CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

213150Orig1s000

CLINICAL MICROBIOLOGY/VIROLOGY REVIEW(S)

Office of Clinical Pharmacology Review

NDA or BLA Number	213150
Link to EDR	\\Cdsesub1\evsprod\NDA213150\213150.enx
Submission Date	02 Jul 2019
Submission Type	505 (b)(2)
Brand Name (Proposed)	ENSOLVI ®
Generic Name	Leuprolide acetate injectable suspension
Dosage Form and Strength	Injection (extended release), 45 mg
Route of Administration	Subcutaneous
Proposed Indication	Treatment pediatric patients 2 years age and older with central precocious puberty
Applicant	Tolmar International Limited
Associated IND	IND 123631
OCP Review Team	*Suryanarayana Sista, PhD; Jaya Vaidyanathan, PhD*

Table of Contents

List of Tables

List of Figures

1. EXECUTIVE SUMMARY

This is an original 505(b)(2) NDA submitted by Tolmar International Limited on 02 July 2019, seeking marketing approval for leuprolide acetate for injectable suspension (proposed name – FENSOLVI) for the treatment of pediatric patients 2 years of age and older with central precocious puberty (CPP). FENSOLVI 45 mg is available as an injectable suspension of leuprolide acetate, and is the same product as Tolmar's currently marketed Eligard 45mg (NDA 021731) for the palliative treatment of advanced prostate cancer. It differs only in terms of labeling and intended use. Based on an agreement with the Agency (pre-NDA meeting preliminary comments dated January 9, 2019 and pre-NDA written responses to additional questions dated January 23, 2019), the Sponsor submitted only the drug substance and drug product specifications in Module 3 of the CTD. NDA 021731 (right of reference provided) serves as the reference for all other non-clinical and Chemistry Manufacturing and Controls (CMC) information.

The proposed dosing regimen for FENSOLVI is to administer it as a 45 mg single subcutaneous injection once every six months. The dose of FENSOLVI does not require individualization for each child.

NDA 213150 is primarily supported by a pivotal pharmacokinetic (PK)/ pharmacodynamic (PD) study (TOL2581A) which assessed the effectiveness of leuprolide acetate for injectable suspension, 45 mg for treatment of children with CPP. Apart from the findings of this study, the sponsor is referencing Lupron Depot-Ped (NDA 20263) with respect to class labeling language used for the pediatric indication of CPP and some metabolism data under section 12.3 of the product label. However, Tolmar does not have right of reference for NDA 20263. While the package insert for Lupron Depot-Ped (NDA 20263) also includes reproductive toxicity and carcinogenicity data with respect to the active ingredient leuprolide acetate, the original data for reproductive toxicity and carcinogenicity in NDA 20263 were derived from Lupron Depot (NDA 019943). Tolmar is able to bridge to both the Lupron Depot and Lupron Depot-Ped information based on the fact that their product has the same active moiety (leuprolide acetate) as those products.

It is to be noted that FENSOLVI and Lupron Depot-Ped differ in the route of admininistration (subcutaneous versus intramuscular), and in the recommended dosage to be administered. However, reference to Lupron Depot-Ped is not needed for clinical pharmacology and efficacy/safety relevant sections in the proposed label since the Sponsor can report the results from Study TOL2581A or use their own leuprolide product, Eligard for class labeling.

1.1 Recommendations

The Office of Clinical Pharmacology/Division of Cardiometabolic and Endocrine Pharmacology (OCP/DCEP) has reviewed the information contained in NDA 213150 and found it acceptable to support approval of leuprolide acetate injection for the treatment of pediatric patients 2 years of age and older with CPP. Key review issues with specific recommendations and comments are summarized below:

Review Issues	Recommendations and Comments
Supportive evidence of effectiveness	The primary endpoint of leutenizing hormone (LH) suppression <4 IU/L at week 24 was met in ≥80% of subjects evaluated in TOL2581A, and it serves as as evidence for effectiveness in the treatment of children with CPP. The PK and PD (LH suppression, and Gonadotropin Releasing Hormone Agonist (GnRHa) stimulation test for serum FSH, estradiol and testosterone hormone concentration) of leuprolide in pediatric subjects who had been diagnosed with CPP but had not yet received GnRHa therapy provide supportive evidence for effectiveness.
General dosing instructions	From a Clinical Pharmacology perspective, the proposed treatment regimen of administering leuprolide acetate as a 45 mg single subcutaneous injection once every six months for the treatment of pediatric patients 2 years of age and older with CPP is acceptable.
Dosing in patient subgroups	The dose of Fensolvi does not require individualization for each child.
Bridge between the "to-be-marketed" and clinical trial formulations	The formulation used in the pivotal Phase 1 study is the proposed commercial formulation.

1.2 Post-Marketing Requirements and Commitments

None.

