6 | CYP2C9 | CYP2C19 | CYP2D6 | CYP2E1 | CYP3A |
|---|---|---|---|---|---|---|---|
| 0.5 μM | 5 μM | 5 μM | 10 μM | 20 μM | 10 μM | 100 μM | 100 μM |

The probe substrates were incubated at concentrations around the Km values in the absence and presence of the following concentrations of TMC125: 1.15, 2.3, 11.5 and 50 μM in pooled human liver microsomes.

333

Results:

Table 1. Evaluation of TMC125 an Inhibitor of CYP Activities in Pooled Human Liver Microsomes

| P450 enzyme | Selected TMC125 concentration (μM) | Type of inhibition | Inhibition constant Ki (μM) |
|---|---|---|---|
| CYP1A2 | 11.5 | competitive | 7.0 ± 1.1 |
| CYP2B6 | 50 | non-competitive | 83 ± 13 |
| CYP2C9 | 1.15 | competitive | 0.58 ± 0.09 |
| CYP2C19 | 11.5 | non-competitive | 22 ± 4 |
| CYP2D6 | 50 | competitive | 15 ± 1 |
| CYP3A | 11.5 | competitive | 6.7 ± 1.1 |

Conclusions:

Considering the Ki values and the average plasma level of TMC125 in vivo (0.451 μg/mL or ~1 μM), TMC125 is likely an inhibitor of CYP2C9, a possible inhibitor of CYP1A2 and CYP3A but TMC125 is unlikely an inhibitor of other CYP enzymes tested in this study.

APPEARS THIS WAY
ON ORIGINAL

An *in-vitro* study on the inhibition of CYP2C8 mediated paclitaxel 6-α-hydroxylase activity by TMC125 (TMC125- NC360)

Methods:
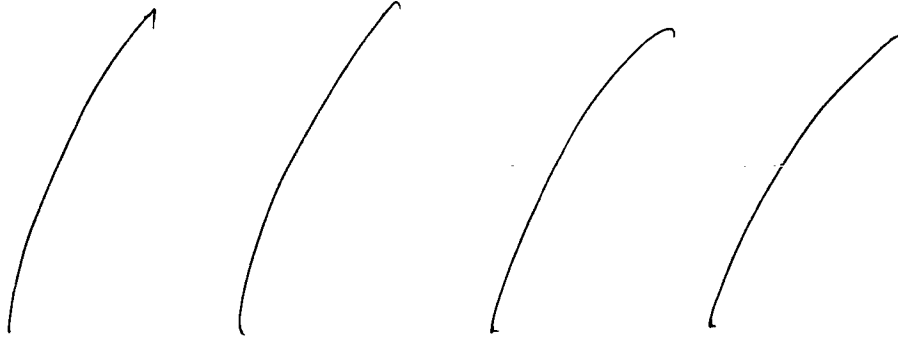
Results:

Figure 1. Inhibition of paclitaxel 6-α-hydroxylation by TMC125



Michaelis-Menten
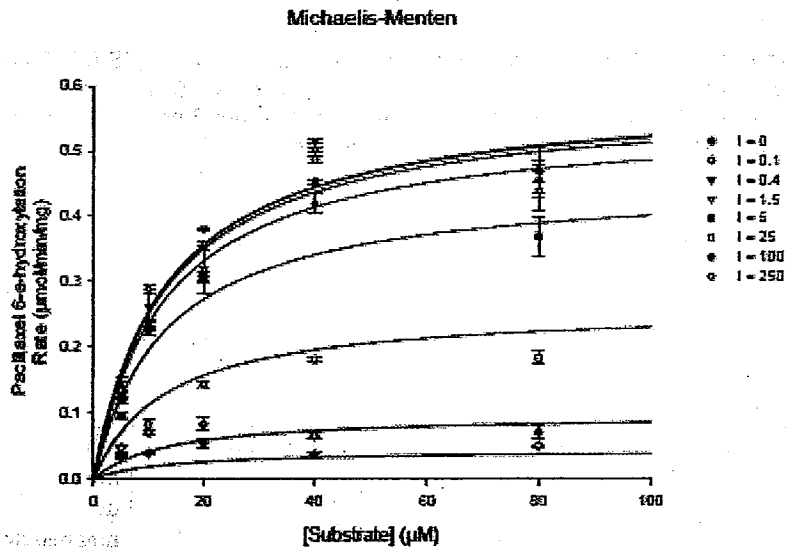
Conclusion:

The apparent inhibition constant Ki for the inhibition of the CYP2C8 mediated paclitaxel 6-α-hydroxylation by TMC125, calculated from the noncompetitive inhibition model, amounted to 19.6 ± 2.0 µM. Taking into account a Cmax-value of 0.45 µg/ml (~1.03 µM) for TMC125 in human plasma, inhibition of CYP2C8 by TMC125 is clinically rather unlikely.

The CYP2C8 selective inhibitor montelukast proved to be strongly inhibitory in the paclitaxel 6-α-hydroxylation assay with an IC50-value of 0.68 ± 0.14 µM.

335

An in vitro study to assess the potential of TMC125 to induce CYP enzyme activities in cryopreserved human hepatocytes (TMC125-NC238)

Methods:
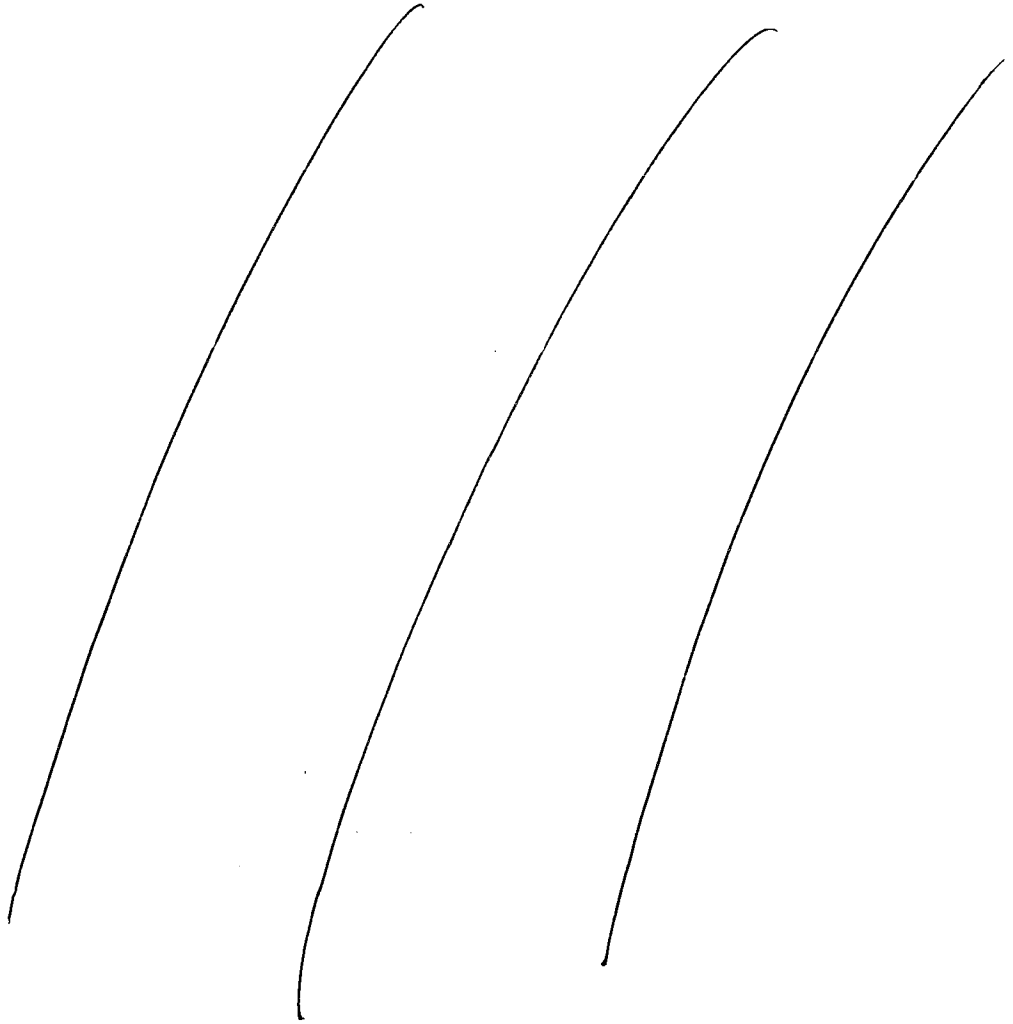
Results:

Table 1. The potential of TMC125 to induce CYP enzyme activities in cryopreserved human hepatocytes

| Test Condition | Mean fold induction | | | |
|---|---|---|---|---|
| | CYP1A2 | CYP2B6 | CYP2C19 | CYP3A4 |
| Control (Vehicle) | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 | 1.00 ± 0.00 |
| TMC125 (1.0 µM) | 0.86 ± 0.06 | 1.13 ± 0.50 | 0.33 ± 0.25 | 5.48 ± 1.39 |
| TMC125 (25 µM) | 1.09 ± 0.07 | 0.99 ± 0.35 | 0.29 ± 0.31 | 2.42 ± 0.62 |
| Rifampicin (50 µM) | 1.09 ± 0.17 | 3.01 ± 0.52 | 4.39 ± 0.80 | 10.62 ± 4.55 |
| Omeprazole (25 µM) | 3.95 ± 2.45 | 1.45 ± 0.41 | 0.56 ± 0.23 | 3.44 ± 1.22 |
| Rifampicin (50 µM) + TMC125 (25 µM) | 1.41 ± 0.47 | 1.92 ± 0.81 | 0.29 ± 0.30 | 2.26 ± 0.81 |

APPEARS THIS WAY
ON ORIGINAL

Figure 1. Induction profile of CYP1A2 in cryopreserved human hepatocytes.
The fold induction of CYP1A2 in different batches of human hepatocytes (Top). Mean fold induction of CYP1A2 activity, calculated as the mean + SD from the three different lots of cryopreserved human hepatocytes (Bottom).



