CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761116Orig1s000

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology Review	
BLA	761116
Type/Category	NME
Submission Date	6/21/2018
PDUFA Goal Date	2/21/2019
Applicant	Stemline Therapeutics Inc
Brand Name	ELZONRIS
Generic name	Tagraxofusp
Formulation and Strength	1mg/mL in a single-use vial
Route of Administration	Intravenous infusion
Proposed Indication	Treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN)
Proposed Dosing Regimen	12 µg/kg/day over 15 minutes once daily on days 1-5 of a 21-day cycle.
Recommended Indication	Treatment of BPDCN in adults and in pediatric patients 2 years and older
OCP Divisions	Division of Clinical Pharmacology V (DCPV) Division of Pharmacometrics (DPM)
OND Division	Division of Hematology Products (DHP)
OCP Primary Reviewers	Liang Li, Ph.D.; Luning Zhuang, Ph.D.
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Executive Summary

ELZONRIS (tagraxofusp) is a CD123-directed cytotoxin, which is composed of recombinant human interleukin-3 (IL-3) and truncated diphtheria toxin (DT) fusion protein that inhibits protein synthesis and causes cell death in CD123-expressing cells. The Applicant is seeking an approval for the treatment of blastic plasmacytoid dendritic cell neoplasm (BPDCN) in adults and in pediatric patients 2 years and older at a proposed dosing regimen of 12 mcg/kg administered intravenously over 15 minutes once daily on days 1 to 5 of a 21-day cycle. To support the registration of this Biologics License Application (BLA), the Applicant conducted a multi-stage, nonrandomized, open-label, multicenter, registrational Trial STML-401-0114 (hereinafter referred to as "Trial 0114") in 32 treatment-naïve patients and 15 relapsed/ refractory (R/R) patients with BPDCN.

The Clinical Pharmacology Section of the BLA is supported by single and repeat dose pharmacokinetics (PK) characterization of tagraxofusp, population pharmacokinetics (popPK) and exposure-response (ER) analyses, and immunogenicity assessment. The key review questions focus on: 1) the appropriateness of the proposed dosing regimen for the treatment of BPDCN in adults and in pediatric patients 2 years and older; 2) the effect of anti-drug antibody (ADA) on the PK, efficacy and safety of tagraxofusp for BPDCN; and 3) the requirement of an alternative dosing regimen and/or management strategy for subpopulations based on intrinsic patient factors.

In general, the proposed dose regimen of ELZONRIS is approvable based on a complete response/clinical complete response (CR/CRc) rate of 53.8% (95% confidence interval [95% CI]: 25.1, 80.8) in 13 treatment -naïve patients and one CR and one CRc in 15 R/R patients in Trial 0114. In addition, ELZONRIS appears to have a tolerable and manageable safety profile. The presence of anti-drug antibodies (ADAs) had an effect on the PK of tagraxofusp-xxx; however, dose adjustment is not recommended based on ADAs due to the life-threatening nature of the disease, lack of standard of care, and high childhood and booster vaccination rate in the population. Additionally, no dose modification is needed for specific populations based on other intrinsic and extrinsic factors.

1.1.Recommendations

The Office of Clinical Pharmacology has reviewed the information contained in BLA 761116. This BLA is approvable from a clinical pharmacology perspective. The key review issues with specific recommendations and comments are summarized below:

Review Issues	Recommendations and Comments
Evidence of effectiveness	A non-randomized, open-label, multi-stage, dose-escalation and expansion Trial 0114 provides primary evidence. Exposure-response relationship for best overall response (BOR) provides supportive evidence.

General Dosing instructions	Administer ELZONRIS at 12 mcg/kg intravenously over 15 minutes once daily on days 1 to 5 of a 21-day cycle. The dosing period may be extended for dose delays up to day 10 of the cycle. Continue treatment with ELZONRIS until disease progression or unacceptable toxicity.
Dosing in patient subgroups (intrinsic and extrinsic factors)	No dose modification is needed for specific populations of age (22 to 84 years), sex, mild to moderate renal impairment (eGFR 30 to 89 mL/min/1.73 m^2, estimated by MDRD), mild (total bilirubin ≤ ULN and AST >ULN, or total bilirubin 1 to 1.5 times ULN and any AST) or moderate (total bilirubin >1.5 to 3 times ULN and any AST) hepatic impairment or body weight after adjusting dose by body weight. The effect of severe renal impairment (eGFR 15 to 29 mL/min/1.73 m^2), or severe hepatic impairment (total bilirubin >3 times ULN and any AST) on tagraxofusp pharmacokinetics is unknown.

1.2.Post-Marketing Requirements and Commitments

There is no Post-Marketing Requirement (PMR) or Post-Marketing Commitment (PMC) from clinical pharmacology perspective.

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2. SUMMARY OF CLINICAL PHARMACOLOGY ASSESSMENT

2.1.Pharmacology and Clinical Pharmacokinetics

Tagraxofusp is a CD123-directed cytotoxin (molecular weight 58 kDa) composed of recombinant human interleukin-3 (IL-3) and truncated diphtheria toxin (DT) fusion protein that inhibits protein synthesis and causes cell death in CD123-expressing cells.

Following the administration of tagraxofusp 12 mcg/kg via 15-minute infusion in patients with BPDCN, the mean (standard deviation [SD]) area under the plasma concentration over time curve (AUC) was 231 (123) hr·mcg/L, the maximum plasma concentration (C_{max}) was 162 (58.1) mcg/L, the volume of distribution was 5.1 (1.9) L, the clearance was 7.1 (7.2) L/hr, and the terminal half-life was 0.7 (0.3) hours.

Among 130 patients treated with ELZONRIS in 4 clinical trials (STML-401-0114, STML-401-0214, STML-401-0314, and STML-401-0414):

- 96% (115/120) of patients evaluable for the presence of pre-existing anti-drug antibodies (ADAs) at baseline before treatment were confirmed positive with 21% being positive for the presence of neutralizing antibodies. The high prevalence of ADAs at baseline was anticipated due to diphtheria immunization.
- 99% (107/108) of patients evaluable for treatment-emergent ADA tested positive with most patients showing an increase in ADA titer by the end of Cycle 2 of ELZONRIS.
- 85% (86/101) of ADA-positive patients evaluable for the presence of neutralizing antibodies were neutralizing antibody-positive.
- 68% (73/108) of patients evaluable for treatment-emergent anti-IL-3 antibodies tested positive with most patients testing positive by Cycle 3 of ELZONRIS.