2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1 Pharmacology and Clinical Pharmacokinetics

The following is a summary of the clinical pharmacokinetics of leuprolide acetate injection:

Absorption:	• Following a subcutaneous injection of FENSOLVI 45 mg in children 4 to 9 years of age with CPP, peak leuprolide (C_{max}) was (b) (4) ng/mL occurring at 4 hours postdose. • Absorption of leuprolide occurred in two phases, a burst phase followed by a plateau phase. • The mean trough serum leuprolide level (C_{trough}) from 4 to 48 weeks was approximately 0.37 ng/mL with a range of 0.18 to 0.63 ng/mL. • There was no accumulation of leuprolide following the second dose.
Distribution:	• The mean steady-state volume of distribution of leuprolide following intravenous bolus administration to healthy male volunteers has been reported to be approximately 27 L. • The distribution of leuprolide following Fensolvi administration was not evaluated in children. • In vitro binding of leuprolide to human plasma proteins has been reported to range from 43% to 49%.
Elimination:	• Leuprolide is a peptide and is expected to be catabolized to smaller inactive peptides. Based on a two-compartment model, following a 1 mg bolus of leuprolide administered intravenously to healthy male volunteers, the mean systemic clearance was 8.34 L/h, with a terminal elimination half-life of approximately 3 hours.
Metabolism:	• Upon administration with different leuprolide acetate formulations, the major metabolite of leuprolide acetate is an inactive pentapeptide (M-1) metabolite.

2.2 Dosing and Therapeutic Individualization

2.2.1 General dosing

The proposed dosing recommendation is for FENSOLVI to be administered as a 45 mg single subcutaneous injection once every six months.

2.2.2 Therapeutic individualization

The dose of FENSOLVI does not require individualization for each child.

2.3 Outstanding Issues

None.

2.4 Summary of Labeling Recommendations

The Office of Clinical Pharmacology recommends the following labeling language to be included in the final package insert:

Label Section	Recommendation
12.2 Pharmacodynamics	In the clinical trial evaluating FENSOLVI in pediatric patients with CPP, there was a transient surge in circulating levels of LH, FSH, estradiol and testosterone following the first administration. A sustained decrease in basal and GnRH agonist-stimulated LH and FSH levels along with marked reductions in basal estradiol and testosterone were observed after repeat administration.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1 Overview of the Product and Regulatory Background

The regulatory history regarding communications for the 505(b)(2) submission of FENSOLVI is summarized below:

Dates	Meeting Type	Key Communication Points
09/29/2014	Pre-IND Written Response	**Nonclinical Studies:** • Agency concurred that no further nonclinical studies are necessary prior to conducting the proposed clinical study. **Clinical:** • Agency agreed that the proposed primary and secondary efficacy endpoints, and recommended that the Sponsor conduct the study in treatment naïve patients. • The Agency recommended the Sponsor to characterize the maximum leuprolide acetate concentrations from the initial burst phase (e.g., 4 hours after the administration of Eligard 45 mg as shown in adults), and explore PK (e.g., C_{max} and the baseline concentrations) - PD relationship. • TheAgency agreed that submitting a request for a pediatric waiver for children under 2 years of age was acceptable. The Sponsor was told that this request should be as part of a Pediatric Study Plan. • Regarding whether the proposed development plan supported the Sponsor's plan for a 505(b)(2) NDA submission, the Agency reiterated that in their plan to submit as part of the 505(b)(2) application, the Sponsor will need to clarify to what information they do not have the right of reference, and that they may cross-reference information already submitted to NDA 021731.
01/09/2019	Pre-NDA Meeting Preliminary Comments	**Label:** • The Sponsor was informed that impairment of fertility information is understandably lacking in the current label for treatment of advanced prostate cancer. However, such information is pertinent to an adolescent CPP population and should be included in the drug label for a product with this indication. TheSponsor was asked to include this information for leuprolide in draft labeling for the CPP indication.

3.2 General Pharmacological and Pharmacokinetic Characteristics

FENSOLVI is a single dose product. It is supplied in two prefilled syringes (Syringe A and Syringe B) with a sterile safety needle. Prior to administration, the contents of Syringes A and B are mixed together until homogenous to yield the reconstituted drug product. The drug product is administered subcutaneously where it solidifies and releases the drug over a six month period.

Syringe A contains the liquid ATRIGEL Delivery System and Syringe B contains the lyophilized leuprolide acetate powder. ATRIGEL is a polymeric delivery system consisting of a biodegradable polymer, 85:15 poly(DL-lactide-co-glycolide) (PLG), dissolved in the biocompatible solvent, N-methyl-2-pyrrolidone (NMP).

Table 1 Composition of FENSOLVI Reconstituted Drug Product

Reconstituted Drug Product	Leuprolide acetate delivered	45 mg
	Approximate leuprolide free base equivalent	42 mg
	PLG polymer delivered	165 mg
	NMP delivered	165 mg
	Approximate administered formulation weigh	375 mg
	Approximate injection volume	0.375 mL

(Source eCTD module 1.14.1.3. 04006141 Draft Labeling Text rx0619, Table 3)

3.2.1 Mechanism of Action:

Leuprolide acetate, a GnRH agonist, acts as a potent inhibitor of gonadotropin secretion (LH and follicle stimulating hormone (FSH)) when given continuously in therapeutic doses. Following an initial stimulation of GnRH receptors, chronic administration of leuprolide acetate results in downregulation of GnRH receptors, reduction in release of LH, FSH and consequent suppression of ovarian and testicular production of estradiol and testosterone respectively. This effect is reversible upon discontinuation of drug therapy.