Fold induction of CYP1A2 with TMC125 in different batches of hepatocytes (Ethoxyresorufin O-deethylase)

082    BDF    NLR



Induction profile of CYP1A2 with TMC125 and positive control (Ethoxyresorufin O-deethylase)
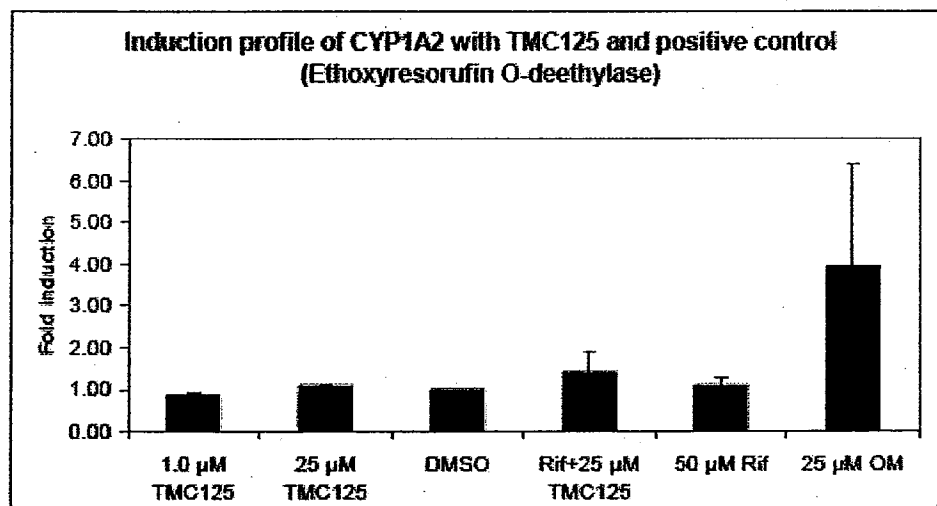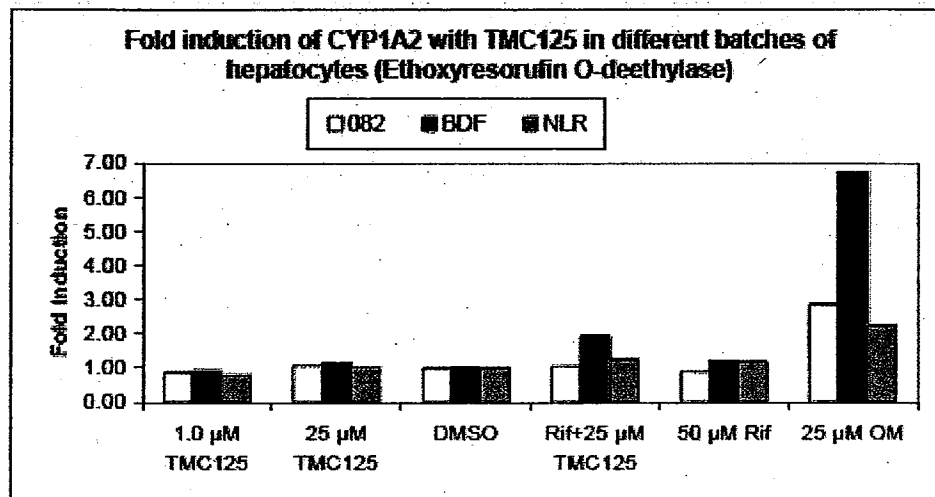
Figure 2. Induction profile of CYP2B6 in cryopreserved human hepatocytes.
The fold induction of CYP2B6 in different batches of human hepatocytes (Top). Mean fold induction of
CYP2B6 activity, calculated as the mean + SD from the three different lots of cryopreserved human
hepatocytes (Bottom).
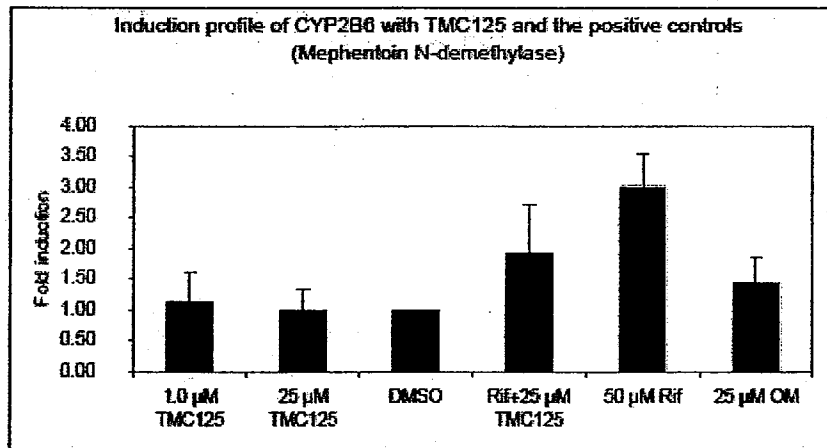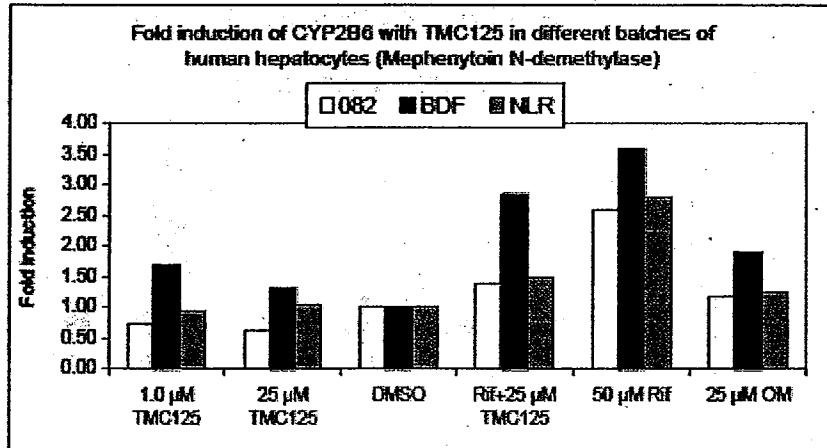
Figure 3. Induction profile of CYP2C19 in cryopreserved human hepatocytes.
The fold induction of CYP2C19 in different batches of human hepatocytes (Top). Mean fold induction of
CYP2C19 activity, calculated as the mean + SD from the three different lots of cryopreserved human
hepatocytes (Bottom).



Fold induction of CYP2C19 with TMC125 in different batches of human hepatocytes ( Mephenytoin 4-hydroxylase)



Induction profile of CYP2C19 with TMC125 and the positive controls ( Mephenytoin 4-hydroxylase)

340
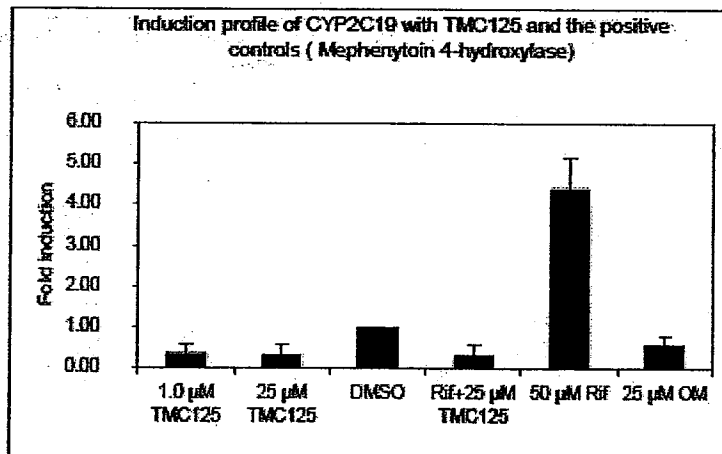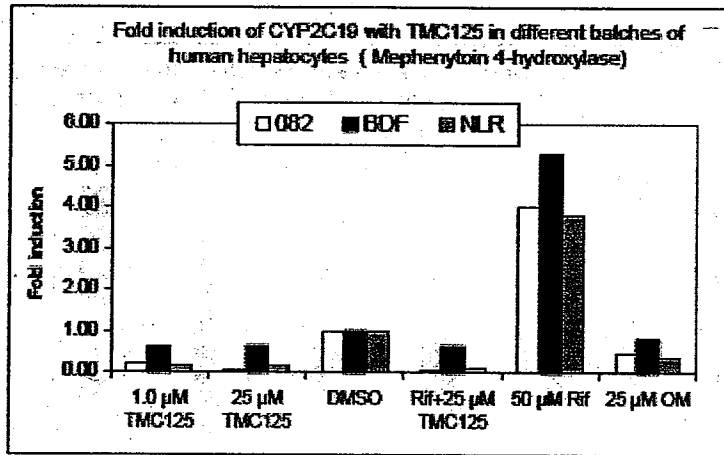
The fold induction of CYP3A4 in different batches of human hepatocytes (Top). Mean fold induction of CYP3A4activity, calculated as the mean + SD from the three different lots of cryopreserved human hepatocytes (Bottom).
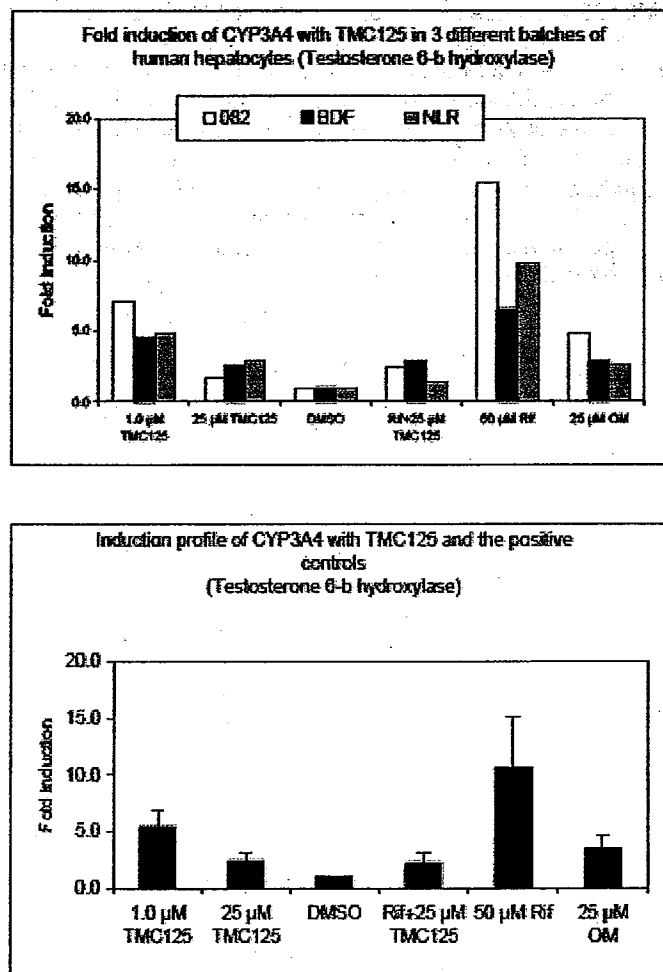