The presence of ADAs had a meaningful effect on the pharmacokinetics of tagraxofusp-xxx. Following administration of tagraxofusp 12 mcg/kg via 15-minute infusion in patients with pre-existing ADA, the mean (SD) AUC of tagraxofusp was 151 (89.2) hr·mcg/L, the C_{max} was 80.0 (82.2) mcg/L, the volume of distribution was 21.2 (25.4) L, and the clearance was 13.9 (19.4) L/hr. The AUC and C_{max} were reduced by 34.6% and 50.6%, respectively, and clearance was increased by 1.96-fold in patients with pre-existing ADA versus the patients without pre-existing ADA.

2.2.Dosing and Therapeutic Individualization

2.2.1. General dosing

The Applicant proposed an ELZONRIS dosing regimen of 12 mcg/kg intravenously over 15 minutes once daily on days 1 to 5 of a 21-day cycle. The dosing period may be extended for dose delays up to day 10 of the cycle. Continue treatment with ELZONRIS until disease progression or unacceptable toxicity. Tagraxofusp at the proposed dosing regimen appears to be effective and has a manageable safety profile in 29 treatment-naïve patients and 15 relapsed and/or refractory patients with BPDCN in Trial STML-401-

0114. The use of body weight-based dosing regimen for ELZONRIS appears to be acceptable, as the population PK analysis indicated that clearance and volume of distribution increased with baseline body weight.

2.2.2. Therapeutic individualization

No therapeutic individualization for intrinsic or extrinsic factors is recommended by the population PK analysis. The positive effect of baseline body weight on clearance and volume of distribution supports the body-weight-based dosing regimen. Although mild to moderate renal function (eGFR 30 to 89 mL/min/1.73 m^2, estimated by MDRD) was identified as a statisitically significant covariate on clearance of free tagraxofusp, the parameter characterizing the renal effect was estimated with large uncertainty. Therefore, no definitive conclusion about the impact of mild to moderate renal impairment on tagraxofusp exposure could be made. The population PK analysis also showed that other factors, e.g., age (22 to 84 years), mild (total bilirubin ≤ ULN and AST >ULN, or total bilirubin 1 to 1.5 times ULN and any AST) or moderate (total bilirubin >1.5 to 3 times ULN and any AST) hepatic impairment were not significant covariates on PK parameters after adjusting dose by body weight.

2.2.3. Outstanding Issues

There are no outstanding issues at this time.

2.2.4. Summary of Labeling Recommendations

Section 6.2 Immunogenicity: The impact of immunogenicity on the pharmacokinetics of tagraxofusp is recommended stated in this section as follows:

The presence of ADA had a clinically significant effect on the pharmacokinetics of tagraxofusp.

Section 12.2 (b) (4)

(b) (4) Pharmacokinetics: This section was revised in light of current labeling practices and new guidance document "Clinical Pharmacology Section of Labeling for Human Prescription Drug and Biological Products - Content and Format". The PK parameters were recommended focusing on the BPDCN patient population (b) (4) The impact of ADAs on the PK of tagraxofusp is recommended stated in detail in this section. The PK parameters in patients with pre-existing ADAs are recommended stated separately in this section.

3. COMPREHENSIVE CLINICAL PHARMACOLOGY REVIEW

3.1.Overview of the Product and Regulatory Background

ELZONRIS (tagraxofusp) is a CD123-directed cytotoxin composed of recombinant human IL-3 and truncated DT fusion protein that inhibits protein synthesis and causes cell death in CD123-expressing cells, indicated for the treatment of BPDCN in adults and in pediatric patients 2 years and older at a proposed dosing regimen of 12 mcg/kg administered intravenously over 15 minutes once daily on days 1 to 5 of a 21-day cycle.

From a clinical pharmacology perspective, the Applicant submitted the following clinical study results in this original BLA:

- Pivotal PK, efficacy and safety data from the registrational Trial 0114: a multi-stage, nonrandomized, open-label, multicenter trial in 32 treatment-naïve patients and 15 relapsed/ refractory (R/R) patients with BPDCN.
- Supportive PK and safety data from the following trials:
  - Trial STML-401-0214 (Trial 0214), a Phase 1/2 non-randomized, open-label, 2 stage dose-escalation study of tagraxofusp as monotherapy in patients with adverse risk AML in first or second CR, with or without evidence of minimal residual disease in first CR.
  - Trial STML-401-0314 (Trial 0314), a Phase 1/2 non-randomized, open-label, 2 stage dose-escalation study of tagraxofusp as monotherapy in patients with advanced, high-risk myeloproliferative neoplasms.
  - Trial STML-401-0414 (Trial 0414), a Phase 1/ 2 non-randomized, open-label, 2 stage dose-escalation study of tagraxofusp in combination with pomalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma.
- Pharmacometic analysis reports:
  - Study STM0102F: population PK analysis of free tagraxofusp in patients with hematological cancers.
  - Study STM0103F: evaluation of the tagraxofusp exposure-ECG relationship in patients with hematological cancers
  - Study STM0104F: exposure-response relationships for tagraxofusp in patients with BPDCN and other hematologic malignancies.

3.2.General Pharmacological and Pharmacokinetic Characteristics

The summary of clinical pharmacology, pharmacokinetics and ADME information of tagraxofusp is listed below.

Pharmacology	
Mechanism of Action	Tagraxofusp is a CD123-directed cytotoxin composed of recombinant human interleukin-3 (IL-3) and truncated diphtheria toxin (DT) fusion