3.2.2 Pharmacokinetics:

The Sponsor conducted a single PK/PD study (TOL2581A) in support of this 505(b)(2) application. The pharmacokinetics (absorption, distribution, metabolism and elimination) of leuprolide are covered in details in Section 3.3.

3.2.2.1 Drug-drug Interactions

The Sponsor did not conduct any drug-drug interaction studies. Drug-drug interactions are not anticipated because leuprolide acetate is degraded primarily by peptidases rather than cytochrome P450 enzymes and is weakly bound to plasma proteins.

3.2.2.2 Special Populations

3.2.2.2.1 Renal Impairment

No PK or PD studies were conducted with leuprolide acetate for injectable suspension, 45 mg in renally impaired patients.

3.2.2.2.2 Hepatic Impairment

No PK or PD studies were conducted with leuprolide acetate for injectable suspension, 45 mg in hepatically impaired patients.

3.2.2.2.3 Pediatric

Leuprolide acetate injection 45 mg was studied in pediatric patients in Study TOL2581A. Females age 2 to 8 years (inclusive) or males age 2 to 9 years (inclusive), with a confirmed diagnosis of CPP within 12 months of baseline visit (Day 0) but had not received prior GnRH agonist treatment for CPP were eligible to be included in the study. Details of the findings of the study are discussed in Section 3.3.

3.2.2.2.4 Effects of Age, Body Weight, Gender, Ethnicity and Race

The Sponsor did not evaluate the intrinsic factors on the pharmacokinetics of leuprolide acetate injection. Over the 48-week duration of Study TOL2581A, several steady-state leuprolide trough concentrations were available. Regression of leuprolide trough concentrations at week-24 (end of treatment period) from Study TOL2581A as a function of age or body weight did not show any correlation, indicating that these factors do not affect the PK of leuprolide (**Figure 1**). Literature reported population PK/PD modeling showed BMI and age had no effect on variability in PK parameters for a different leuprolide product, leuprorelin[1] in adults.

