Office of Clinical Pharmacology Review

NDA or BLA Number	206229/S-008
Link to EDR	\\CDSESUB1\evsprod\NDA206229\206229.enx
Submission Date	December 26, 2018 (SDN439); February 28, 2019 (SDN456)
Submission Type	Efficacy supplement / 505(b)(2)
Brand Name	Liletta®
Generic Name	levonorgestrel-releasing intrauterine system
Dosage Form and Strength	Intrauterine system, 52 mg, initial release rate of levonorgestrel is approximately 20 μg/day
Route of Administration	Intrauterine administration
Proposed Indication	Prevention of pregnancy
Applicant	Medicines360
Associated IND	IND 105836
OCP Review Team	Peng Zou, PhD; Yanhui Lu, PhD
OCP Final Signatory	Yanhui Lu, PhD

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1. EXECUTIVE SUMMARY

Liletta®, a levonorgestrel-releasing intrauterine system (LNG-IUS), was approved on 26 February 2015 for prevention of pregnancy for up to 3 years, and the duration of use was extended to 4 years and then 5 years upon approval of S-004 (03 August 2017) and S-007 (15 October 2018), respectively. In this supplemental New Drug Application (sNDA), Medicines360 (the Applicant) seeks to extend the duration of use for Liletta to 6 years.

Medicines360 is conducting Study M360-L102, an ongoing, multicenter, open-label, Phase 3 study, to evaluate long-term reversible contraception with the use of Liletta for up to 10 years. To support the proposed extension of use to 6 years, in the current Supplement-008 (S-008), the Applicant updated the efficacy and safety data. Pharmacokinetic (PK) report and ex vivo LNG release data were updated through 7 years of use. Pharmacodynamic (PD) data (menstrual bleeding) was updated through 6 years of

use. Exposure-response analyses for return to menses (RTM), return to fertility (RTF), and endometrial thickness (ET) were updated through 6 years of use.

1.1 Recommendations

The Office of Clinical Pharmacology Division of Clinical Pharmacology-3 has reviewed the information contained in NDA 206229, efficacy supplement 008 and recommends approval of this supplement from a clinical pharmacology perspective. The key review issues with specific recommendations/comments are summarized in the table below:

Review Issue	Recommendations and Comments
General dosing instructions	The proposed dosing regimen is appropriate for the prevention of pregnancy for up to 6 years in general population
Dosing regimen for subpopulations	Subject age, body mass index (BMI)/body weight, race and inserter type did not affect the efficacy of Liletta. No alternative dosing regimen and/or management strategy is required for subpopulations.
Labeling	Section 2.1: The release rate of LNG after 6 years is revised. Section 12.3: The number of subjects for each race is updated.
Other	None

1.2 Post-Marketing Requirements and Commitments

None.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

As part of Study M360-L102, plasma LNG concentrations were assessed in a PK substudy from Week 1 through Month 30 of use, and then in all subjects (including those who participated in the PK substudy) beginning at Month 36. PK data from the main study cohort starting at the Month 36 submitted in this supplement were obtained from 894 Liletta subjects, with 191 of these subjects providing PK data for Month 72. Plasma LNG concentrations decreased from 101 ± 43 pg/mL at Month 60 to 93 ± 45 at Month 72.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

Liletta contains 52 mg of levonorgestrel (LNG). Initially, LNG is released at a rate of approximately 20 mcg/day. The release rate of LNG decreases progressively to approximately 8.6 mcg/day after 6 years. The average in vivo release rate of LNG is approximately 14.3 mcg/day over a period of 6 years. Liletta can be removed at any time but must be removed by the end of the sixth year.

2.2.2 Therapeutic individualization

N.A.

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

None.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

Liletta® contains 52 mg of LNG for which Medicines360 (the Applicant) claims that the initial release rate and release rate of LNG after 5 years are 19.5 mcg/day and 9.8 mcg/day, respectively. In this supplemental NDA, Medicines360 seeks to extend the duration of use for Liletta to 6 years.