**Conclusions:**

The results showed that TMC125 has no inducing effect on CYP1A2 or CYP2B6 or CYP2C19 activities in human hepatocytes, but the compound appears to be an inducer of CYP3A4.

<u>In vitro study on the potential of TMC125 to induce CYP mRNA in cryopreserved human hepatocytes (TMC125 NC164)</u>

Methods:

The potential of TMC125 to induce cytochromes P450 (CYPs) was determined in primary hepatocyte cultures established on ——— well plates from cryopreserved human hepatocytes originating from 3 different donors that retained acceptable attachment characteristics. After the establishment of the hepatocyte cultures, human hepatocytes were treated for two consecutive days either with vehicle, with various concentrations of TMC125 (1, 5, 10 and 25 $\mu$M) or with the positive control compounds omeprazole (25 $\mu$M), rifampicin (25 $\mu$M), phenobarbital (100 $\mu$M), or efavirenz (5 $\mu$M). Induction of CYP enzymes was assessed at the end of the 48 h treatment period, by measurement of the mRNA expression with ——— quantitative RT-PCR. In addition, possible cytotoxicity due to the treatment of the cells was determined by measurement of the intracellular ATP content.

Results:

## Figure 1. Induction of CYP1A2 mRNA expression

**A**



**B**
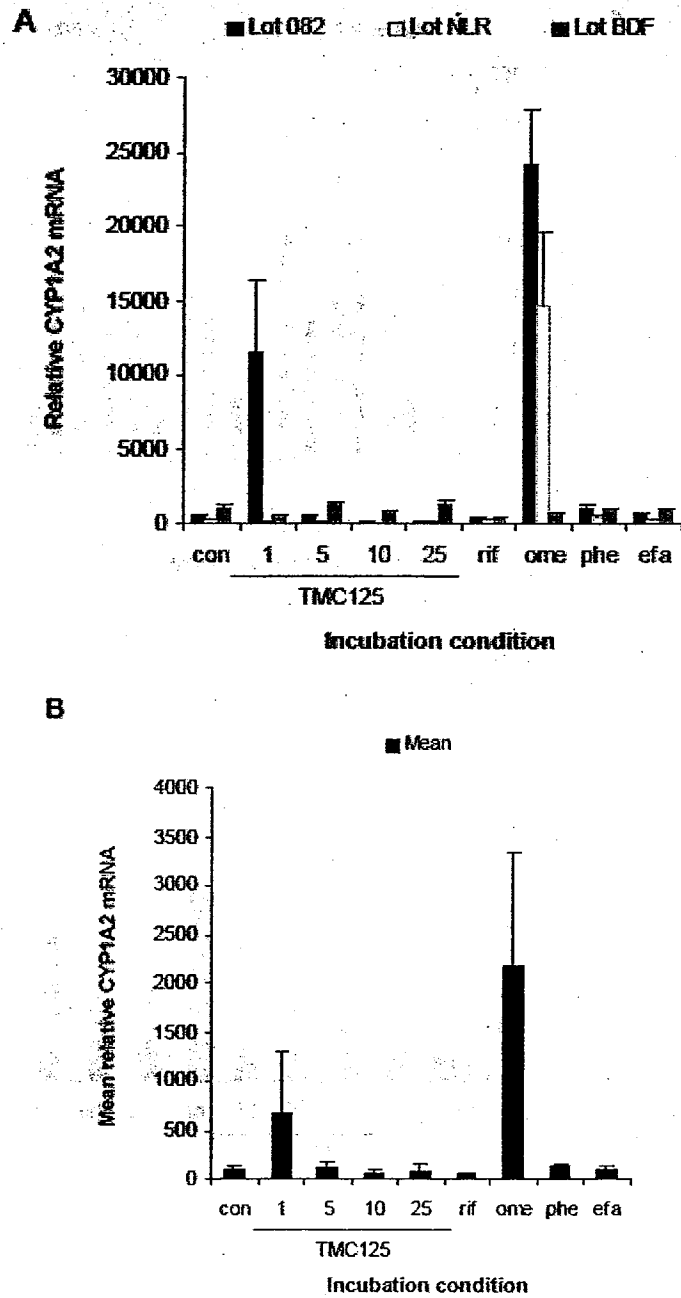


(A) CYP1A2 mRNA expression in cells from lot 082 (black bars), lot NLR (white bars), and lot BDF (grey bars) treated with vehicle (con), 1, 5, 10, or 25 µM TMC125, 25 µM rifampicin (rif), 25 µM omeprazole (ome), 100 µM phenobarbital (phe), or 5 µM efavirenz (efa). Values were calculated as the mean of three independent measurements and the error bars represent the SEM. (B) CYP1A2 mRNA expression in control and treated cells, calculated as the mean + SEM from the three different lots of cryopreserved human hepatocytes. * p-value < 0.05, n = 3.

343

# Figure 2. Induction of CYP2B6 mRNA expression
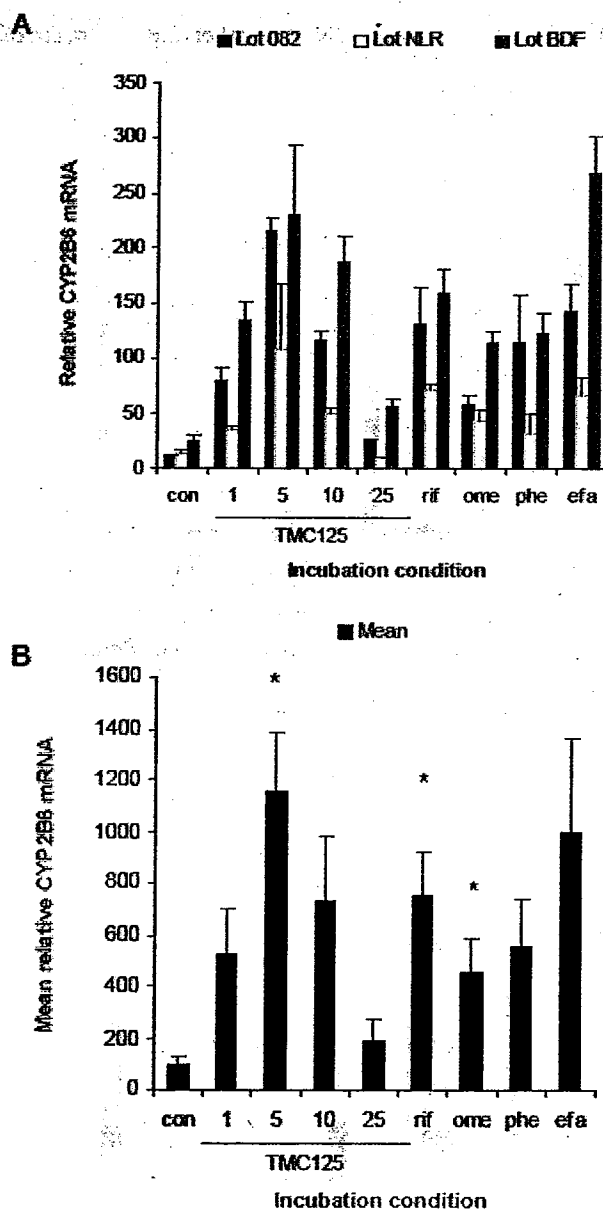


(A) CYP2B6 mRNA expression in cells from lot 082 (black bars), lot NLR (white bars), and lot BDF (grey bars) treated with vehicle (con), 1, 5, 10, or 25 µM TMC125, 25 µM rifampicin (rif), 25 µM omeprazole (ome), 100 µM phenobarbital (phe), or 5 µM efavirenz (efa). Values were calculated as the mean of three independent measurements and the error bars represent the SEM. (B) CYP2B6 mRNA expression in control and treated cells, calculated as the mean + SEM from the three different lots of cryopreserved human hepatocytes. * p-value < 0.05, n = 3.