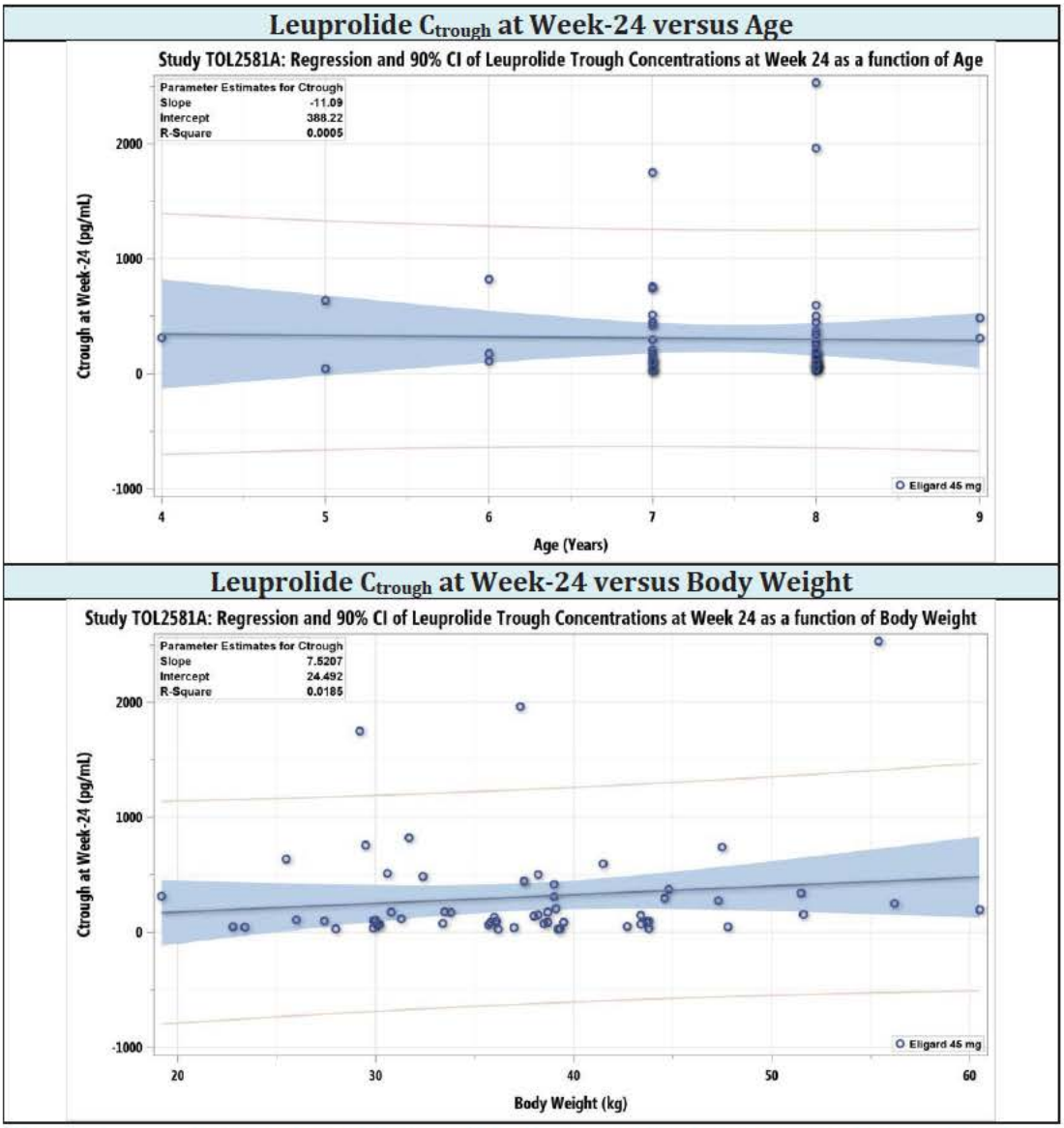

Figure 1 Regression of Leuprolide C_{trough} at Week-24 versus Age and Body Weight (n=60)

(Source: Reviewer generated plots)

[1] Lim, CN and Salem, AH . A Semi-Mechanistic Integrated Pharmacokinetic/Pharmacodynamic Model of the Testosterone Effects of the Gonadotropin-Releasing Hormone Agonist Leuprolide in Prostate Cancer Patients. *Clin Pharmacokinet*, 2015; **54** (9), 963-73

3.2.3 Pharmacodynamics:

The pharmacodynamic effects of leuprolide on GnRH receptors in pituitary, on basal LH and FSH, and on serum estradiol and testosterone are covered in details in Section 3.3.

3.2.4 QT Prolongation:

QT studies are not applicable in this age group.

3.3 Clinical Pharmacology Questions

3.3.1 Does the clinical pharmacology information provide supportive evidence of effectiveness?

Yes. The standard of care for patients with CPP is to treat with with GnRH agonists (GnRHa) such as leuprolide acetate. Treatment with leuprolide results in suppression of abnormal hormone release and pubertal development, and normalization of growth and skeletal maturation rates.

The primary objective of the study to achieve a post-GnRHa stimulation test serum LH levels below 4 IU/L was achieved for 80% of subjects. Basal serum LH anf FSH levels decreased by greater than 70% from baseline at week 4 and remained at this level for the duration of the trial. During the trial, sustained exposure to leuprolide resulted in mean basal serum estradiol levels to remain low and below the level indicative of suppression (73.4 pmol/L).

Study schematics for Study TOL2581a is shown in Figure 2.

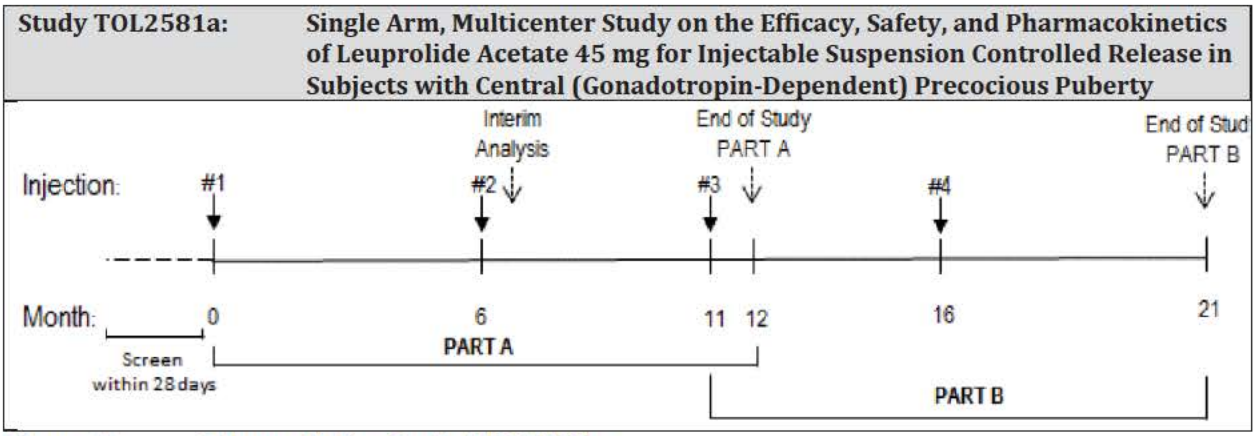

Figure 2 Schematic for Study TOL2581a

(Source: eCTD for NDA 213150, Module 5.3.5.1, CSR for Study TOL2581a, Figure 1, Page 31)

Pharmacokinetics:

Blood samples for quantitating serum leuprolide concentrations, were collected on Day 1 at 0 (pre-Dose), 1, 4, and 6 hours and at Weeks 4, 12, 20 and 24 post-administration. The second dose was administered at Week 24, and blood samples were collected at Weeks 36, 44 and 48 (final visit) for analyses of leuprolide and hormone levels.