3.2 General Pharmacology and Pharmacokinetic Characteristics

Pharmacology								
Mechanism of Action	The local mechanism by which continuously released LNG provides contraception has not been conclusively demonstrated. Studies of LNG-releasing IUSs suggest several mechanisms for pregnancy prevention: prevention of fertilization due to the thickening of the cervical mucus, which inhibits sperm passage through the cervix, and inhibition of sperm mobility and function (capacitation), and alteration of the endometrium.							
Active Moieties	Levonorgestrel							
General Information								
Bioanalysis	A LC-MS/MS method (AM-999-715-B) was used to determine levonorgestrel in human plasma with sodium heparin.							
Healthy vs. Patients	No dedicated comparative PK study between healthy subjects and patients was conducted.							
Drug exposure (Plasma LNG Concentrations, pg/mL) (Mean ± SD)	7 Days (n = 40)	6 Months (n = 36)	12 Months (n = 33)	24 Months (n = 30)	36 Months (n = 894)	48 Months (n = 737)	60 Months (n = 531)	72 Months (n = 191)
	252 ± 123	195 ± 68	168 ± 51	150 ± 47	132 ± 54	114 ± 52	101 ± 43	93 ± 45
Maximally tolerated dose or exposure	Maximally tolerated dose was not established.							
Variability	Inter-subject variability in plasma LNG concentrations: Day 7, 49%; Month 6, 35%; Month 12, 30%; Month 24, 31%; Month 36, 41%; Month 48, 46%; Month 60, 43%; Month 72, 48%							
Absorption								
Bioavailability	N.A.							
Intrauterine LNG release	The initial in vivo release rate is 20.1 mcg/day and decreases to 17.5 mcg/day at 1 year, 15.2 mcg/day at 2 years, 13.2 mcg/day at 3 years, 11.4 mcg/day at 4 years, 9.9 mcg/day at 5 years, and 8.6 mcg/day at 6 years.							
Distribution								
Volume of distribution	1.8 L/kg, 98.9% plasma protein binding							
Elimination								
Terminal elimination half-life (Mean ± SD)	13.9 ± 3.2 hours							
Metabolism and Excretion								

Primary metabolic and excretion pathway(s)	Oxidative metabolism of LNG is catalyzed by CYP enzymes, especially CYP3A4. Both LNG and its phase I metabolites undergo sulfate conjugation and, to a less extent, glucuronide conjugation.

3.3 Clinical Pharmacology Review Questions

3.3.1 To what extent does the available clinical pharmacology information provide pivotal or supportive evidence of effectiveness?

Plasma PK data were submitted to support the effectiveness of Liletta for up to 6-year use. No dedicated clinical pharmacology studies were conducted with Liletta. PK assessment was conducted as part of Phase 3 trial (Study M360-L102). Plasma LNG concentrations for all subjects in Study M360-L102 from Month 36 to Month 84 are presented in Table 1. PK data for the main study cohort starting at the Month 36 time point includes data from 894 Liletta subjects, with 191 of these subjects providing PK data for Month 72. Plasma LNG concentrations decreased from 101 ± 43 pg/mL at Month 60 to 93 ± 45 pg/mL at Month 72.

A comparison of plasma LNG concnetrations between Liletta and Mirena treatment groups was conducted to support the effectiveness of Liletta. Consistent with the findings in the original submission, the LNG plasma concentrations measured from Month 36 through Month 60 in Study M360-L102 were similar between Liletta and Mirena groups (Figure 1 and Table 1).

Table 1. Mean ± SD (pg/mL) Plasma Levonorgestrel Concentrations for All Subjects Beginning at Month 36 (Data source: Summary of Clinical Pharmacology Studies, Table 7)

	36	**42**	**48**	**54**	**60**	**66**	**72**	**78**	**84**
Liletta	132 ± 54.3 (n = 894)[a]	122 ± 56.6 (n = 793)[a]	113.7 ± 51.9 (n = 737)[a]	109.6 ± 51.5 (n = 662)[a]	101.2 ± 42.8 (n = 531)[a]	96.5 ± 37.1 (n = 305)[a]	92.7 ± 45 (n = 191)[a]	86.5 ± 33.4 (n = 172)[a]	86.9 ± 34.2 (n = 155)[a]
Mirena	141.2 ± 60.4 (n = 60)[a]	142 ± 73.3 (n = 53)[a]	134.2 ± 98.8 (n = 46)[a]	111 ± 44 (n = 37)[a]	111.7 ± 49.4 (n = 34)[a]	NA	NA	NA	NA

[a]Concentration values at early discontinuation were assigned to the nearest protocol-specified time point.

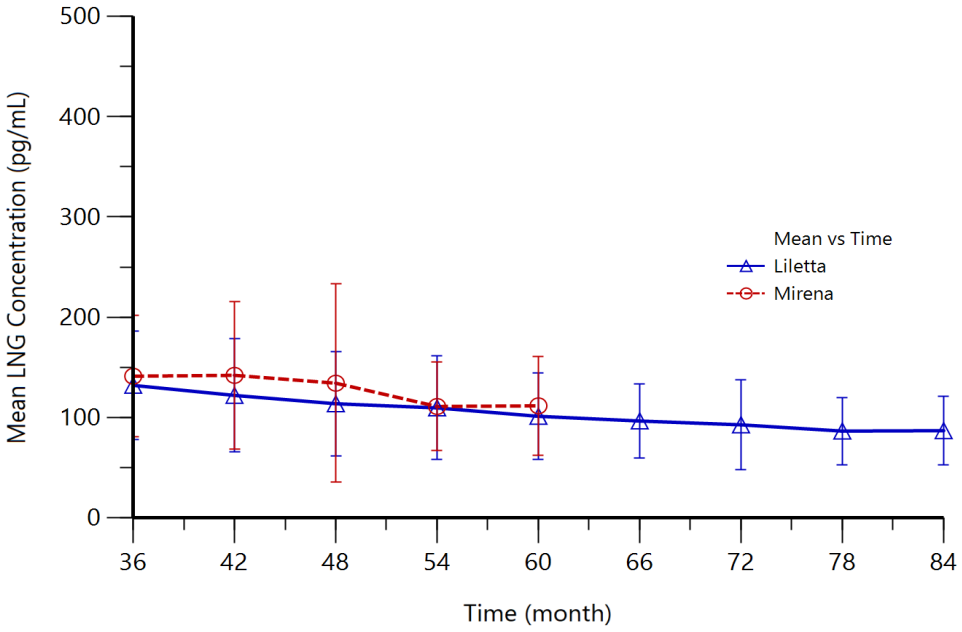

Figure 1. Mean (± SD) Plasma Levonorgestrel Concentration-Time Profiles for All Subjects Beginning at Month 36 (Data source: Summary of Clinical Pharmacology Studies, Figure 4)