## Figure 3. Induction of CYP2C8 mRNA expression

**A**
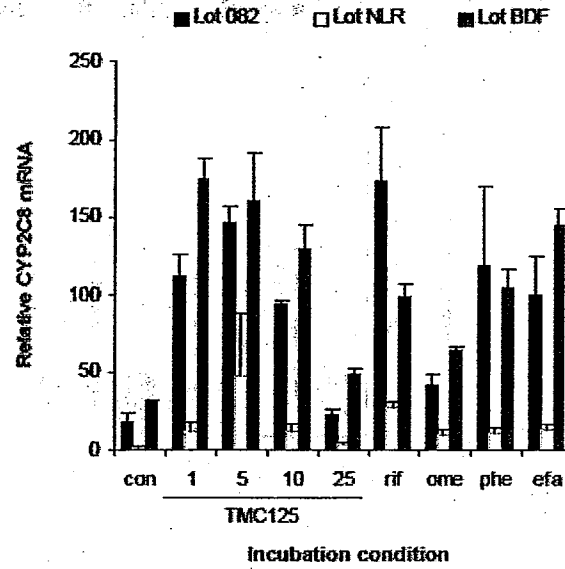


**B**
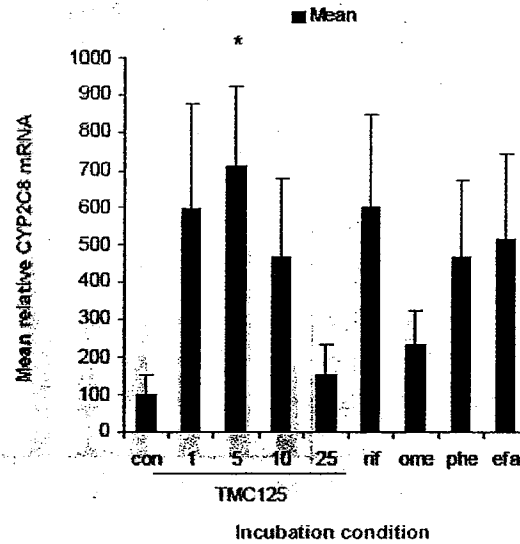


(A) CYP2C8 mRNA expression in cells from lot 082 (black bars), lot NLR (white bars), and lot BDF (grey bars) treated with vehicle (con), 1, 5, 10, or 25 µM TMC125, 25 µM rifampicin (rif), 25 µM omeprazole (ome), 100 µM phenobarbital (phe), or 5 µM efavirenz (efa). Values were calculated as the mean of three independent measurements and the error bars represent the SEM. (B) CYP2C8 mRNA expression in control and treated cells, calculated as the mean + SEM from the three different lots of cryopreserved human hepatocytes. * p-value < 0.05, n = 3.

# Figure 4. Induction of CYP2C9 mRNA expression

## A
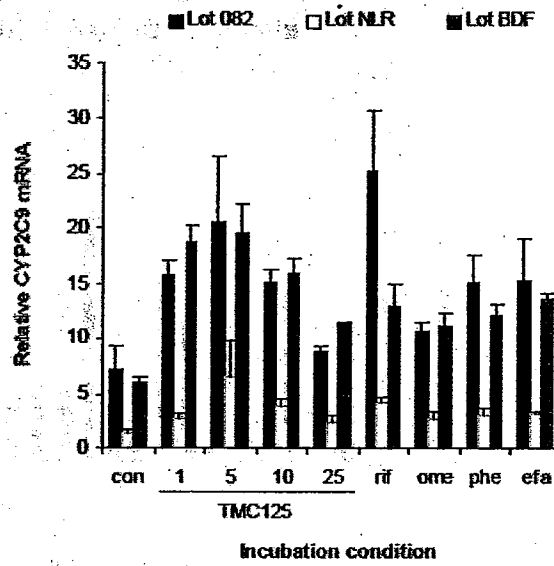


## B
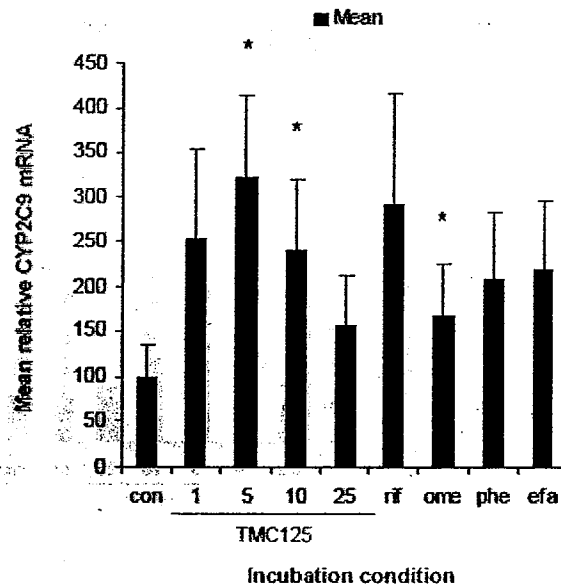


(A) CYP2C9 mRNA expression in cells from lot 082 (black bars), lot NLR (white bars), and lot BDF (grey bars) treated with vehicle (con), 1, 5, 10, or 25 µM TMC125, 25 µM rifampicin (rif), 25 µM omeprazole (ome), 100 µM phenobarbital (phe), or 5 µM efavirenz (efa). Values were calculated as the mean of three independent measurements and the error bars represent the SEM. (B) CYP2C9 mRNA expression in control and treated cells, calculate as the mean + SEM from the three different lots of cryopreserved human hepatocytes. * p-value < 0.05, n = 3.

## Figure 5. Induction of CYP2C18 mRNA expression

**A**



**B**
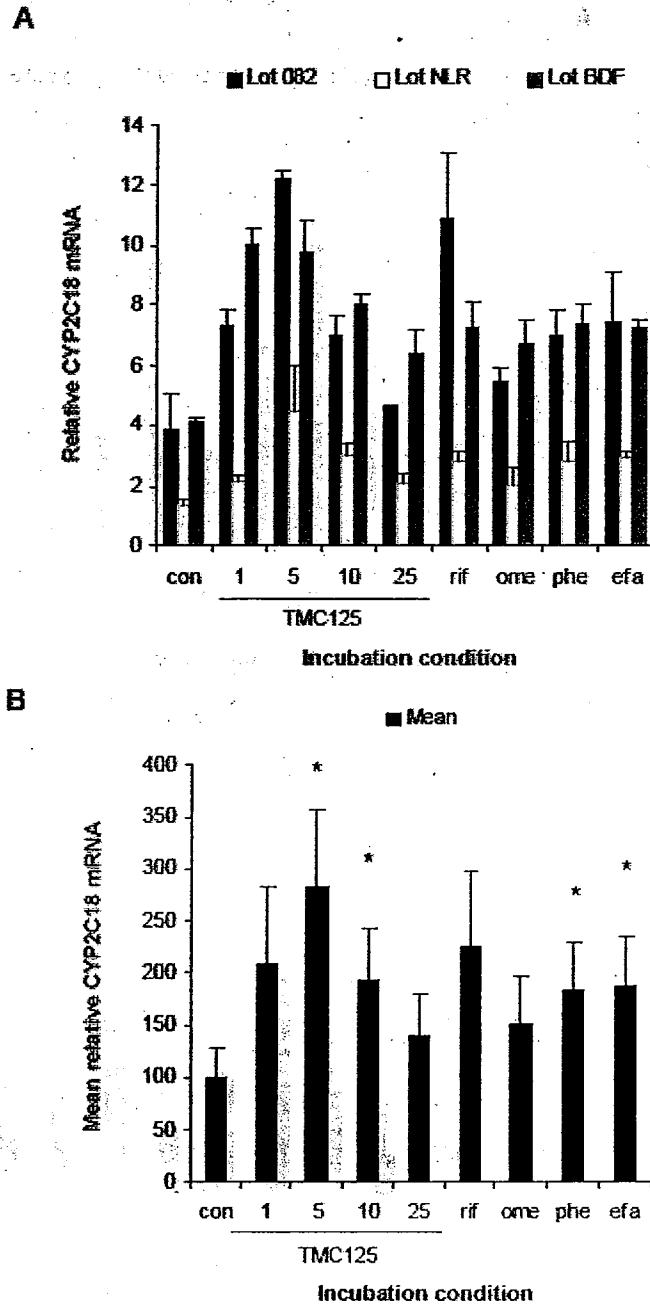


(A) CYP2C18 mRNA expression in cells from lot 082 (black bars), lot NLR (white bars), and lot BDF (grey bars) treated with vehicle (con), 1, 5, 10, or 25 µM TMC125, 25 µM rifampicin (rif), 25 µM omeprazole (ome), 100 µM phenobarbital (phe), or 5 µM efavirenz (efa). Values were calculated as the mean of three independent measurements and the error bars represent the SEM. (B) CYP2C18 mRNA expression in control and treated cells, calculated as the mean + SEM from the three different lots of cryopreserved human hepatocytes. * p-value < 0.05, n = 3.