Peak (C_{max}) leuprolide concentrations of 215.74 ± 163.24 ng/mL were achieved at 4 hours after the injection (T_{max}). Mean serum leuprolide concentration declined to 0.627 ± 0.553 ng/mL at 4 weeks post-injection, (Figure 3). At 12 weeks, mean serum leuprolide concentrations were 0.353 ± 1.47 ng/mL and remained steady until administration of the second dose at Week 24. After the second dose, mean serum concentrations of leuprolide were 0.317 ± 0.830 ng/mL at Week 36 (12 weeks after second dose), 0.411 ± 0.722 ng/mL at Week 44 (20 weeks after second dose) and 0.177 ± 0.240 (end of the second dosing interval, Week 48), which were similar to the serum leuprolide concentrations after the first dose, indicating that there was no accumulation of leuprolide from repeat administration.

Evaluation of the initial burst release (Day 0, 0-6 hours after the first dose of leuprolide) indicated that the area under the leuprolide concentration-time curve during the initial burst release ($AUC_{0-6\ hr}$) of 39.71 ± 29.54 day·ng/mL accounted for only a small portion (1.5%) of the overall AUC of 2720 ± 2602 day•ng/mL during the first dosing interval ($AUC_{0-169\ days}$). The $AUC_{Day\ 7-6\ mo.}$ which excluded first 7 days was estimated to be 1,771 ± 2,055 day·ng/mL. Mean plateau concentrations of leuprolide from Week 4 to the end of treatment at Week 48 ranged from 0.177 to 0.627 ng/mL.

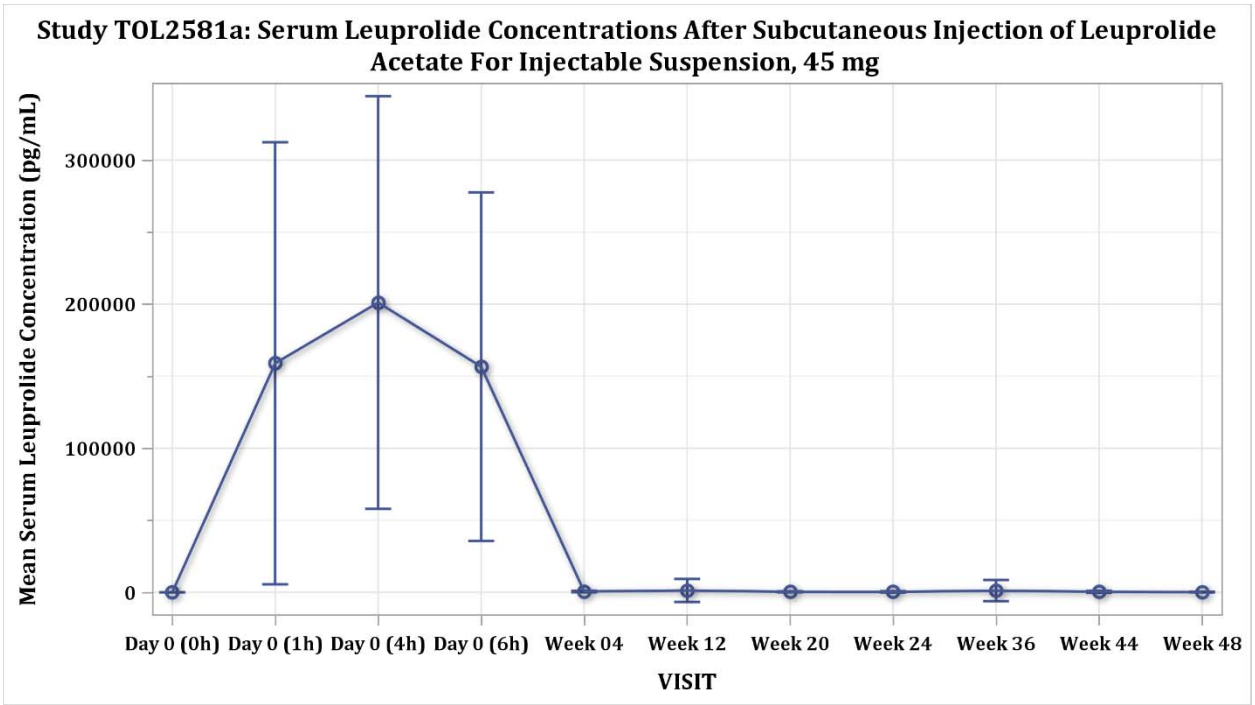

Figure 3 Mean (±SD) Serum Leuprolide Concentrations after Subcutaneous Injection of Leuprolide Acetate for Injectable Suspension, 45 mg (n=60)

(Source: Reviewer generated plot)

Pharmacodynamics

Serum Leutenizing Hormone Concentrations:

Following subcutaneous administration of leuprolide acetate injection, serum leuprolide levels showed an immediate rapid increase (burst phase). Mean serum LH levels at baseline were 3.4 ± 9.7 IU/L, and increased by nearly 12-fold above basal levels 4 hours post injection to a mean of 43.4 ± 43.7 IU/L and then began to decline after 6 hours. Mean basal serum LH levels were 0.8 ± 1.601 IU/L at Week 4 and the levels remained more or less constant for the rest of the treatment (Figure 4).

Fifty-two (52) of the 59 subjects (88%) from the ITT population with LH values at 6 months showed LH suppression with serum levels below 4 IU/L after the GnRHa stimulation test.