	protein that inhibits protein synthesis and causes cell death in CD123-expressing cells.
Active Moieties	Tagraxofusp is the active moiety
QT Prolongation	At the approved recommended dosage, ELZONRIS does not prolong the QTc interval, nor does it prolong QTc in a concentration dependent manner.
General Information	
Bioanalysis	Tagraxofusp was measured using a dual antibody sandwich immunoassay (IA) that employs an electrochemiluminescence (ECL) detection label.
Drug total exposure following the therapeutic dosing regimen	The mean (SD) area under the plasma drug concentration over time curve (AUC) was 231 (123) hr·mcg/L and maximum plasma concentration (C_{max}) was 162 (58.1) mcg/L after first dose (Cycle 1, Day 1). Tagraxofusp exposure was below lower limit of quantitation (LLOQ) at steady state in most of patients due to meaningful effect of ADAs.
Minimal effective dose or exposure	12 mcg/kg over 15 minutes once daily on days 1 to 5 of a 21-day cycle. There was no efficacy data below 12 mcg/kg dosing regimen.
Dose Proportionality	The dose proportionality of tagraxofusp exposure could not be fully assessed due to the significant impact of pre-existing and treatment-emergent ADAs across the dose groups.
Accumulation	Exposures of free tagraxofusp on Cycle 1 Day 5 were generally higher than Cycle 1 Day 1, and were markedly reduced in Cycle 3 as compared with Cycle 1 due to significant effect of pre-existing and treatment-emergent ADAs.
Variability	The %CV was 35.9% for C_{max} and 53.2% for AUC after the first dose (Cycle 1, Day 1). The %CV of C_{max} and AUC was not available due to meaningful effect of ADAs on tagraxofusp exposure.
Immunogenicity	In 130 patients treated with ELZONRIS in 4 clinical trials (0114, 0214, 0314 and 0414): • 96% (115/120) of patients evaluable for the presence of pre-existing ADA at baseline before treatment were confirmed positive with 21% being positive for the presence of neutralizing antibodies. The high prevalence of ADA at baseline was anticipated due to diphtheria immunization.

	• 99% (107/108) of patients evaluable for treatment-emergent ADA tested positive with most patients showing an increase in ADA titer by the end of Cycle 2 of ELZONRIS. • 85% (86/101) of ADA-positive patients evaluable for the presence of neutralizing antibodies were neutralizing antibody-positive. • 68% (73/108) of patients evaluable for treatment-emergent anti-IL-3 antibodies tested positive with most patients testing positive by Cycle 3 of ELZONRIS. Pre-existing and treatment-emergent ADAs had significant impact on the PK of tagraxofusp. Following administration of tagraxofusp 12 mcg/kg via 15-minute infusion in patients with pre-existing anti-drug antibodies, the mean (SD) volume of distribution of tagraxofusp is 21.2 (25.4) L, clearance is 13.9 (19.4) L/hr, AUC is 151 (89.2) hr·mcg/L and Cmax is 80.0 (82.2) mcg
Distribution	
Volume of Distribution	The mean (SD) volume of distribution was 5.1 (1.9) L.
Plasma Protein Binding	Not evaluated. As a fusion protein with a molecular weight of 58 kDa, tagraxofusp is not expected to bind to plasma proteins.
Blood to Plasma Ratio	Not evaluated.
Substrate transporter	Not evaluated. As a fusion protein, tagraxofusp is not expected to be a substrate of metabolic transporters.
Elimination	
Mean terminal elimination half-life	Mean (SD) terminal half-life is 0.7 (0.3) hours.
Metabolism	
Primary metabolic pathway(s)	Not evaluated. As a fusion protein, tagraxofusp is not expected to be metabolized by CYP enzymes.
Inhibitor/Inducer	Not evaluated.
Excretion	

Primary excretion pathways (% dose) ± SD	No evaluated. As a fusion protein with a molecular weight of 58 kDa, tagraxofusp is expected to be eliminated via degradation and renal pathways.

3.3.Clinical Pharmacology Questions

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

Yes. In general, the proposed tagraxofusp dosing regimen is effective and appears to have a tolerable and manageable safety profile.

The median [min, max] time to CR/CRc following tagraxofusp was 2 [0.7, 6.2] cycles in treatment-naïve patients and 1.1 [0.8, 2.1] cycles in relapsed and/or refractory patients. The review team explored whether treatment should be restricted given observed increase in ADA and undetectable exposure in plasmaafter Cycle 3, and whether that may affect response.

However, tagraxofusp exhibited high potent activity in *in-vitro* and *in-vivo studies* with an IC_{50} of around 10 femotomolar, which is approximately 1,000,000 times lower than the LLOQ for the determination of tagraxofusp in plasma. Therefore, it cannot be precluded that patients may benefit from the continuous treatment despite undetectable plasma concentration. Additionally, the median [minimun, maximun] duration of complete response in responders who received treatment > 6 cycles was 9.9 [1.3, 32.2] months, which was slightly longer than that of 8.3 [1.4, 27.2] months in responders who received treatment ≤ 6 cycles. Overall, there is no strong rationale to support restricting treatment duration. Therefore, the proposed dosing regimen is appropriate for the general population for which the indication is being sought.

3.3.2. Does ADA affect the PK, efficacy and safety of tagraxofusp in patients with BPDCN?

Yes. The presence of ADA had a significant effect on the PK, efficacy and safety of tagraxofusp in patients with BPDCN.

Immune response to tagraxofusp was characterized in terms of total ADA reactive to diptheria (DT) or interleukin-3 (IL-3) portion of tagraxofusp, total anti-tagraxofusp neutralizing antibodies (NAbs), anti-IL-3 antibodies reactivity against the IL-3 portion (anti-IL-3 Abs), and anti-IL-3 neutralizing antibodies (anti-IL-3 NAbs). A total of 96% (115/120) of patients with BPDCN had positive ADA at baseline due to immunization against DT; and 21% (18/91) of these patients were positive for the presence of NAbs. Following the treatment of tagraxofusp at the proposed dosing regimen, 99% (107/108) of patients were ADA-positive and 85% (86/101) were NAb-positive. Furthermore, 68% (73/108) of patients evaluable for treatment-emergent anti-IL-3 antibodies tested positive with most patients testing positive by Cycle 3 of tagraxofusp administration.

Effect on PK

Table 1 summarizes free tagraxofusp exposure by ADA titer on Cycle 1 Day 1 (C1D1) and Cycle 3 Day 1 (C3D1) at the proposed dosing regimen in patients with BDPCN or AML. AUC_{last} of tagraxofusp on C1D1

was inversely correlated with ADA titer at baseline. In addition, PK data obtained following doses given in Cycle 3 showed increased titers of ADA and reduced free tagraxofusp concentration in most plasma samples. Following administration of tagraxofusp 12 mcg/kg via 15-minute infusion in patients with pre-existing ADAs, the mean (SD) clearance and volume of distribution of tagraxofusp were 13.9 (19.4) L/hr and 21.2 (25.4) L, respectively, which were greater than those in patients without pre-existing ADAs. The mean (SD) AUC of 151 (89.2) hr·mcg/L and C_{max} of 80.0 (82.2) mcg/L were significantly lower in patients with pre-existing ADAs as compared to those in patients without pre-existing ADAs. Overall, these results suggest that the presence of ADAs is a determinant of exposure to tagraxofusp.