The Applicant reported 9 on-treatment pregnancies in the Liletta modified intention-to-treat (MITT) population through Year 6 of use; 2 occurred in the first year, 4 in the second year, 1 in the third year, 1 in the fourth year, and 1 in the fifth year of use. According to the Applicant, no on-treatment pregnancy was reported in the sixth year of use and thus systemic exposure-efficacy analysis was not conducted in this sNDA.

The Applicant updated bleeding and spotting data collected in Study M360-L102 for up to 72 months. The bleeding and spotting data collected from summary questionnaires through subject interview every 3 months were updated to Month 72 in S-007 (Table 2). The bleeding and spotting data submitted in S-004, S-007, and this supplement demonstrated a consistent bleeding pattern alteration following Liletta placement. Both the plasma PK profile and bleeding data indicated a sustained in vivo release LNG from Liletta. The bleeding and spotting data supported the extension to 6 years of use of Liletta.

Table 2. Bleeding and Spotting Patterns by 90-Day Interval between Month 30 –72 (Data source: Summary of Clinical Pharmacology Studies, Table 13)

	No Bleeding or Spotting	**Main Pattern**		
		Just Spotting	**Irregular Bleeding**	**Regular Bleeding (Periods)**
Data Collected by Recall for the Preceding 3 Months				
Month 30 (n = 1010)	390 (38.6%)	388 (62.6%)	72 (11.6%)	160 (25.8%)
Month 36 (n = 981)	359 (36.6%)	354 (56.9%)	84 (13.5%)	184 (29.6%)
Month 42 (n = 897)	334 (37.2%)	333 (59.1%)	71 (12.6%)	159 (28.2%)
Month 48 (n = 840)	312 (37.1%)	310 (58.7%)	80 (15.2%)	138 (26.1%)
Month 54 (n = 768)	287 (37.4%)	272 (56.5%)	63 (13.1%)	146 (30.4%)
Month 60 (n = 707)	284 (40.2%)	244 (57.7%)	45 (10.6%)	134 (31.7%)

Month 66 (n = 563)	220 (39.1%)	199 (58.0%)	40 (11.7%)	104 (30.3%)
Month 72 (n = 349)	141 (40.4%)	115 (55.3%)	27 (13.0%)	66 (31.7%)

LNG release rate was determined by residual drug content analysis of 128 IUS samples that removed or expelled over from 1 day through 7.3 years in Study M360-L102. The new revised initial in vivo release rate is 20.1 mcg/day. Consitent with the findings in orginal NDA and supplements, this LNG release rate decreased in an exponentially manner. The release rates at 1, 2, 3, 4, 5, 6, and 7 years after insertion were approximately 17.5, 15.2, 13.2, 11.4, 9.9, 8.6, and 7.5 mcg/day, respectively. The average in vivo release rate was approximately 14.3 mcg/day over a period of 6-year duration of use. Refer to Biopharmaceutics review for more details.

3.3.2 Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes, the proposed dosing regimen appropriate for prevention of pregnancy in general population.

Exposure-response (E-R) data for the secondary endpoints of return to menses (RTM), return to fertility (RTF), and endometrial thickness (ET) were submitted in original NDA submission to support the safety of Liletta after removal. E-R analysis for safety through 6 years of Liletta use was provided in the current sNDA.

Upon discontinuation of IUS use, Liletta subjects were followed to assess the time to RTM. A total of 223 Liletta subjects with a discontinuation plasma LNG concentration available were included in the E-R analysis. No obvious correlation between plasma LNG concentration and RTM was observed (Figure 2).

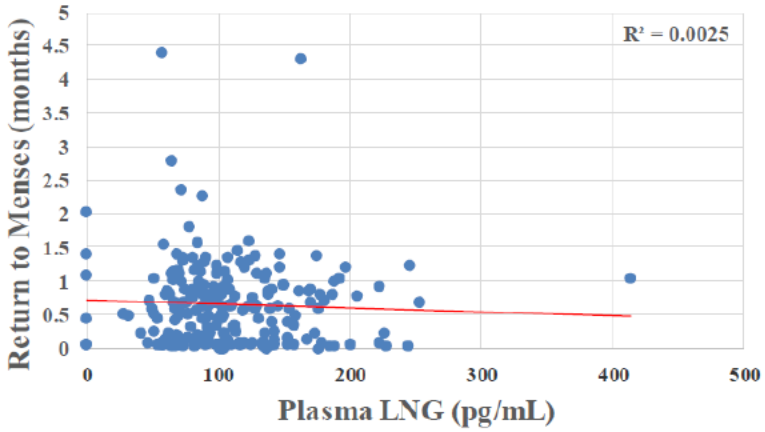

Figure 2. Time to Return to Menses vs. Plasma Levonorgestrel Concentration at Discontinuation (N = 223) (Data source: Summary of Clinical Pharmacology Studies, Figure 9)