The GnRHa stimulation test at screening and after the burst in serum leuprolide concentrations following the first administration of leuprolide acetate for injectable suspension, 45 mg, resulted in substantial increases in gonadotropin concentrations for a short time in GnRHa-naïve subjects. Release of LH and FSH were suppressed once subjects received sustained delivery of leuprolide acetate.

Serum FSH:

Mean serum FSH levels at baseline were 3.9 ± 2.5 IU/L, and reached a maximum level of 26.3 ± 12.5 IU/L 6 hours post injection and began to decline to concentrations between 0.99 and 1.45 IU/L from Weeks 4 to 48. At 36 weeks, FSH levels (1.4 ± 0.8 IU/L) remained below baseline until the end of treatment at 48 weeks (1.5 ± 0.9 IU/L) (Figure 4).

Serum estradiol and testosterone:
Serum estradiol was measured using two different assays, the first method used chemiluminescent microparticle immunoassay and the second method used a LC-MS/MS high sensitivity assay. Values obtained using the LC-MS/MS assay only will be reported in this section. Mean serum estradiol levels at baseline were 92.5 ± 93.9 pmol/L and dropped to 54.2 ±94.0 pmol/L. Mean basal serum estradiol levels remained low for the duration of the study and were below the level indicative of suppression (73.4 pmol/L) (Figure 4).

There were only 2 males in the study. Average (n = 2) basal testosterone concentration decreased from a baseline value of 9.90 nmol/L to 0.85 nmol/L at Week 4. Basal testosterone levels remained low through Week 48 of treatment.

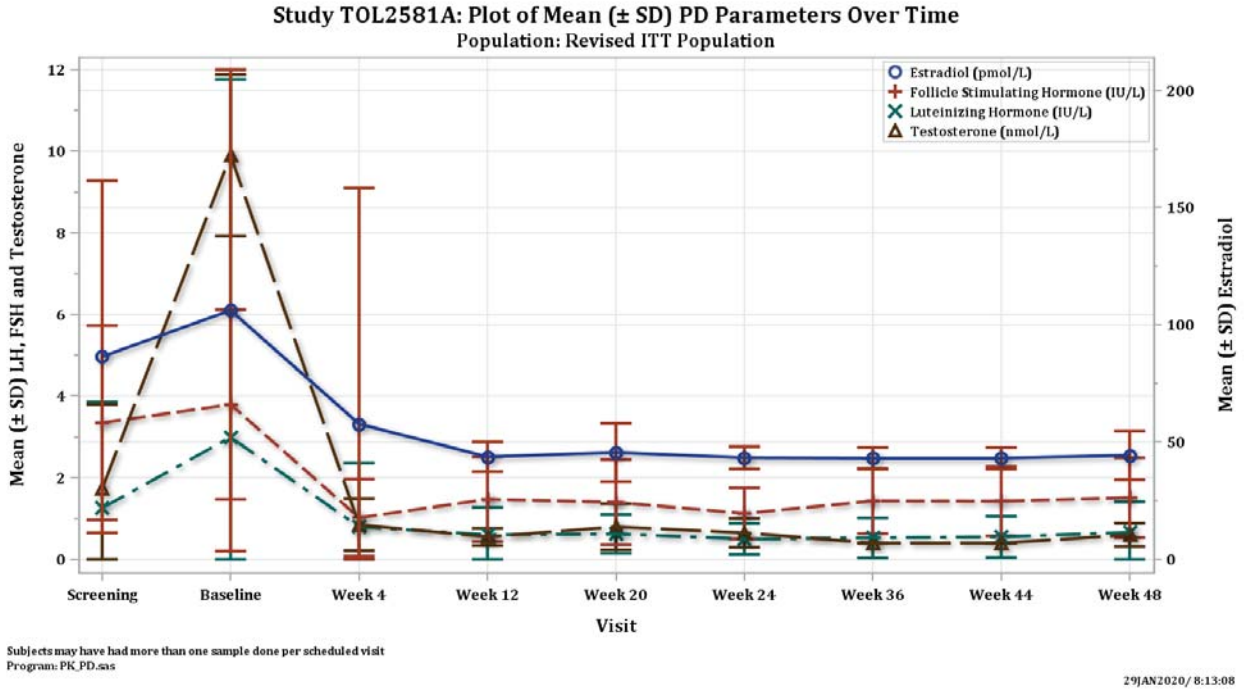

Figure 4 Mean (±SD) Serum LH, FSH, Estradiol and Testosterone concentrations after Subcutaneous Injection of Leuprolide Acetate for Injectable Suspension, 45 mg (n=60)

(Source: Reviewer generated plot)

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

Yes, the proposed general dosing regimen of FENSOLVI administered as a 45 mg single subcutaneous injection once every six months is appropriate for children with CPP. The dose of FENSOLVI does not require individualization for each child.

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

The Sponsor did not evaluate the intrinsic factors on the pharmacokinetics of leuprolide acetate injection. Literature reported population PK/PD modeling (see citation in Section 3.2.2.6.4) showed BMI and age had no effect on variability in PK parameters for a different leuprolide product, leuprorelin. However, the population PK/PD modeling that the Sponsor cited is based on adult data from prostrate cancer patients (age range 56-92 years, BMI range 22.6 – 35.3 kg/m^2) and healthy adults (age range 23-31 years, BMI range 21.0 – 30.0 kg/m^2). Exploratory analysis of observed leuprolide trough concentrations in Study TOL2581a did not show any correlation to age or body weight (Section 3.2.2.2.4).