Table 1 Free Tagraxofusp Exposure by ADA Titer on Cycle 1 Day 1 and Cycle 3 Day 1, 12 **µg/kg/day.**

	AUC_{last} (h•µg/L)					
	Cycle 1 Day 1 (N = 77)			Cycle 3 Day 1(N = 31)		
ADA Titer	**N**	**Mean**	**StD**	**N**	**Mean**	**StD**
Not Detected	5	183	130	1	0.246	--
1	1	1.42	--	--	--	--
8	3	208	109	--	--	--
80	12	162	57.3	--	--	--
800	37	66.6	69.7	1	6.14	--
8000	16	32.9	57.4	2	0	--
80000	3	3.96	2.84	9	1.71	2.64
800000	--	--	--	16	1.11	2.30
8000000	--	--	--	2	0	--

Abbreviations: AUC_{last} = AUC from time 0 to last quantifiable observation; StD = standard deviation
Note: (Study 0114; May 7, 2017 Data Cutoff)
Source: Summary of Clinical Pharmacology Studies, Figure 1.

Effect on Efficacy and Safety

The efficacy and safety in treatment-naïve and relapsed/refractor patients with BPDCN with anti-tagraxofusp NAb at baseline and post-treatment are summaried in Table 2.

Table 2 Summary of Efficacy and Safety in Patients with BPDCN with Anti-tagraxofusp Neutralizing Antibodies at Baseline and Post-treatment.

	Treatment-naïve BPDCN (N = 25)		Relapsed/refractory BPDCN (N = 13)		
	Baseline NAb+ to post-trt NAb+ (n = 6)	Baseline NAb- to post-trt NAb+ (n = 19)	Baseline NAb+ to post-trt NAb+ (n = 3)	Baseline NAb- to post-trt NAb+ (n = 8)	Baseline NAb- to post-trt NAb- (n = 2)
CR+CRc Rate % [95% CI]	17 [0.4, 64]	74 [49, 91]	0	25 [3, 65]	50 [1, 99]
Grade ≥ 3 TEAEs % [95% CI]	67 [22, 96]	81 [58, 95]	67 [9, 99]	88 [47, 100]	100 [16, 100]

Source: Reviewer's analysis.

The rate [95% CI] of complete response (CR) plus complete response with minimal residual skin abnormality (CRc) was 74% [49%, 91%] in 19 treatment-naïve patients who had negative NAb at baseline and developed positive NAbs post-treatment, compared to 17% [0.4%, 64%] in 6 treatment-naïve patients who had positive NAbs at baseline and post-treatment. A similar trend was also observed in relapsed and/or refractory patients. The CR+CRc rate was 50% in 2 patients with negative NAb at baseline and post-treatment; 25% in 8 patients with negative NAb at baseline and positive NAbs post-treatment; and 0% in 3 patients with positive NAbs at baseline and post-treatment. These results indicate that the efficacy of ELZONRIS is affected by pre-existing NAbs, and may also affected by treatment-emergent NAbs in patients with BPDCN.

On the other hand, the rate of at least one Grade ≥3 TEAE was higher in patients without NAbs compared to those with NAbs at baseline and/or post-treatment.

Although there were effects of pre-existing and treatment-emergent ADAs on the PK, efficacy and safety of ELZONRIS in patients with BPDCN, there is no dose adjustment recommended for patients with pre-existing and treatment-emergent ADAs, given the life-threatening nature of the disease, lack of standard of care, and high childhood and booster vaccination rate in the population.

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

No. Population PK analysis (n = 127) showed that patient demographics such as sex, age (range: 22 - 84 years), albumin (29 - 44 g/L), and mild (total bilirubin ≤ ULN and AST >ULN, or total bilirubin 1 to 1.5 times ULN and any AST) or moderate (total bilirubin >1.5 to 3 times ULN and any AST) hepatic impairment were not found to have a clinically meaningful influence on the PK parameters of tagraxofusp after adjusting dose by body weight. The effect of severe hepatic impairment (total bilirubin >3 times ULN and any AST) on the PK of tagraxofusp is unknown. Clearance and volume of distribution increased with baseline body weight (47 - 162 kg); however, the effect of body weight on exposure is inconclusive due to large uncertainty in covariate effect estimates (Figure 1).

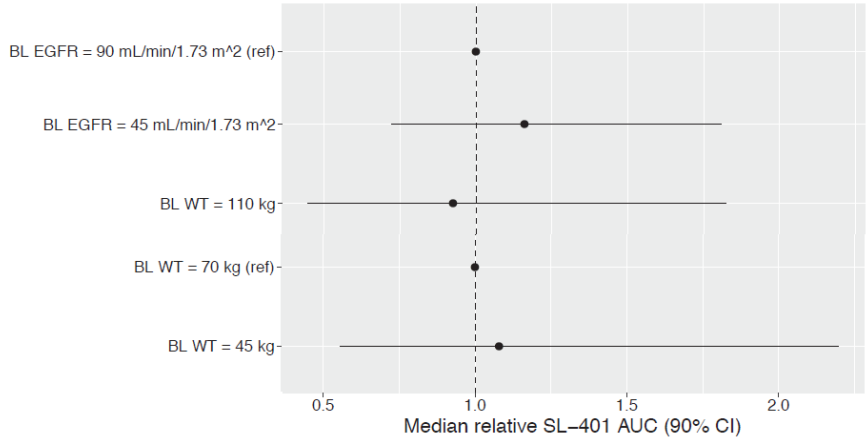


Figure 1 Relative weight and EGFR effects on AUC (BL = baseline)

Source: Applicant's population PK reports, Page 173, Figure 121

Renal impairment

Tagraxofusp has a molecular weight of <69 kDa, suggesting that it may be renally excreted. The population PK analysis indicated that tagraxofusp exposure decreased with increasing renal function (eGFR >30 mL/min/1.73 m^2, estimated by MDRD) in patients with normal renal function, mild or moderate renal impairment. However, the parameter characterizing the renal effect was estimated relatively small and imprecisely (Error! Reference source not found.). Therefore, dose adjustment is not recommended for patients with mild or moderate renal impairment (eGFR 30 to 89 mL/min/1.73 m^2, estimated by MDRD). Regarding severe renal impairment (eGFR 15 to 29 mL/min/1.73 m^2, estimated by MDRD), it would be difficult to conduct a dedicated renal impairment study to assess the PK and safety in patients with severe renal impairment given the rarity of BPDCN. As such, a PMR regarding a dedicated severe renal impairment study will not be required at this moment. (b) (4)