Subjects in the RTM analysis group who indicated that they desired pregnancy were followed for 12 months to assess RTF (i.e., pregnancy). A total of 104 Liletta subjects were included in the exposure-RTF response analysis. Pregnancy occurred for 85 subjects and pregnancy did not occur for 19 subjects within the 12-month assessment period. The analysis showed that there was no correlation between plasma LNG concentration and RTF (Figure 3). Subject (b) (6), Subject (b) (6), Subject (b) (6) Subject (b) (6) Subject (b) (6), and Subject (b) (6) reported pregnancy 4 days, 9 days, 10 days, 12 days, 13 days, and 14 days post IUS removal, respectively. Refer to the clinical review regarding evaluation of the six pregnancies observed immediately post IUS removal.

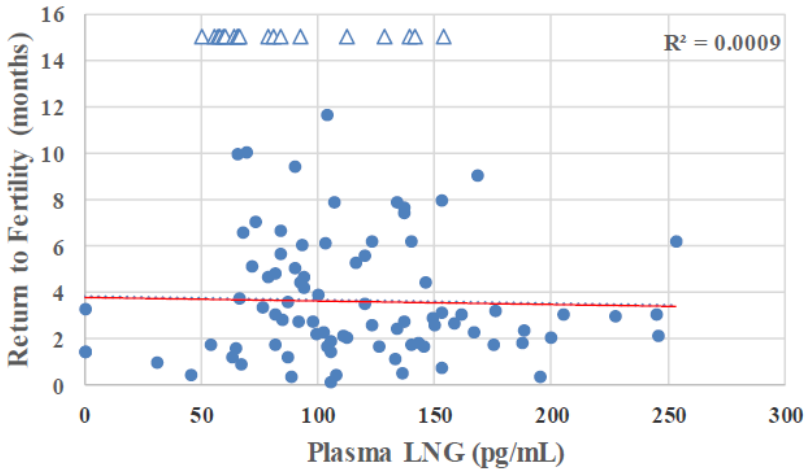

Figure 3. **Time to Return to Fertility vs. Plasma Levonorgestrel Concentration at Discontinuation (N = 104).** Note: Filled circles = pregnancy occurred; Open triangles = pregnancy did not occoured. (Data source: Summary of Clinical Pharmacology Studies, Figure 10)

The plasma LNG concentrations and ET measured at Month 60 in nineteen Liletta subjects were used for E-R analysis. The analysis showed that there was no correlation between plasma LNG concentration and ET (Figure 4). Because ET data collection was not synchronized to the secretory (luteal) phase of the menstrual cycle, the clinical significance of this ER analysis results cannot be determined.

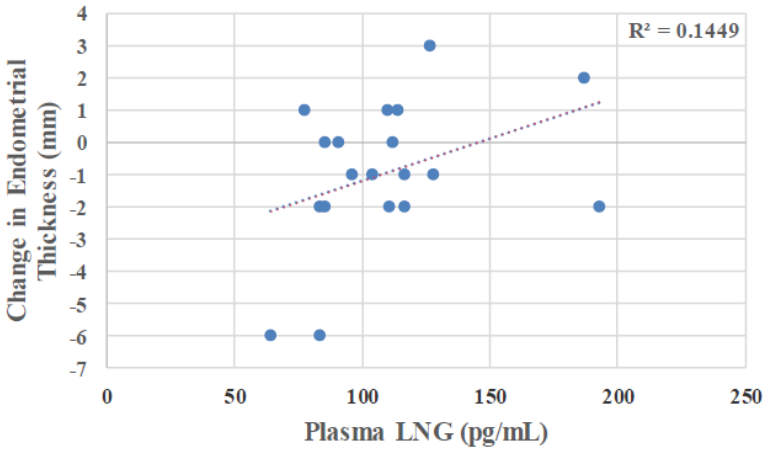

Figure 4. **Change in Endometrial Thickness vs. Plasma Levonorgestrel Concentration at Month 60 (N = 19)** (Data source: Summary of Clinical Pharmacology Studies, Figure 11)

3.3.3 Is an alternative dosing regimen and/or management strategy required for subpopulations based on intrinsic or extrinsic factors?

The Applicant assessed the effect of subject age, BMI/body weight, race, and inserter type on the plasma LNG concentrations. No additional alternative dosing regimen and/or management strategy is required for subpopulations.