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

The Sponsor did not conduct any drug-drug interaction studies. Drug-drug interactions are not anticipated because leuprolide acetate is degraded primarily by peptidases rather than cytochrome P450 enzymes and is weakly bound to plasma proteins.

3.3.5 Is the to-be-marketed formulation the same as the clinical trial formulation, and if not, are there bioequivalence data to support the to-be-marketed formulation?

Leuprolide acetate for injectable suspension, 45 mg is the same product as Tolmar's currently marketed ELIGARD® 45mg (NDA 021731) for the palliative treatment of advanced prostate cancer. It differs only in terms of labeling and intended use. Study TOL2581a used ELIGARD for evaluation of PK/PD of leuptolide in children with CPP.

4. APPENDICES

TOL2581A was the only study conducted by Tolmar in support of this application.

4.1 Summary of Bioanalytical Method Validation

4.1.1 How are leuprolide, LH, FSH, estradiol and testosterone identified and what are the analytical methods used to measure them in serum?

Leuprolide in serum was quantitated using a validated high-performance LC-MS/MS method and automated extraction. Commercial diagnostic kits were used for analysis of the LH, FSH, and testosterone. Two methods were used for estradiol analysis: a commercial diagnostic kit and a high sensitivity liquid chromatography tandem mass spectrophotometric (LC-MS/MS) method. All assays were validated in accordance to appropriate regulatory guidances.

Leuprolide:
The method for analysis of leuprolide in serum was developed and validated by (b) (4) using a validated high-performance LC-MS/MS method and automated extraction. The initial validation range for the LC-MS/MS method was over a range of 100 pg/mL to 100,000 pg/mL. The method was modified slightly to decrease the lower limit of quantitation (LLOQ) when it was determined that the original method was not sufficiently sensitive. The modified method for leuprolide was validated over a range of 25 pg/mL to 50,000 pg/mL. Both methods were validated according to predefined criteria for precision, accuracy and stability of the analyte.

Estradiol:
The method for analysis of estradiol in serum was developed and validated by (b) (4) using a liquid chromatography with tandem mass spectrophotometric detection (LC-MS/MS) using a solid phase extraction procedure to remove interferences. Human serum samples (200 µL) containing the analyte and internal standard were processed through the extraction process, and subsequently were evaporated to dryness and derivatized with dansyl chloride before being analyzed by LC-MS/MS. The validated range of the assay was 10–1000 pg/mL (36.71-3671 pmol/L). Estradiol concentrations at or below the LLOQ of 36.713 pmol/L were reported as "< 36.713 pmol/L." A few aliquots had sample volumes less than 200 µL. For these samples, 100 µL aliquots were diluted 1:2 and LLOQ values were reported as "< 73.426 pmol/L."

Leutenizing Hormone:
Serum luteinizing hormone levels were quantitated using the sandwich principle in which a biotinylated monoclonal LH-specific antibody and a monoclonal LH-specific antibody labeled with ruthenium complex reacted to form a sandwich complex (first incubation). Subsequent to this step, streptavidin coated microparticles were added to bind the complex to the solid phase via interaction of biotin and streptavidin. Microparticles were magnetically captured on the surface of the electrode and unbound materials removed. A photomultiplier measured and amplified the induced chemiluminescent emission after voltage was applied to the electrode. According to (b) (4) the kit manufacturer, the method was validated over a linear range of 0.100 IU/L to 200 IU/L.

FSH:
Serum FSH concentrations were determined using a sandwich principle with monoclonal FSH-specific antibodies. According to (b) (4) the kit manufacturer, the method was was validated over a linear range of 0.100 IU/L to 200 IU/L.

Testosterone:
Serum testosterone levels in male subjects were measured using the competitive test principle with a monoclonal antibody specific for testosterone using the cobas e-immunoassay analyzer. Testosterone was released from the sample with the aid of 2-bromoestradiol. Endogenous testosterone competed with added testosterone-ruthenium complex for sites on microparticles and was analyzed in a similar manner as described above for estradiol. According to (b) (4) the kit manufacturer, the method was was validated over a linear range of 0.087 nmol/L to 52.0 nmol/L.

A summary of the method used for leuprolide and estradiol is presented in Table 4.1.1-1.

Table 4.1.1-1: Summary of Leuprolide and Estradiol Validated Analytical Methods

Method Validation Report	Laboratory	Compound	LLOQ	Linear Range	Matrix
115194AETX	(b) (4)	Leuprolide (LC/MS/MS)	25 pg/mL	25.00 – 50000.00 pg/mL	Human Serum
V/E2/HS		Estradiol	10 pg/mL	10 - 1000 pg/mL	Human Serum

(Source: eCTD for NDA 209803, Module 2.7.1 Summary of Biopharmaceutic Studies and Associated Analytical Methods, Table 5, Page 18)

4.1.2 What was the performance of bioanalytical methods?

The analytical methods were found to be selective, sensitive, precise, and accurate for the determination of leuprolide and estradiol in human serum. The effective analytical ranges were as follows:

Leuprolide: 25.0 – 50000 pg/mL

Estradiol: 10.0 – 1000 pg/mL

The between run precision of the assay, as determined by the percent coefficient of variation were as follows:

Leuprolide: 2.90 % to 10.78 %

Estradiol: 3.6 % to 9.5 %

Performance details of the assays are presented in Table 4.1.2-1.