4. Appendices

4.1.Summary of Bioanalytical Method Validation and Performance

4.1.1. Bioanalytical Method for Detection of Tagraxofusp

Bioanalytical method for the quantitative determination of "free" tagraxofusp in human plasma was developed and validated using a dual antibody sandwich immunoassay (IA) that employs an electrochemiluminescence (ECL) detection label. Free tagraxofusp denotes drug not bound to serum pre-existing or treatment-emergent antibodies. The ECL-IA-based method was originally developed and validated by (b) (4) in accordance with the Agency's Draft Bioanalytical Methods Validation Guidance for Therapeutic Proteins (FDA, 2013). In 2016, the validated method was transferred to (b) (4) Table 3 summaries validation results for selected analytical performance characteristics of the validated (b) (4) ECL-IA methods, which indicates good agreement between two analytical methods for quantification of free tagraxofusp in human plasma.

Table 3 Inter-lab Comparison of Selected Analytical Characteristics for Quantification of Free Tagraxofusp in Human Plasma

Assay Characteristics	Test Method GCL-318 (b) (4)	Test Method TM-TUF-0001 (b) (4)
Standard curve range (ng/mL)	0.39 – 400	0.391 – 200
Curve Fit model and weighting	5PL (Marquardt) and $1/y^2$	5PL (Marquardt) and $1/y^2$
LLOQ / ULOQ (ng/mL)	1.56 / 200	1.56 / 160
QCL, QCM and QCH (ng/mL)	4, 40, and 160	4, 40, and 125
Inter-assay Precision (%CV)	10.2 to 17.1	5.0 to 15.7
Inter-assay Accuracy (%RE)	-1.3 to -15.4	-3.5 to 13.1
Total Error	12.5 to 25.6	9.4 to 28.8
Dilution Linearity Limit (ng/mL)	20,000	10,000
Selectivity (% acceptable)	80%	86.7% agreement in results for blinded plasma samples
Freeze/Thaw Stability (cycles)	Up to 4	Up to 5
Short Term Stability (ambient)	Up to 4 hours	5 hours 24 minutes

Abbreviations: (b) (4) LLC; CV = coefficient of variation; LLOQ =lower limit of quantitation; QCH = quality control high; QCL = quality control low; QCM = quality control medium; RE = Relative error; ULOQ = upper limit of quantitation

1 (b) (4)

2

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 2.

Cross-validation was also conducted to evaluate the acceptability of transferring the ECL-IA assay from (b) (4) (Site 1) to (b) (4) (Site 2) and finally to (b) (4) (Site 3). Of the 56 samples from Trial 0114 that were previously analyzed at (b) (4) (Site 1), 91.1% (51/56) had an absolute % difference of ≤ 30.0% when transferred from Site 1 to Site 2, 85.2% (46/54) had an absolute % difference of ≤ 30.0% when transferred from Site 1 to Site 3, and All of the results (54/54) had an absolute % difference of ≤ 30.0% when transferred from Site 2 to Site

3. In general, the experimental results met the pre-defined acceptance criteria of 80.0% of samples having an absolute % difference of ≤ 30.0% between each site, which indicated that determination of plasma concentrations of tagraxofusp was generally consistent among 3 analytical sites.

4.1.2. Bioanalytical Methods for Detection of Anti-Drug Antibodies

Bioanalytical methods for the assessment of tagraxofusp immunogenicity (ADA, NAb, and anti-hIL-3 antibodies) were developed and validated by (b) (4) in accordance with test methods GCL-319, GCL-315, and GCL-321. Anti-hIL-3 NAb was tested for plasma samples from the Stage 3 (registrational cohort) of Trial 0114 by (b) (4) according to test method TM-TUF-0006. (Table 4).

Table 4 Overview of Bioanalytical Methods for Immunogenicity Assessments

Antibody Assay	CRO	Bioanalytical Method #	Method Development Report or Study #	Method Validation Report or Study #
Anti-tagraxofusp Antibodies	(b) (4)	GCL-319	11-145-DR	11-145-VR
		TM-TUF-0004	TUF-17-083	TUF-17-084-VR02
Anti-tagraxofusp NAb		GCL-315	13-060-DR	13-060-VR
		TM-TUF-0005	TUF-17-087	TUF-17-088-VR01
Anti-hIL-3 Antibodies		GCL-321	13-100-DR	13-100-VR
		TM-TUF-0006	TUF-17-085	TUF-17-086-VR02
Anti-hIL-3 NAb		TM-TUF-0003	TUF-17-055	TUF-17-056-VR01

Abbreviations: (b) (4) CRO = Contract Research Organization; hIL-3 = human interleukin 3; NAb = neutralizing antibodies.

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 6.

Detection of Antidrug Antibodies

Bridging ECL-IA assays were conducted for detection and characterization of tagraxofusp ADA, which consisting of 3 components, a confirmatory assay to both detect and confirm the presence of reactive antibodies, a domain specificity assay to determine which portion of the drug the antibodies are reacting with, and a titration assay to generate a titer value for confirmed positive samples. The summaries of selected assay performance characteristics for Test Method GCL-319 developed by (b) (4) and for Test Method TM-TUF-0004 developed by (b) (4) are presented in Table 5 and Table 6, respectively. The developed methods met the acceptance criteria of the Agency's Guidance for Industry "Bioanalytical Method Validation (May 2001)", demonstrating that the analytical methods are suitable for the assay of ADAs in human plasma samples.

Table 5 Summary of Selected Analytical Characteristics of Method GCL-319 Validation (b) (4)

Assay Characteristics	Results
Tier 1 screening cut point	NA
Tier 2 confirmatory cut point (specificity)	77.1% inhibition.
Intra-assay %CV	≤19.4% CV.
Inter-assay %CV	Negative, low, and high controls: ≤29.5% CV. The Mid control had a CV of 31.1% (slightly above the acceptance criteria of ≤30%).
Sensitivity	3.39 ng/mL
Drug Tolerance	No interference noted from tagraxofusp with surrogate anti-DT antibody. For 500 ng/mL of anti-hIL-3 surrogate, drug tolerance level was 253.9 ng/mL.
Tier 3 titer cut point	3-times assay buffer response.