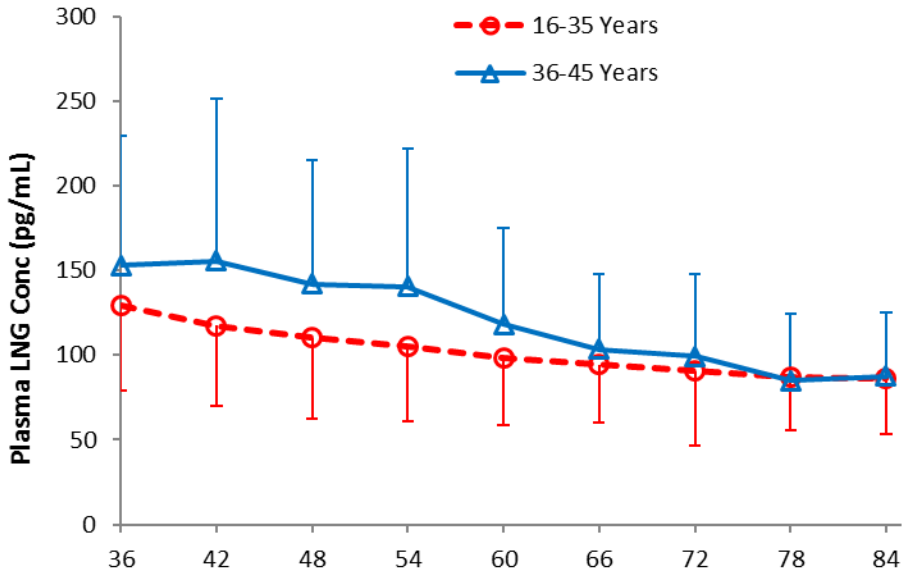

Figure 5. **Effect of Age on Plasma Levonorgestrel Concentrations** (Data source: Summary of Clinical Pharmacology Studies, Table 8)

Age effect:
The effect of age on LNG exposure was assessed in 791 subjects aged 16-35 years and 103 subjects aged 36-45 years from Month 36 through Month 84 in Study M360-L102. As shown in Figure 5, 36-45 years group had a higher mean LNG systemic exposure than 16-35 years group between Month 36 to 72. Comparable mean plasma LNG concentrations were observed in the two groups at Month 72 and Month 84. Despite a higher systemic exposure to LNG in the 36-45 years group, there was no significant difference in the contraceptive efficacy between the two age groups. Subjects aged 16-35 years for Liletta efficacy analysis were further divided into 3 subgroups: < 18 years, 18-30 years, and 31-35 years. Seven pregnancies occurred in subjects aged 18–30 years (N = 1230), while 2 pregnancy occurred in subjects aged 31–35 years (N = 304). No pregnancies occurred in subjects < 18 years of age (N = 11). Life table pregnancy rates through Year 6 of use were comparable between 18-30 years group (0.94) and 31–35 years group (0.78). The lack of impact of lower plasma LNG on efficacy in younger subjects may be attributable to the local action of Liletta in the uterus. No age-based dose adjustment is recommended.

BMI/body weight effect:
The effect of BMI on LNG exposure was assessed in 673 non-obese (BMI $\leq$ 30 kg/m^2) and 219 obese women (BMI $\geq$ 30 kg/m^2) from Month 36 through Month 84 in Study M360-L102. As shown in Figure 6, obese women have a lower LNG systemic exposure, which is consistent with the finding in the original submission. Plasma LNG concentrations were about 23% – 32% lower in obese subjects than those in non-obese subjects. Regression analysis showed that body weight has a significant effect on the plasma LNG concentration at Month 72 (Figure 7). Similar effect of body weight on plasma LNG concentration was observed at Month 36, 42, 48, 54, 60, 66, 78, and 84. For every 1-kg increase in weight, there was approximately a 1% decrease in the LNG plasma concentration observed over time through Month 84. These results are consistent with the results reported for original NDA approval. According to the Applicant, seven pregnancies occurred in subjects with normal weight (N = 795, BMI $\leq$ 24.9 kg/m^2), while 1 pregnancy occurred in an overweight subject (N = 373, BMI 25.0 – 29.9 kg/m^2), no pregnancy occurred in obese subjects (N = 297, BMI 30.0 – 39.9 kg/m^2) and 1 pregnancy occurred in a extremely

obese subject (N = 77, BMI ≥ 40.0 kg/m^2). BMI- or body weight-based dose adjustment is not recommended.