**Figure 6. Induction of CYP2C19 mRNA expression**

**A**
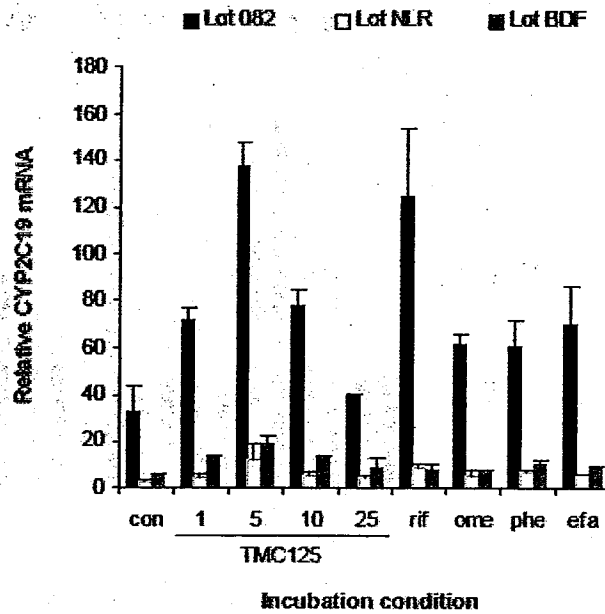
**B**
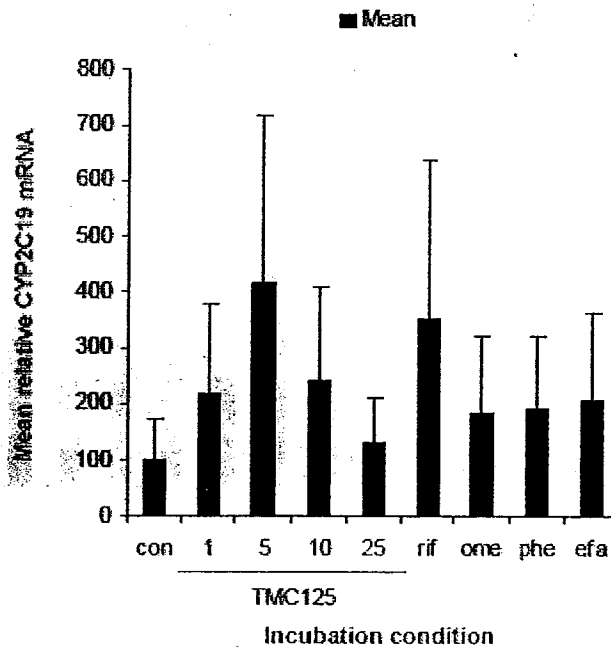
(A) CYP2C19 mRNA expression in cells from lot 082 (black bars), lot NLR (white bars), and lot BDF (grey bars) treated with vehicle (con), 1, 5, 10, or 25 µM TMC125, 25 µM rifampicin (rif), 25 µM omeprazole (ome), 100 µM phenobarbital (phe), or 5 µM efavirenz (efa). Values were calculated as the mean of three independent measurements and the error bars represent the SEM. (B) CYP2C19 mRNA expression in control and treated cells calculated as the mean + SEM from the three different lots of cryopreserved human hepatocytes. * p-value < 0.05, n = 3.

348

# Figure 7. Induction of CYP3A4 mRNA expression

**A**



**B**
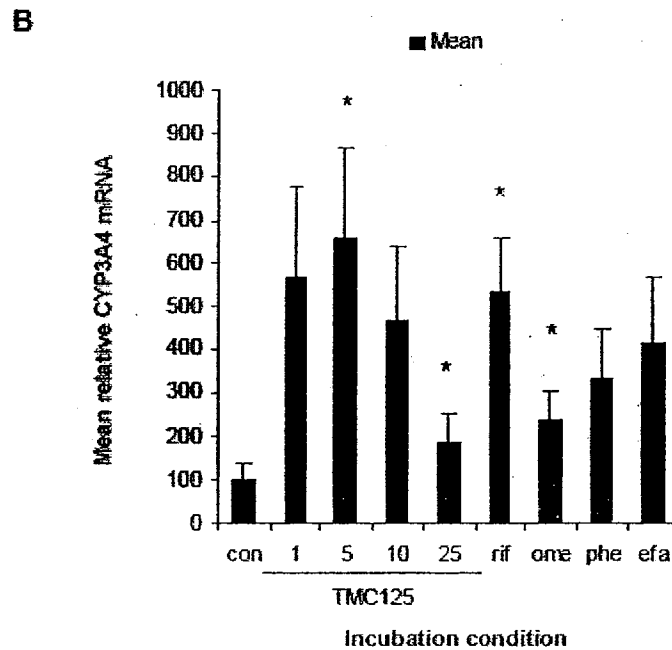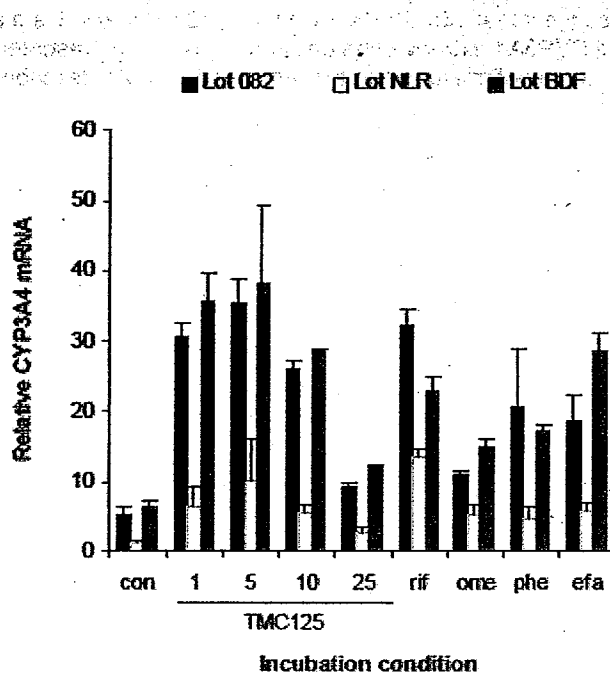


(A) CYP3A4 mRNA expression in cells from lot 082 (black bars), lot NLR (white bars), and lot BDF (grey bars) treated with vehicle (con), 1, 5, 10, or 25 μM TMC125, 25 μM rifampicin (rif), 25 μM omeprazole (ome), 100 μM phenobarbital (phe), or 5μM efavirenz (efa). Values were calculated as the mean of three independent measurements and the error bars represent the SEM. (B) CYP3A4 mRNA expression in control and treated cells, calculated as the mean + SEM from the three different lots of cryopreserved human hepatocytes. * p-value < 0.05, n = 3.

**Conclusions:**

The study results suggest that TMC125 is not a CYP1A2 inducer but is a potent inducer of CYP2B6, CYP2C-family, and CYP3A4. However the enzyme activity data presented in Study Report TMC125-NC238 should be more confirmatory than the results with mRNA described in this study.

## Determination of the in vitro transport characteristics of TMC125, evaluation of the possible role of P-glycoprotein in TMC125 transport and assessment of possible inhibition of P-glycoprotein activity by TMC125: a study in Caco-2 monolayers (TMC125-NC183)

**Methods:**

*Transepithelial transport of TMC125 across cell monola layer.*
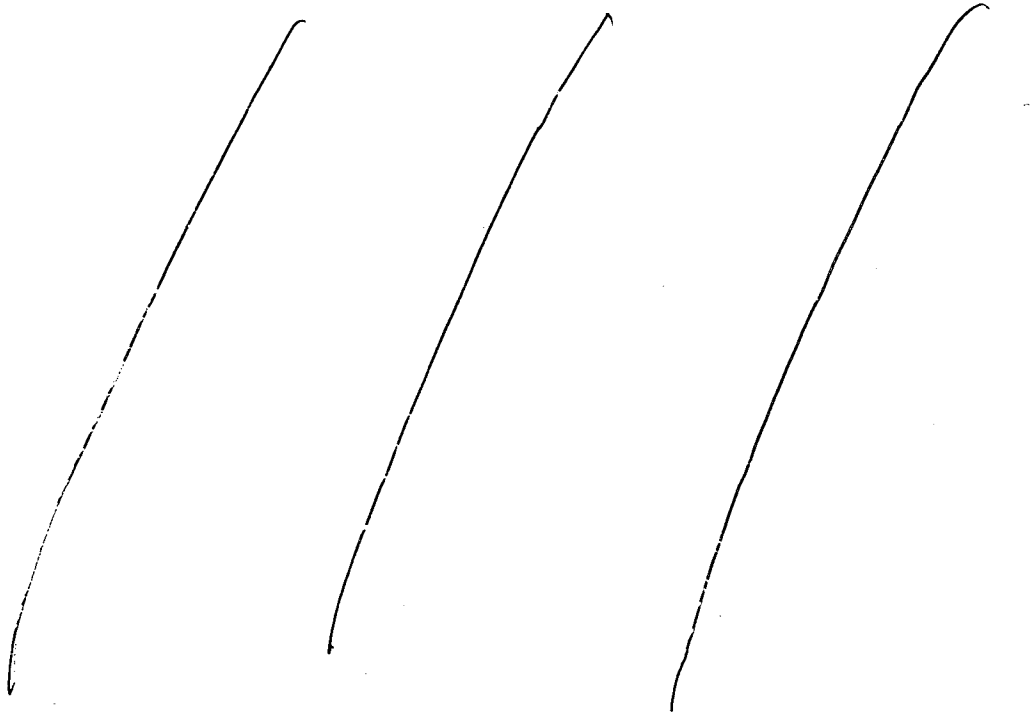
*Effect of TMC125 on bi-directional transport of the Pglycoprotein substrate taxol across Caco-2 monolayers*
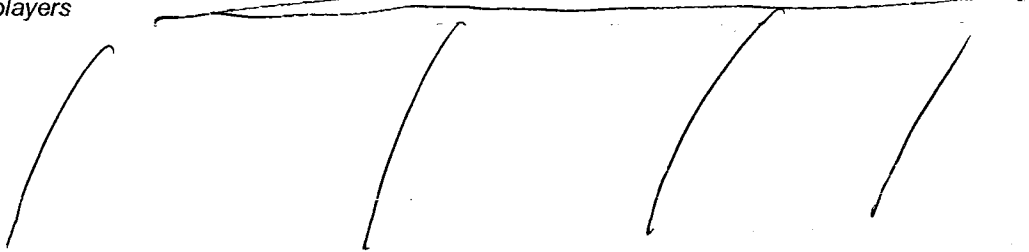
Results:

## 1. Bi-directional transport characteristics of TMC125 across Caco-2 monolayers

Figure 1. Time courses for average (± sd; n=4) absorptive (APBL; open symbols) and secretory (BL-AP; closed symbols) transport of TMC125 across Caco-2 monolayers at nominal initial concentrations of 3, 10 and 30 µM. Note that the AP incubation medium consisted of HBSS at pH 6.5 with 2.5 mM MES and the BL incubation medium of HBSS at pH 7.4