Abbreviations: CV = coefficient of variation; DT = diphtheria toxin; hIL-3 = human interleukin-3; NA = not applicable.

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 8.

Table 6 Summary of Selected Analytical Characteristics of Method TM-TUF-0004 Validation (b) (4)

Assay Characteristics	Method TM-TUF-0004 Validation Results
Tier 1 screening cut point	NA
Tier 2 confirmatory cut point (% inhibition specificity)	64.7%
Tier 3 titer cut point	3-times assay buffer response
Sensitivity	<7.8 ng/mL
Intra-assay %CV (positive QCs)	3.5% – 4.9%
Inter-assay %CV (positive QCs)	19.0% – 22.0%
Drug Tolerance (Low QC)	1000 ng/mL

Abbreviations: (b) (4) CV = coefficient of variation; NA = not applicable; QC = quality control

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 9.

Detection of Neutralizing Antibodies

The developed and validated bioanalytical Method GCL-315 (Table 7) developed by (b) (4) and Method TM-TUF-0005 (Table 8) developed by (b) (4) using a cell-based viability assay for the detection of tagraxofusp NAb in human plasma also met the acceptance criteria of the US FDA Guidance for Industry "Guidance for Industry: Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products (FDA 2016)", demonstrating that the analytical methods are suitable for detecting NAb in human plasma samples.

Table 7 Summary of Selected Analytical Characteristics of Method GCL-315 Validation (b) (4)

Assay Characteristic	Results
NAb cut point	Fixed Cut Point of 8.5% (1% FPER)
Sensitivity	2,850 ng/mL
Selectivity	89% (eight of 9 samples met acceptance criteria)
Robustness	Cells may be used for 26 passages and plates can be incubated from 68-77 hours
Intra-assay %CV (positive QCs)	3.5% – 4.9%
Inter-assay %CV (positive QCs)	19.0% – 22.0%
Drug Tolerance	>1000 ng/mL

Abbreviations: CV = coefficient of variation; FPER = False positive error rate; NAb = neutralizing antibodies; QC = quality control

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 10.

Table 8 Summary of Selected Analytical Characteristics of Cell-based NAb Method TM-TUF-0005 Validation (b) (4)

Assay Characteristics	Results
NAb cut point	Ratio cut point = 2.07 (1% FPER), normalized to drug control
Sensitivity	2,610 ng/mL
Selectivity	100% (10/10) of unspiked samples classified as negative 100% (10/10) of samples spiked at LPC classified as positive
Intra-assay %CV (positive QCs)	4.5% – 7.9%
Inter-assay %CV (positive QCs)	18.4% – 19.2%
Drug Tolerance	LPC can tolerate 100 ng/mL of tagraxofusp HPC can tolerate >1000 ng/mL of tagraxofusp

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 11.

Detection of Human IL-3 Antibodies

The developed and validated bioanalytical Method GCL-321 (Table 7) developed by (b) (4) and Method TM-TUF-0006 (Table 8) developed by (b) (4) using a direct binding ELISA assay for the detection of tagraxofusp anti-IL3 antibodies in human plasma met the acceptance criteria of the Agency's Guidance for Industry "Guidance for Industry: Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products (FDA 2016)", demonstrating that the analytical methods are suitable for detecting tagraxofusp anti-IL3 antibodies in human plasma samples.

Table 9 Summary of Selected Analytical Characteristics of Method GCL-321 Validation (b) (4)

Assay Characteristics	Results
Tier 1 screening cut point	1.59 (multiplicative CP factor)
Tier 2 confirmatory cut point (specificity)	37.9% inhibition
Intra-assay %CV	≤ 9.9% CV
Inter-assay %CV	≤ 13.1% CV
Sensitivity	68 ng/mL
Drug Tolerance	At 100 ng/mL of anti-hIL-3 Ab = < 7.8 ng/mL At 500 ng/mL of anti-hIL-3 Ab = 228.5 ng/mL
Tier 3 titer cut point	3.85 (multiplicative CP factor)

Abbreviations: CV = coefficient of variation; hIL-3 = human interleukin-3

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 12.

Table 10 Summary of Selected Analytical Characteristics of Method TM-TUF-0006 Validation (b) (4)

Assay Characteristics	Results
Tier 1 screening cut point	2.57 (multiplicative CP factor)
Tier 2 confirmatory cut point (specificity)	20.6% inhibition
Intra-assay %CV	≤ 2.1% CV (positive controls)
Inter-assay %CV	≤ 19.4% CV (positive controls)
Sensitivity	31.3 ng/mL
Drug Tolerance	At 100 ng/mL of anti-hIL-3 Ab = 31.3 ng/mL At 500 ng/mL of anti-hIL-3 Ab = 250 ng/mL At 1500 ng/mL of anti-hIL-3 Ab > 1000 ng/mL
Tier 3 titer cut point	3.72 (multiplicative CP factor)

Abbreviations: CV = coefficient of variation; hIL-3 = human interleukin-3

Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 13.

Detection of Neutralizing Antibodies to Human IL-3

The developed and validated bioanalytical Method TM-TUF-0003 (Table 8) developed by (b) (4) using a cell-based assay for the detection of tagraxofusp anti-hIL-3 NAbs in human plasma met the acceptance criteria of the Agency's Guidance for Industry "Guidance for Industry: Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products (FDA 2016)", demonstrating that the analytical methods are suitable for detecting tagraxofusp anti-hIL-3 NAbs in human plasma samples.