Table 4.1.2-1: Bioanalytical Methods Summary

Full Validation Report No.:	115194AETX (SOP ANI 10350)
Full Validation Report Title:	Validation of a High Performance Liquid Chromatographic Method using Tandem Mass Spectrometry Detection and Automated Extraction for the Determination of Leuprolide (100 to 100000 pg/mL) in Human Serum
Full Validation Report Effective Date:	16-JAN-2014
Validation Calibration Range:	99.60 to 99600.00 pg/mL (refer to the validation report in section 16.2.5.3 including the raw numerical data) The difference between the validation and study sample analysis calibration ranges is due to the use of different analyte stock solution preparations
Between-Run Accuracy and Precision:	Biases: 1.27 to 3.76% CV: 1.79 to 5.27%
Within-Run Accuracy and Precision:	Biases: -0.06 to 5.12% CV: 1.27 to 9.34%
Freeze and Thaw Stability:	4 cycles at -20°C
Short-Term Stability of Analyte in Matrix:	23h30min at room temperature
Long-Term Stability of Analyte in Matrix:	968 days at -20°C
Post-Preparative Stability:	118h35min at room temperature
Maximum Run Size:	192 samples

Full Validation Report No.:	115194AMDW (SOP ANI 10938)
Full Validation Report Title:	Validation of a High Performance Liquid Chromatographic Method using Tandem Mass Spectrometry Detection and Automated Extraction for the Determination of Leuprolide (25 to 50000 pg/mL) in Human Serum
Full Validation Report Effective Date:	24-MAR-2016
Validation Calibration Range:	25.00 to 50000.00 pg/mL (refer to the validation report in section 16.2.5.3 including the raw numerical data)
Between-Run Accuracy and Precision:	Biases: -2.23 to 1.86% CV: 2.90 to 10.78 %
Within-Run Accuracy and Precision:	Biases: 0.13 to 3.95% CV: 0.76 to 4.62%
Freeze and Thaw Stability:	4 cycles at -20°C
Short-Term Stability of Analyte in Matrix:	24h40min at 4°C
Long-Term Stability of Analyte in Matrix:	563 days at -20°C
Post-Preparative Stability:	97h25min at room temperature
Maximum Run Size:	192 samples

(Source: eCTD for NDA 213150, Module 5.3.1.4 Bioanalytical Report LA, pp 23-24)

The parameters and validation metrics used for the LC-MS/MS assay are presented in Table 4.1.2-2.

Table 4.1.2-2: Parameters and Validation Metrics for LC-MS/MS Assay (No. B1529008)

VALIDATION SUMMARY			
Analyte		Estradiol	
Internal standard		Estradiol-D_5	
Matrix (Anticoagulant)		Human serum	
SOP Number		SOP 5-132.0	
Analytical Method		High performance liquid chromatography with tandem mass spectrometric detection	
Detector		AB Sciex API 4000 and Sciex QTRAP 6500	
Human Serum Volume Required		200 µL	
Standard Curve Range		10.0 – 1000 pg/mL	
QC concentrations		18.4 or 30.0, 118 or 120, 800 or 818 pg/mL(variable due to endogenous levels)	
Regression Type		Linear (weighted 1/concentration^2)	
Quantification Method		Peak area ratio	
Selectivity		No interfering peaks noted in blank serum samples	
LLOQ Validation Samples		Precision (%)	Accuracy (%)
Inter-batch		8.4	94.2
Intra-batch		3.6 to 9.5	88.8 to 97.5
Quality Control Samples		Precision (%)	Accuracy (%)
Inter-batch	Low	5.7	87.1
	Medium	5.3	98.1
	High	6.7	100.7
Intra-batch	Low	5.7 to 7.7	86.7 to 87.4*
	Medium	2.9 to 5.5	95.3 to 101.9
	High	1.8 to 2.5	93.5 to 108.6
Recovery		Recovery (%)	
Analyte	Low	71.2	
	Medium	64.2	
	High	53.3	
Long-term Stability		417 days at -20°C	
Short-term Stability		24 hours at room temperature and 5°C	
Freeze and Thaw Stability		4 cycles at -20°C	
Primary Stock Solution Stability		428 days at -20°C and 6 hours at room temperature	
Diluted Stock Solution Stability		427 days at -20°C and 6 hours at room temperature	
Internal Standard Stock Stability		Inferred from analyte	
Processed Sample Stability		12 days at 5°C	
Processed Sample Reinjection Stability		4 days at room temperature and 5°C	

(Source: eCTD for NDA 213150, Module 5.3.1.4 Validation Report E2HS, page 10)

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/

SURYANARAYANA M SISTA
03/24/2020 04:31:30 PM

JAYABHARATHI VAIDYANATHAN
03/24/2020 04:44:44 PM