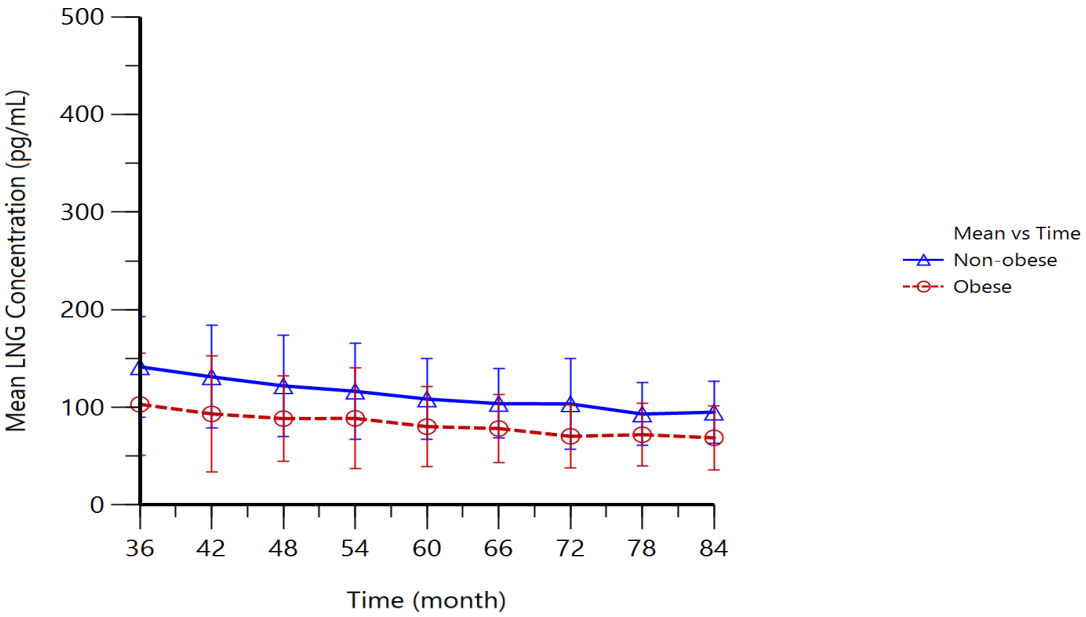

Figure 6. **Effect of Body Mass Index on Plasma Levonorgestrel Concentrations** (Data source: Summary of Clinical Pharmacology Studies, Figure 5)

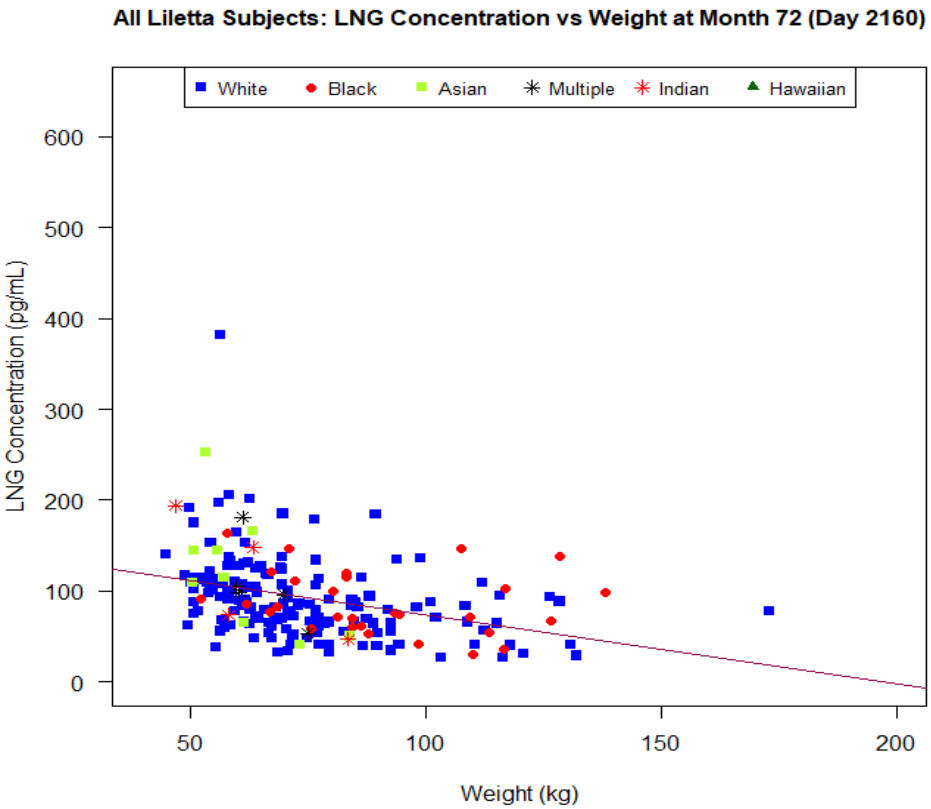

Figure 7. Plasma Levonorgestrel Concentrations (pg/mL) vs Weight for All Subjects in the Liletta Group at Month 72 (Data source: Summary of Clinical Pharmacology Studies, Figure 6)

Race effect:

Assessment of the effects of race on LNG exposure was performed using data from 909 subjects at Month 36 and 176 subjects at Month 84. Among the 909 subjects included in analysis for Month 36, there were 731 (80%) Caucasian subjects, 106 (12%) Black subjects, 40 (4%) Asian subjects, 8 (1%) American Indian/Alaska Native subjects, 21 (2%) multiple-race subjects and 3 Native Hawaiian/Other Pacific subjects. The three Native Hawaiian/Other Pacific subjects were not included in the analysis due to the insufficiency of sample size. Statistical analysis showed that the effect of race (relative to White subjects) on LNG concentration was not significant at any of the time points assessed (*P*-values > 0.05) except for African American at Month 36 (Table 3). The mean plasma LNG concentration in African American subjects at Month 36 was significantly lower than that in Caucasian subjects (*P*-values = 0.0392). The mean plasma LNG concentrations in African American subjects at other time points were slightly lower than that in Caucasian subjects but the differences (2 - 9%) were not statistically significant. Compared with Caucasian subjects, Asian subjects showed higher mean plasma LNG concentrations at all time points but the the differences (3 - 32%) were not statistically significant. The effect of race on LNG PK can be explained with the race difference in body weight (BMI: Caucasian 26.6 ± 6.4 kg/m^2, African American 31.9 ± 8.7 kg/m^2, and Asian 22.9 ± 3.0 kg/m^2). According to the Applicant, over the 6-year use of Liletta, 8 pregnancies occurred in Caucasian subjects (N = 1,255), while 1 pregnancy occurred in a non-white subject (N = 286). No race difference in the efficacy of Liletta was observed. Race-based dose adjustment is not recommended.

Table 3. Plasma LNG Concentrations (pg/mL) by Race at Month 36 – 84 (Data Source: Summary of Clinical Pharmacology Studies, Table 28)

Race		Time (Month)								
		36	**42**	**48**	**54**	**60**	**66**	**72**	**78**	**84**
Caucasian	**N**	731	659	628	574	470	251	155	140	137
	Mean	133.6	123.3	114.3	109.7	102.1	97.1	91.1	86.1	85.9
	SD	55.1	58.6	52.6	50.4	42.5	37.9	44.6	34.3	32.6
	***P* value**	NA	NA	NA	NA	NA	NA	NA	NA	NA
Black/African American	**N**	106	100	90	72	61	39	29	21	20
	Mean	122.0	112.3	107.2	104.3	96.8	89.3	87.0	79.2	84.0
	SD	46.1	43.5	41.5	49.2	39.1	30.2	34.6	30.1	32.9
	***P* value**	0.0392	0.0718	0.2203	0.3905	0.3557	0.2214	0.6397	0.3843	0.8082
Asian	**N**	40	35	34	28	26	13	9	8	10
	Mean	137.3	128.2	124.3	125.2	105.5	106.0	120.6	103.6	106.2
	SD	59.3	50.9	64.9	76.2	54.7	40.9	66.2	29.6	51.5
	***P* value**	0.6805	0.6278	0.2869	0.1229	0.6962	0.4116	0.0627	0.1600	0.0711
American Indian/Alaska Native	**N**	8	8	8	6	8	4	4	5	4
	Mean	123.0	127.5	104.8	120.1	94.8	99.3	115.7	90.5	92.8
	SD	41.7	62.4	55.3	62.0	36.2	40.8	67.5	34.4	47.4
	***P* value**	0.5878	0.8405	0.6121	0.6161	0.6295	0.9085	0.2836	0.7785	0.6807
Multiple Races	**N**	21	18	20	14	13	7	5	5	5
	Mean	123.3	115.8	109.7	97.3	84.4	93.7	106.9	94.3	80.1
	SD	57.5	57.1	48.5	48.2	52.7	39.7	46.2	27.4	34.2
	***P* value**	0.3992	0.5921	0.6997	0.3630	0.1418	0.8153	0.4372	0.5984	0.6970
Native Hawaiian/Other Pacific Islander	**N**	3	3	2	2	1	1	NA	NA	NA
	Mean	111.5	112.3	109	103.6	66.8	96.1	NA	NA	NA
	SD	53.7	60.9	11.3	23.2	NA	NA	NA	NA	NA
	***P* value**	0.4883	0.7458	0.8868	0.8643	NA	NA	NA	NA	NA

Inserter type effect:
Two different inserters (THI-001 and SHI-001) were used in Study M360- L102. The original two-handed inserter (THI-001) was used for the first 760 women. Enrollment was temporarily suspended due to reports from investigators of difficult placements, placement failures, and the need for cervical dilation. A single-handed inserter (SHI-001) was developed and used in 991 women enrolled from March 2012. In current submission, the effect of inserter on plasma LNG concentration was assessed. Among the 911 subjects with LNG plasma concentration available at Month 36, the IUS of 380 subjects were placed with THI-001 and the IUS of 531 subjects were placed with SHI-001. For THI-001 group, the plasma LNG concentrations for Months 36 – 84 are available. For SHI-001 group, the plasma LNG concentrations for Months 36 – 78 are currently available. It is noted that there are only 2 and 1 subjects in the SHI group that have data at Months 72 and 78, respectively. Therefore, comparisons between inserter groups are limited to Months 36 through 66. Figure 8 shows that the mean plasma LNG concentrations of THI-001 group were 20 – 37% higher than that of SHI-001 group at Month 36 – 66. The reason for the different mean plasma LNG concentrations between the two groups is unknown. For both groups, the life table-derived pregnancy rates during the 6 years of use of Liletta were low (Table 4). Inserter type did not affect the efficacy of Liletta and no dose adjustment is needed.