Table 11 Summary of Selected Analytical Characteristics of Method TM-TUF-0003 Validation (b) (4)

Assay Characteristics	Results
Tier 1 screening cut point	1.30
Tier 2 confirmatory cut point (specificity)	1.13
Intra-assay %CV	≤ 4.0% CV (positive controls)
Inter-assay %CV	≤ 10.9% CV (positive controls)
Sensitivity	130 ng/mL
Drug Tolerance	At 200 ng/mL of anti-hIL-3 Ab = 100 ng/mL At 2000 ng/mL of anti-hIL-3 Ab = > 1000 ng/mL At 20,000 ng/mL of anti-hIL-3 Ab > 1000 ng/mL

Abbreviations: Ab = antibody (b) (4) CV = coefficient of variation; hIL-3 = human interleukin-3

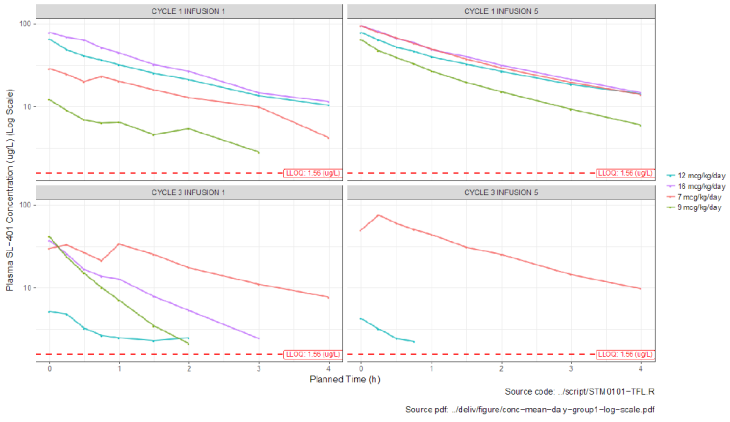
Source: Biopharmaceutic Studies and Associated Analytical Methods, Table 14.

4.2.Clinical PK and Immunogenicty Assessments

4.2.1. PK Assessment

The PK of tagraxofusp was characterized by non-compartmental analysis (NCA) based on intensive PK data on Cycle 1 Day 1 (C1D1), Cycle 1 Day 5 (C1D5), Cycle 3 Day 1 (C3D1), and Cycle 3 Day 5 (C3D5), from 96 patients in Trial 0114 with BPDCN or AML given 15-minute intravenous infusions of 7, 9, 12 and 16 µg/kg/day.

Plasma concentrations of tagraxofusp generally declined rapidly in a monoexponential manner post end of infusion (Figure 2). Many patients had free tagraxofusp-xxx concentrations below the limit of quantitation (LOQ) by 4 hours post dose on C1D1, across all dose levels ranging from 7 µg/kg/day to 16 µg/kg/day. The key exposure metrics, C_{max} and AUC, in patients with BPDCN and AML are summarized in Table 12. The PK linearily could not be fully assessed due to the significant impact of pre-existing and treatment-emergent ADA across the dose groups. Exposures of free tagraxofusp on C1D5 were generally higher than C1D1, and were markedly reduced in Cycle 3 as compared with Cycle 1.



Abbreviations: LLOQ = lower limit of quantification

Figure 2 Mean Free Tagraxofusp Plasma Concentration vs Time by Dose and Period.

Source: Summary of Clinical Pharmacology Studies, Figure 1.

Table 12 Free Tagraxofusp Exposure Following Intravenous Doses of 7, 9, 12, and 16 **µg/kg/** day on Days 1 and 5 of Cycles 1 and 3.

		Tagraxofusp Daily Dose							
		7 µg /kg		9 µg /kg		12 µg /kg		16 µg /kg	
Exposure	**Period**	**N**	**Mean (StD)**	**N**	**Mean (StD)**	**N**	**Mean (StD)**	**N**	**Mean (StD)**
C_{max}	C1D1	6	29.1 (25.1)	3	12.2 (10.8)	79	66.4 (70.8)	7	78.5 (66.2)
µg/L	C1D5	6	94.7 (39.5)	3	64.3 (29.3)	39	77.1 (72.9)	1	93.8 (--)
	C3D1	4	22.4 (35.5)	2	20.8 (29.5)	36	2.42 (3.56)	1	37.3 (--)
	C3D5	3	33.1 (50.4)	1	0 (--)	39	1.52 (2.37)	0	-- (--)
AUC*	C1D1	6	44.8 (36.9)	3	13.2 (15.5)	79	82.8 (88.1)	7	118 (103)
h•µg/L	C1D5	6	164 (90.2)	3	89.6 (4.01)	39	115 (111)	1	162 (--)
	C3D1	4	29.0 (54.2)	2	14.3 (20.2)	36	1.42 (2.46)	1	38.9 (--)
	C3D5	3	46.6 (79.8)	1	0 (--)	39	0.648 (1.24)	0	-- (--)

Abbreviations: AUC = area under the plasma concentration-time curve; C = Cycle, C_{max} = maximum concentration; D = Day; StD = standard deviation
*AUC is AUC_{last}. -- indicates not calculable
Concentrations below the quantifiable limit were replaced with a value of zero to show the impact of ADA on average exposure.

Source: Summary of Clinical Pharmacology Studies, Table 2.

The PK characterization of tagraxofusp in patients with BPDCN (N = 43) following 15-minute intravenous doses of 12 µg/kg/day are summaried in Table 13 and Table 14.

Table 13 Free Tagraxofusp Exposure in Patients with BPDCN Following Intravenous Doses of 12 **µg/kg/day** on Days 1 and 5 of Cycles 1 and 3.

Exposure	**Period**	**N**	**Mean**	**StD**
C_{max}	C1D1	43	89.7	82.5
µg/L	C1D5	18	60.4	60.7
	C3D1	32	2.33	3.59
	C3D5	35	1.29	2.16
AUC*	C1D1	43	106	98.5
h•µg/L	C1D5	18	93.3	102
	C3D1	32	1.42	2.52
	C3D5	35	0.581	1.15

Abbreviations: AUC = area under the plasma concentration-time curve; BPDCN = blastic plasmacytoid dendritic cell neoplasm; C = Cycle, C_{max} = maximum concentration; D = Day; StD = standard deviation
*AUC is AUC_{last}. -- indicates not calculable

Source: Summary of Clinical Pharmacology Studies, Table 4.

Table 14 Free Tagraxofusp PK Parameters in Patients with BPDCN Following Intravenous doses of 12 **µg/kg/day,** Cycle 1, Days 1 and 5.

		$t_{1/2}$ (h)		CL (L/h)		Vz (L)	
Period	**N***	**Mean**	**StD**	**Mean**	**StD**	**Mean**	**StD**
C1D1	29	1.12	0.584	13	18.3	19	24.2
C1D5	14	0.989	0.325	38	39.9	39.8	32.9

Abbreviations: BPDCN = blastic plasmacytoid dendritic cell neoplasm; C = Cycle, CL = clearance; D = Day; StD = standard deviation; $t_{1/2}$ = terminal half-life; V_z = volume of distribution
*Number of patients for whom a terminal phase slope could be estimated

Source: Summary of Clinical Pharmacology Studies, Table 5.