**100 µM TMC125**

**300 µM TMC125**
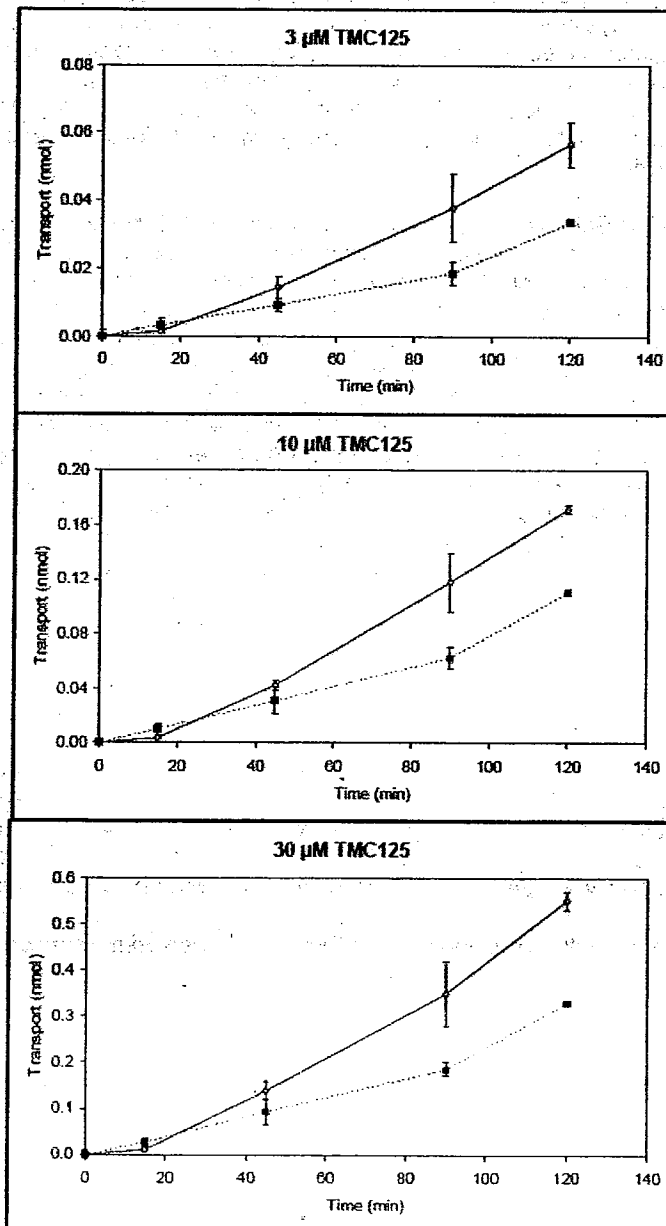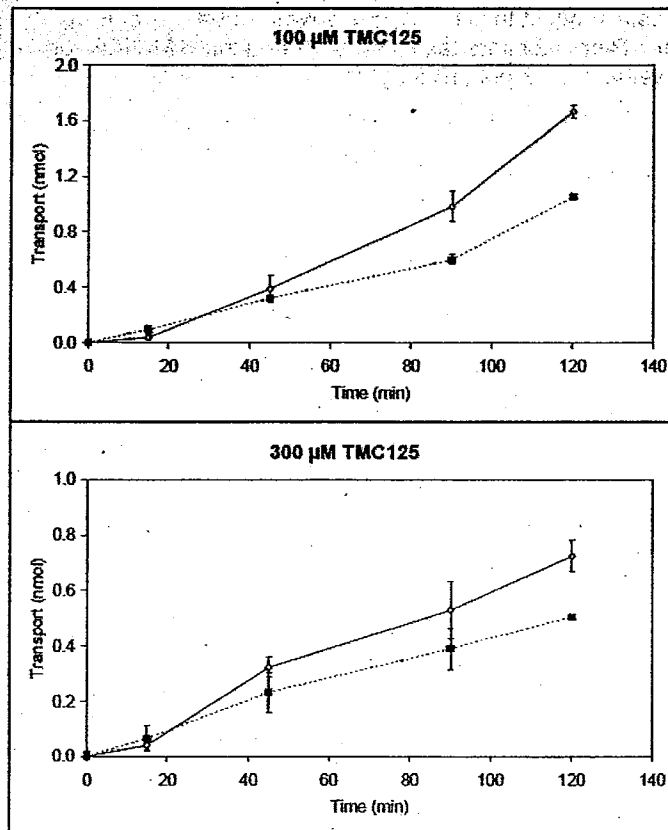
2. Effect of TMC125 on bi-directional transport of the Pglycoprotein substrate taxol across Caco-2 monolayer

Figure 2. Effect of various TMC125 concentrations (1-100 µM) on absorptive (AP-BL) and secretory (BL-AP) transport of the P-glycoprotein substrate ^{3}H-taxol (75 nM) across Caco-2 monolayers. Bars represent average (± sd; n=3) ^{3}H-taxol permeability coefficients obtained following 120-min incubation periods. The effect of the P-glycoprotein inhibitor verapamil (100 µM) was also measured as positive control treatment
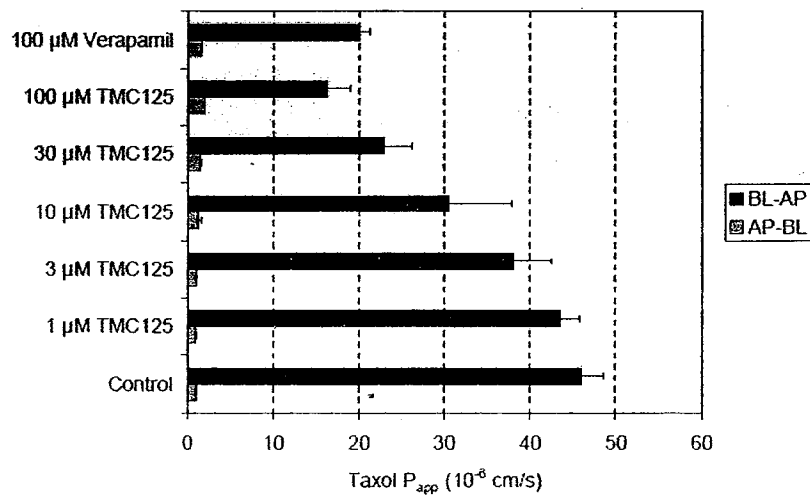
Conclusions: The data suggest that TMC125 is a weak substrate of P-gp . Bi-directional transport experiments with the P-gp substrate taxol demonstrated that TMC125 has P-gp inhibitory properties with an apparent IC50 value of 24.2 μM (10.5 μg/mL).

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

## Plasma Protein Binding and Blood-to-Plasma Partitioning (TMC125-NC143)

### Methods:

The plasma protein binding of TMC125 was studied by equilibrium dialysis of plasma samples from healthy male adult subjects, male beagle dogs, male and female SPF Sprague-Dawley rats, male and female Swiss CD-1 mice and female SPF New Zealand White rabbits after fortification with $^3$H-labelled TMC125. In the above species, the distribution of TMC125 to various compartments of blood was also studied. The binding of TMC125 to purified human serum albumin and α1-acid glycoprotein was also investigated by equilibrium dialysis.
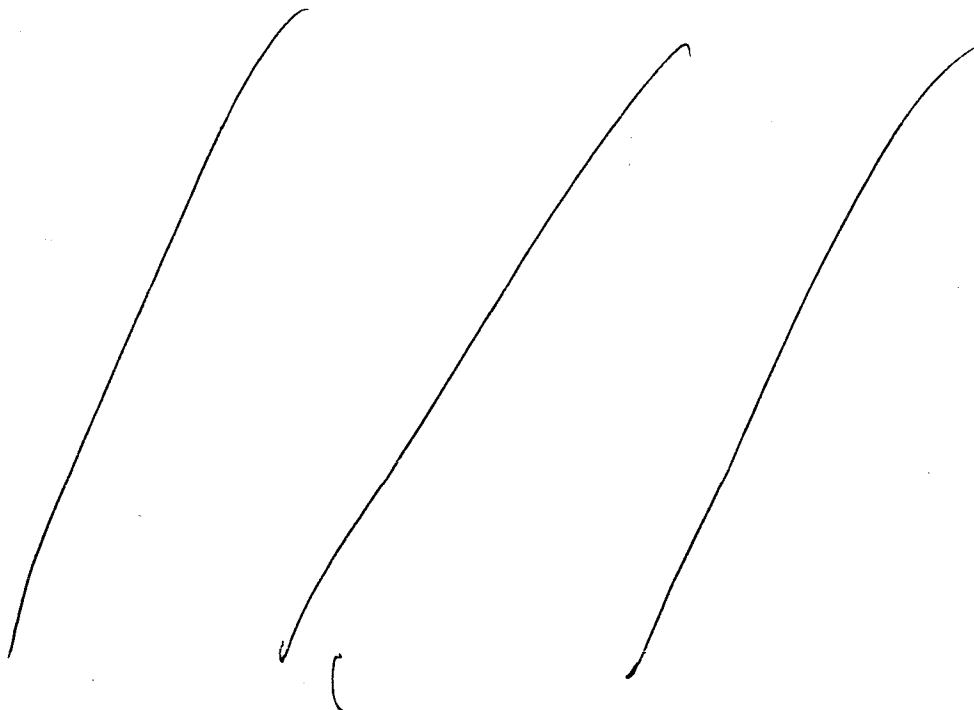
Results:

Table 1. Protein binding of TMC125 in plasma of mouse, rat, rabbit, dog, and man. Each value represents the mean ± SD of three determinations.