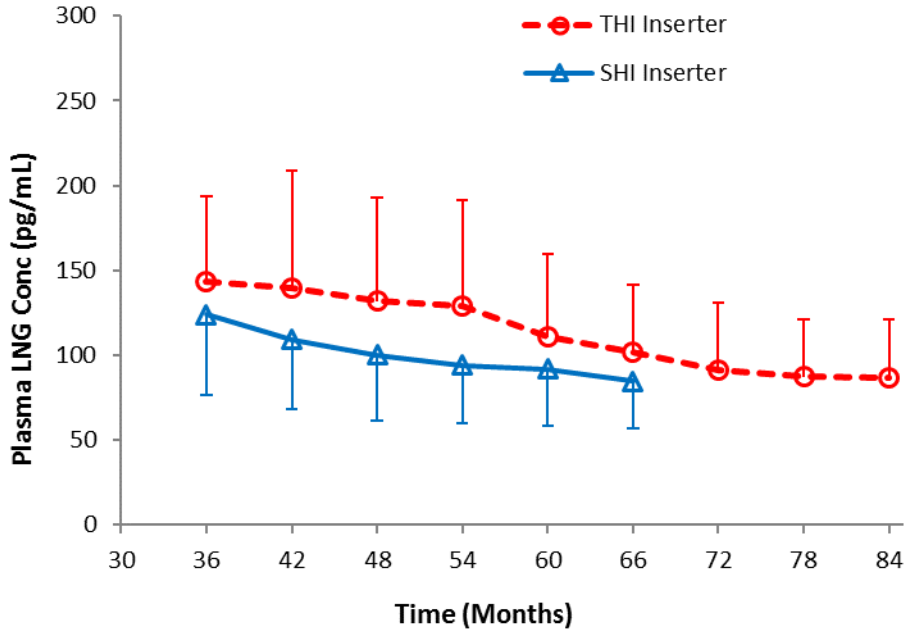

Figure 8. **Effect of Inserter Type on Plasma Levonorgestrel Concentrations** (Data source: Summary of Clinical Pharmacology Studies, Table 29 and Table 30)

Table 4. Life Table-derived Pregnancy Rates by Inserter Type for the Liletta MITT Population (Data source: Summary of Clinical Efficacy, Table 13)

	Cumulative Pregnancy Rates (95% CI)	
	THI-001 Inserter (N= 611)	**SHI-001 Inserter (N = 934)**
Year 1	0.18 (0.03, 1.30)	0.12 (0.02, 0.83)
Year 2	0.18 (0.03, 1.30)	0.68 (0.28, 1.63)
Year 3	0.18 (0.03, 1.30)	0.85 (0.38, 1.89)
Year 4	0.55 (0.13, 2.36)	0.85 (0.38, 1.89)
Year 5	1.01 (0.31, 3.29)	0.85 (0.38, 1.89)
Year 6	1.01 (0.31, 3.29)	0.85 (0.38, 1.89)

*Excluding cycles where other birth control methods were used

3.3.4 Are there clinically relevant food-drug or drug-drug interactions and what is the appropriate management strategy?

No food-drug interaction or drug-drug interaction data were submitted in this sNDA. Contraceptive effect of Liletta is mediated via the direct release of LNG into the uterine cavity and is unlikely to be affected by drug interactions via metabolic enzyme/transporter induction or inhibition.

3.3.5 Does pharmacokinetic data bridge the proposed to-be-marketed product to Phase 3 trial formulation?

The LNG-IUS tested in Phase 3 Study M360-L102 is identical to the commerical product.

4. APPENDICES

4.1 Summary of Bioanalytical Method Validation and Performance

LNG plasma concentrations were determined using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Two validation reports for this method submitted in the original submission were reviewed by Dr. Li Li and found acceptable. The same method was used in current submission. A bioanalytical report amendment dated 30 July 2018 was provided to support bioanalysis of PK samples in the current submission. The method verification results are summarized in Table 5. The acceptable criteria and assay performance were in compliance with the *Bioanalytical Method Validation Guidance* and the bioanalytical method was found to be acceptable.

Table 5. LC-MS/MS Method Validation Results

Parameter	**QC Samples**	**Standard Curve Samples**
Biological matrix	Human plasma with sodium heparin	Human plasma with sodium heparin
Concentration (pg/mL)	75, 400, 800	25.0, 50.0, 100, 250, 500, 900, 1000
QC Dilution Range (pg/mL)	1000 to 10,000	N/A
Interday Precision (% CV)	7.2 to 13.6	5.2 to 9.5 (87 batches)
Interday Accuracy (% RE)	-8.9 to 1.1	-4.0 to 3.0 (87 batches)
Linearity (Range of R^2 values)	N/A	0.9878 to 0.9989 (87 batches)
Linear Range (pg/mL)	N/A	25 to 1000
LOQ (pg/mL)	N/A	25
Storage Stability	The established storage stability (382 days at -70°C) covered sample collection and analysis period.	

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

PENG ZOU
09/10/2019 09:31:49 PM

YANHUI LU
09/10/2019 09:33:59 PM