| Species | Test Concentrations ( ng (base eq.)/ml) | | | | Grand Mean ± S.D. (% bound) |
|---|---|---|---|---|---|
| | 10 | 100 | 1000 | 5000 | |
| | % protein bound (Mean ± S.D) | | | | |
| Mouse (m) | 99.92 ± 0.01 | 99.92 ± 0.01 | 99.93 ± 0.01 | 99.92 ± 0.01 | 99.92 ± 0.01 |
| Mouse (f) | 99.91 ± 0.01 | 99.92 ± 0.01 | 99.92 ± 0.01 | 99.91 ± 0.01 | 99.92 ± 0.01 |
| Rat (m) | 99.84 ± 0.01 | 99.83 ± 0.01 | 99.83 ± 0.01 | 99.84 ± 0.01 | 99.84 ± 0.01 |
| Rat (f) | 99.86 ± 0.00 | 99.85 ± 0.01 | 99.86 ± 0.01 | 99.85 ± 0.01 | 99.86 ± 0.01 |
| Rabbit (f) | 99.86 ± 0.02 | 99.86 ± 0.01 | 99.86 ± 0.01 | 99.86 ± 0.01 | 99.86 ± 0.00 |
| Dog (m) | 99.89 ± 0.01 | 99.90 ± 0.01 | 99.89 ± 0.01 | 99.90 ± 0.01 | 99.90 ± 0.01 |
| Man | 99.91 ± 0.01 | 99.90 ± 0.01 | 99.90 ± 0.01 | 99.90 ± 0.01 | 99.90 ± 0.01 |

(m): male; (f) : female

Table 2. The binding of TMC125 (100 ng (base-eq.)/ml) to purified protein solutions of human serum albumin and α1-acid glycoprotein. Each value represents the mean of two determinations.

| Human serum albumin (g/100 ml) | Percentage bound (%) | Percentage unbound (%) |
|---|---|---|
| 6.0 | 99.69 | 0.31 |
| 4.3 | 99.60 | 0.40 |
| 2.0 | 99.20 | 0.80 |
| 1.0 | 98.33 | 1.67 |
| 0.5 | 97.06 | 2.94 |
| 0.25 | 94.46 | 5.54 |
| 0.1 | 87.92 | 12.08 |

| $\alpha_1$-acid-glycoprotein (g/100 ml) | Percentage bound (%) | Percentage unbound (%) |
|---|---|---|
| 0.20 | 99.02 | 0.98 |
| 0.15 | 98.58 | 1.42 |
| 0.10 | 97.66 | 2.34 |
| 0.05 | 94.48 | 5.52 |
| 0.02 | 69.22 | 30.78 |

356

Figure 1. Blood-to-plasma concentration ratio (Cb/C) of TMC125 at 100 and 1000 ng (base-eq.)/ml
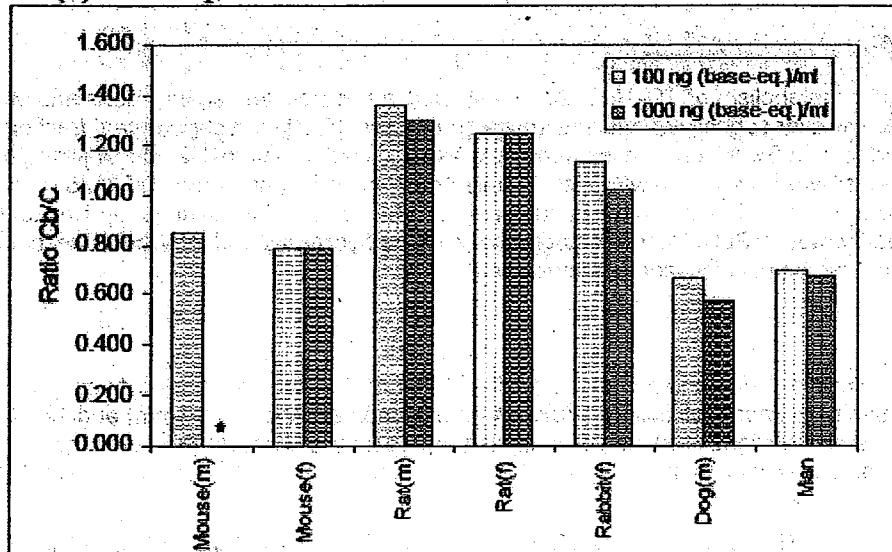
(m): male; (f) : female

Conclusions:

TMC125 was extensively bound to plasma proteins and the plasma protein binding was concentration independent and similar across all species.

APPEARS THIS WAY
ON ORIGINAL

**Methods:**

The *in vitro* metabolism of $^{14}$C-TMC125 was studied in hepatocytes (suspensions and primary cultures) and liver subcellular fractions (microsomes and 12,000 x g supernatant fractions) of male and female Swiss albino mice, male and female black agouti rasH2 microinjected mice, male and female Sprague-Dawley rats, female rabbit, male dog and man. TMC125 (5 µM, 2.18 µg/ml) was incubated in the above matrices at 37°C for various time periods, and incubates were analysed by radio-HPLC. Cochromatography, enzyme hydrolysis and LC-MS/MS techniques were used for the identification of metabolites.

**Results:**

Table 1. Mass balance and metabolite profile of TMC125 in human hepatocyte suspensions (SK, 120 min), primary cell culture (PCK, 24 h), microsomes (MICR, 120 min) and 12,000 x g supernatant fractions (120 min). The figures represent the percentage of the injected sample radioactivity accounted for by TMC125 (unchanged drug, UD) and its metabolites.

| Metabolite | Hepatocytes (Donor 1) | | Hepatocytes (Donor 2) | | Hepatocytes (Donor 3) | | Hepatocytes (Donor 4) | | Hepatocytes (Mean of Donors 1-4)[a] | | Liver subcellular fractions | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SK | PCK | SK | PCK | SK | PCK | SK | PCK | SK | PCK | 12,000 g | MICR |
| 1 | | | | | | | | | 0.4 | 19.8 | ND | ND |
| 4 | | | | | | | | | ND | 0.2 | ND | ND |
| 5 | | | | | | | | | ND | 2.9 | ND | ND |
| 6 | | | | | | | | | 1.3 | 17.1 | ND | ND |
| 8 | | | | | | | | | 1.7 | 5.2 | 5.5 | 3.7 |
| 12 | | | | | | | | | 0.7 | 5.8 | 3.5 | 3.8 |
| 13 | | | | | | | | | ND | 0.3 | 4.4 | 4.9 |
| UD | | | | | | | | | 95.4 | 46.4 | 86.6 | 87.1 |
| Sum | 99.5 | 95.8 | 100.0 | 98.3 | 98.9 | 97.5 | 99.6 | 98.7 | 99.5 | 97.6 | 100.0 | 99.5 |

a) amounts for non-detectable (ND) or non-quantifiable (NQ) metabolites in one or more donor samples were considered 0 for calculation of mean percentages

APPEARS THIS WAY
ON ORIGINAL

Table 2. A summary of the various metabolites with the metabolite identification code, the technique(s) used in the identification along with the metabolic route

| Metabolite code | Identification method | Metabolic route |
|---|---|---|
| UD | LC-MS/MS Co-elution (TMC125) | Unchanged drug (R168335; TMC125) |
| 1 | LC-MS/MS Enzymatic deconjugation | Glucuronidation of metabolite 8 |
| 4 | LC-MS/MS | Glucuronidation of dihydroxylated TMC125 |
| 5 | LC-MS/MS Enzymatic deconjugation | Glucuronidation of metabolite 13 |
| 6 | LC-MS/MS Enzymatic deconjugation | Glucuronidation of metabolite 12 |
| 7 | LC-MS/MS | Monohydroxylation at the pyrimidine moiety and glucuronidation |
| 8 | LC-MS/MS co-elution (TMC125-M21) | di-methyl-hydroxylation on the dimethyl-benzonitrile moiety |
| 9 | LC-MS/MS Enzymatic deconjugation | Glucuronidation of metabolite 15 |
| 12 | LC-MS/MS Co-elution (TMC125-M12) | methyl-hydroxylation at the dimethyl-benzonitrile moiety) |
| 13 | LC-MS/MS | Aromatic mono-hydroxylation at the di-methylbenzonitrile moiety |
| 14 | Co-elution (TMC125-M10) | Aromatic mono-hydroxylation at the benzonitrile moiety |
| 15 | LC-MS/MS Co-elution (TMC125-M11) | Aromatic mono-hydroxylation at the benzonitrile moiety |

359

Figure 1. Proposed in vitro biotransformation pathways for TMC125
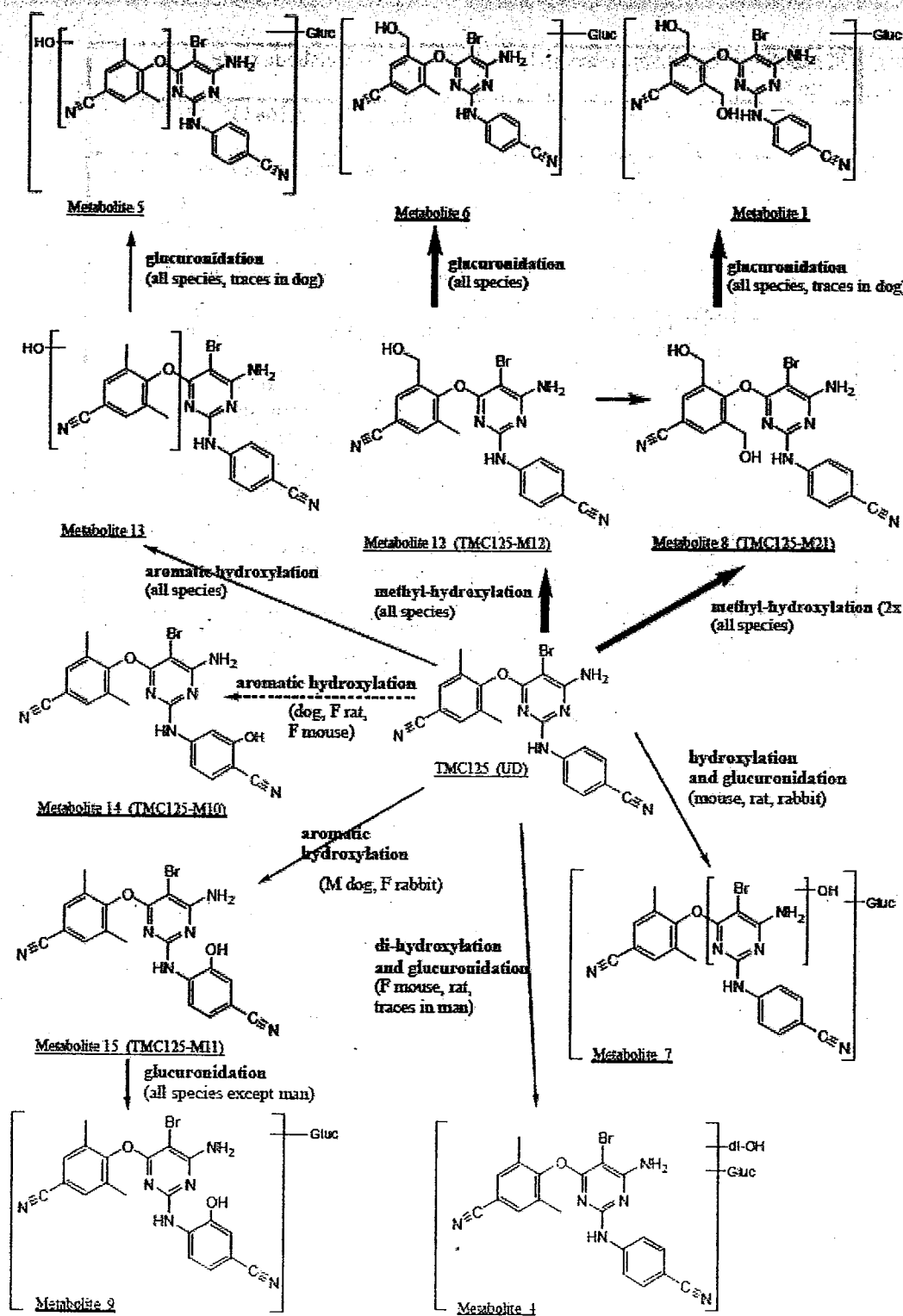
Conclusions: Metabolism occurred to a significantly lower extent in human systems than that of animal species. The major in vitro metabolic pathway of TMC125 in all species was methylhydroxylation at one of the methyl groups of the dimethylbenzonitrile moiety in combination with glucuronide conjugation (Metabolite 12). Hydroxylation at both methyl groups of the dimethylbenzonitrile moiety of TMC125 also occurred in all species (Metabolite 8). A glucuronide conjugate of the latter dihydroxylated metabolite was found to be relatively more important in man compared to other species (Metabolite 1). All TMC125 metabolites that were identified in human test systems used in the present in vitro study, were also found in at least one preclinical species.

APPEARS THIS WAY
ON ORIGINAL

The mass balance of unchanged TMC125 and its metabolites in human urine, faeces and plasma by Radio-HPLC and characterization of TMC125 metabolites (TMC125-NC205)

Methods:

The in vivo metabolism of ^{14}C-TMC125 was studied in faeces, urine and plasma collected from healthy male subjects after a single oral dose of 800 mg ^{14}C-TMC125 (PEG-4000 formulation). Faeces extracts, pooled and concentrated urine of individual subjects and pooled plasma samples were analyzed by radio-HPLC. Co-chromatography of authentic substances, enzyme hydrolysis and LC-MS/MS techniques were used for the identification of metabolites.

Results:

Table 1. A summary of the identity of various metabolites of TMC125

| Metabolite code | Identification method | Metabolic route |
|---|---|---|
| UD | LC-MS/MS Co-elution (TMC125) | Unchanged drug (TMC125) |
| 1 | LC-MS/MS Enzymatic deconjugation | Di-methyl-hydroxylation on the di-methylbenzonitrile moiety (TMC125-M21) and glucuronidation |
| 6 | LC-MS/MS Enzymatic deconjugation | Methyl-hydroxylation on the di-methylbenzonitrile moiety (TMC-M12) and glucuronidation |
| 8 | LC-MS/MS Co-elution (TMC125-M21) | Di-methyl-hydroxylation on the di-methylbenzonitrile moiety |
| 12 | LC-MS/MS Co-elution (TMC125-M12) | Methyl-hydroxylation on the di-methylbenzonitrile moiety |
| 13 | LC-MS/MS | Aromatic mono-hydroxylation on the di-methylbenzonitrile moiety |

APPEARS THIS WAY
ON ORIGINAL

Figure 1. Metabolic pathways of TMC125 in human based on metabolite structures characterised by LC-MS/MS and co-elution of authentic substances in faeces, urine and plasma
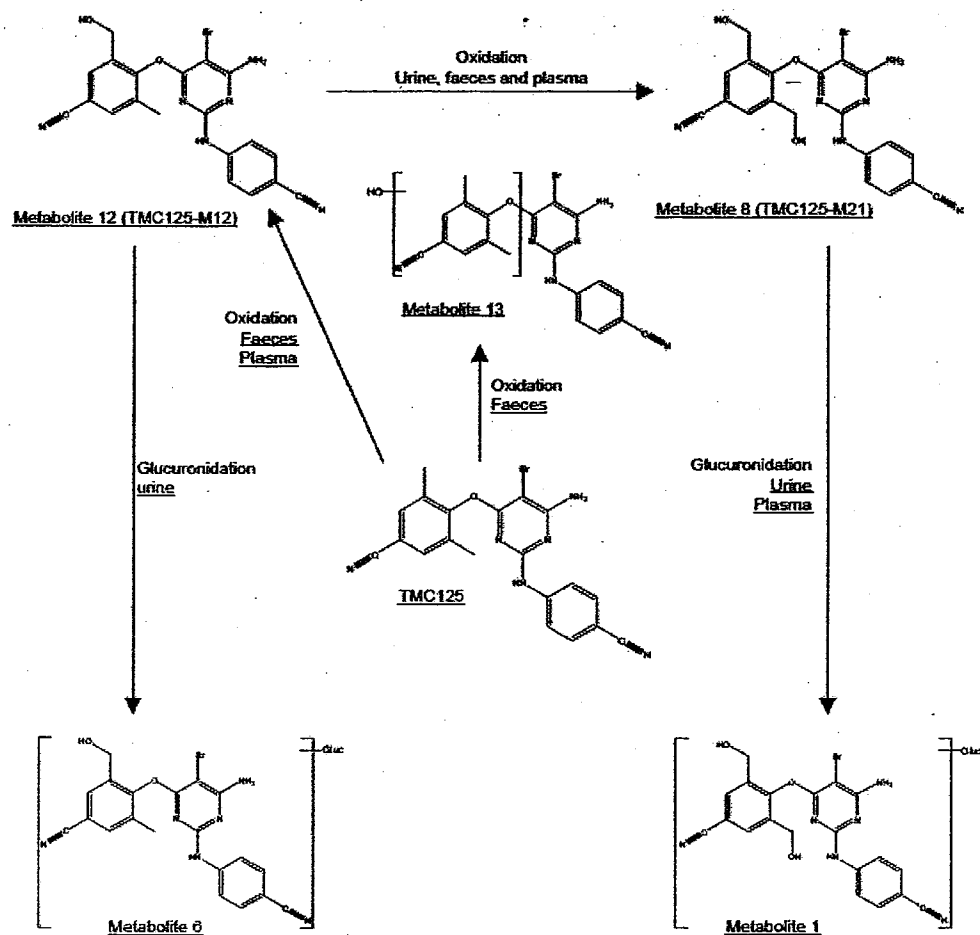


Conclusions:

The major part of orally administered TMC125 was excreted unchanged in the faeces (81.2-86.4 % of the dose). The most important phase-1 metabolic pathway of TMC125 in humans was hydroxylation of the methyl carbons of the dimethylbenzonitrile moiety. Both the mono and di-methyl hydroxylated metabolites (metabolites 12 and 8, respectively) and their glucuronides (metabolites 6 and 1) were formed. Overall methyl hydroxylation accounted for 3.8 -9.5% of the dose. Aromatic hydroxylation at the dimethylbenzonitrile moiety (metabolite 13) was a very minor metabolic pathway in humans. In plasma, TMC125 represented the major fraction of the absorbed radioactivity at the three time points studied (2h, 4 h and 8 h). Metabolite 1, 8 and 12 were detected in plasma from which metabolite 8 amounted to one third to half of the TMC125 concentration.

These in vivo results are in consistent with in vitro results described in Study TMC125K-NC142.

## 4.2    Consult Reviews (including pharmacometric reviews)

APPEARS THIS WAY
ON ORIGINAL

## PHARMACOMETRICS REVIEW

| | |
|---|---|
| **NDA Number:** | 22187 |
| **Generic Name:** | Etravirine (TMC125) |
| **Proposed Indication:** | Treatment experienced subjects infected with HIV-1 |
| **Sponsor:** | Tibotec |
| **Type of Submission:** | NME |
| **Pharmacometrics (PM) Reviewer:** | Pravin Jadhav Ph.D. |
| **Primary Reviewer:** | Vikram Arya Ph.D. |
| **Clinical Pharmacology Team Leader:** | Kellie S. Reynolds Pharm.D. |
| **PM Team Leader:** | Jogarao Gobburu Ph.D. |
| **Proposed Dosage and Administration:** | 200 mg BID |

365

## Table of contents

APPEARS THIS WAY
ON ORIGINAL

## List of Figures

## *List of Tables*