4.2.2. Immunogenicty Assessment

A comprehensive analyses of the immunogenicity data from 4 Trials (0114, 0214, 0314 and 0414) was performed using the bioanalytical methods for detection of anti-drug antibodies stated in Section Error! Bookmark not defined..

The majority of patients (96%; 115/120), regardless of disease diagnosis, were positive for ADAs at baseline; 21% of patients were ADA NAb-positive at baseline. The median baseline titer for the immunogenicity evaluable patients was 800; titers ranged from undetected to 80,000. Of the evaluable patients who were ADA-positive at baseline, 69% (72/104) had a significant increase (⩾100-fold) in titer after drug administration, with the maximum titer of 80,000,000 by the end of Cycle 1 representing a 100,000-fold increase from baseline. By the end of treatment, 99% of patients were ADA-positive, and 85% were ADA NAb-positive. Most patients were positive for ADAs and NAbs by Cycle 2.

In contrast, 99% (119/120) patients were negative for anti-IL-3 antibodies at baseline. However, 68% (73/108) patients developed anti-IL-3 antibody by the end of treatment. The majority (67%; 72/107) of immunogenicity evaluable patients who were anti-IL-3 antibody-negative at baseline had a ⩾ 4-fold increase in anti-IL-3 antibody titer after administration of tagraxofusp, with the peak titer occurring at C3D15, and with continued administration, virtually 100% had a ≥4-fold increase in titer.

4.3.Population PK and/or PD Analysis

A population PK analysis was conducted by Applicant based on the pooled PK data from Study 0114, 0214, 0314, and 0414.

Trial 0114: A 4-stage, standard 3+3 dose escalation and expansion study in patients with treatment-naïve or Relapsed/refractory (R/R) blastic plasmacytoid dendritic cell neoplasm (BPDCN) and (acute myeloid leukemia) AML. In Stage 1, cohorts of 3-6 patients were treated with doses of tagraxofusp of 7, 9, 12, or 16 µg/kg/day for 5 consecutive days every 21 days. The 16 µg/kg/day dose was evaluated only in patients with AML. Stages 2 and 4 were expansion studies in patients receiving maximum tolerate or tested dose from Stage 1. Stage 3 was the pivotal study with a standalone cohort of treatment-naïve BPDCN patients. PK samples were collected at pre-infusion, end of infusion, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 hours post-infusion on days 1 and 5 of cycles 1 and 3. Immunogenicity samples were collected on day 1 (pre-infusion), day 15, and day 21 for cycle 1 and day 1 (pre-infusion) and day 21 of all subsequent cycles. Additionally, if there were clinical manifestations suggesting either an infusion related reaction or drug hypersensitivity, an immunogenicity sample was obtained.

Trial 0214: A 2-stage, standard 3+3 dose escalation study in adult patients with adverse risk AML in first or second CR and/or evidence of minimal residual disease in first CR. In Stage 1, a starting dose of 7 µg/kg/day was given for 5 consecutive days every 28 days, with escalation to 9 and 12 µg/kg/day. Cohorts of 3-6 patients were treated for each dose level. In Stage 2, up to 20 additional patients were treated at the maximum tolerated dose from Stage 1. PK samples were collected at pre-infusion, end of infusion, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 hours post-infusion on days 1 and 5 of cycles 1 and 3. Immunogenicity samples were collected on day 1 (pre-infusion), day 15, and day 28 for cycle 1 and day 1 (pre-infusion) and day 28

of all subsequent cycles. Additionally, if there were clinical manifestations suggesting either an infusion related reaction or drug hypersensitivity, an immunogenicity sample was obtained.

Trial 0314: A 2-stage, standard 3 + 3 dose escalation study in patients with advanced, high-risk myeloproliferative neoplasms (HRMPN). In Stage 1, doses of 7, 9, 12, 16 μg/kg/day were administered for 3 consecutive days at the beginning of each cycle. Cohorts of 3-6 patients were treated for each dose level. In Stage 2, up to 72 additional patients were treated at the maximum tolerated dose from Stage 1. The initial 4 cycles of therapy were 21 days long, with cycles 5-7 being 28 days long. PK samples were collected at pre-infusion, end of infusion, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 hours post-infusion on days 1 and 3 of cycles 1 and 4. Immunogenicity samples were collected on day 1 (pre-infusion), day 15, and day 21 for cycles 1 through 4 and end of cycle for cycles 5-7. Additionally, if there were clinical manifestations suggesting either an infusion related reaction or drug hypersensitivity, an immunogenicity sample was obtained.

Trial 0414: A standard 3 + 3 dose escalation study in combination with pomalidomide (POM) and dexamethasone (DEX) in patients with relapsed or relapsed and refractory multiple myeloma (MM). At least 3 patients received an initial run-in cycle (28 days) of single agent of tagraxofusp with doses of 7, 9, or 12 μg/kg/day followed by combination of tagraxofusp/POM/DEX at standard doses if they did not experience a dose-limiting toxicity during run-in cycle. PK samples were collected at pre-infusion, end of infusion, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 hours post-infusion on days 1 and 5 of the run-in cycle and cycle 2. Immunogenicity samples were collected prior to the infusion during run-in cycle 1 on days 1 and 5 and on day 1 pre-infusion at the beginning of all subsequent cycles, and at end-of-therapy. Additionally, if there were clinical manifestations suggesting either an infusion related reaction or drug hypersensitivity, an immunogenicity sample was obtained.

The total dataset was divided into three data windows: cycle 1, day 1 (C1D1) data; cycle 1, day 3 or 5 (C1D3-5) data, with day 3 data coming solely from study 0314; cycle 3 or 4 (C3-4) data (days 1, 3, or 5). C1D3-5 data was used as the reference dataset, given the assumption that ADA was less impactful during this treatment period, assuming: 1) baseline or pre-existing ADA has been diminished by drug binding and other elimination mechanisms and 2) anamnestic and/or treatment-induced immune response has not yet begun producing large amounts of new ADA.

The PK data set was comprised of 127 patients (n = 96 in study 0114; n = 11 in study 0214; n = 15 in study 0314, n = 5 in study 0414), contributing a total of 2920 observations. There was a large number of BLQ observations across the four studies (1360 records total, 1049 records in study 0114, 144 records in study 0214, 149 records in study 0314, and 18 records in study 0414), which is almost half of the total number of PK observations. The majority of BLQs occurred in treatment cycles 3 or 4 for study 0314 (Figure 3). M3 method was used in the population PK analysis for BLQ data.

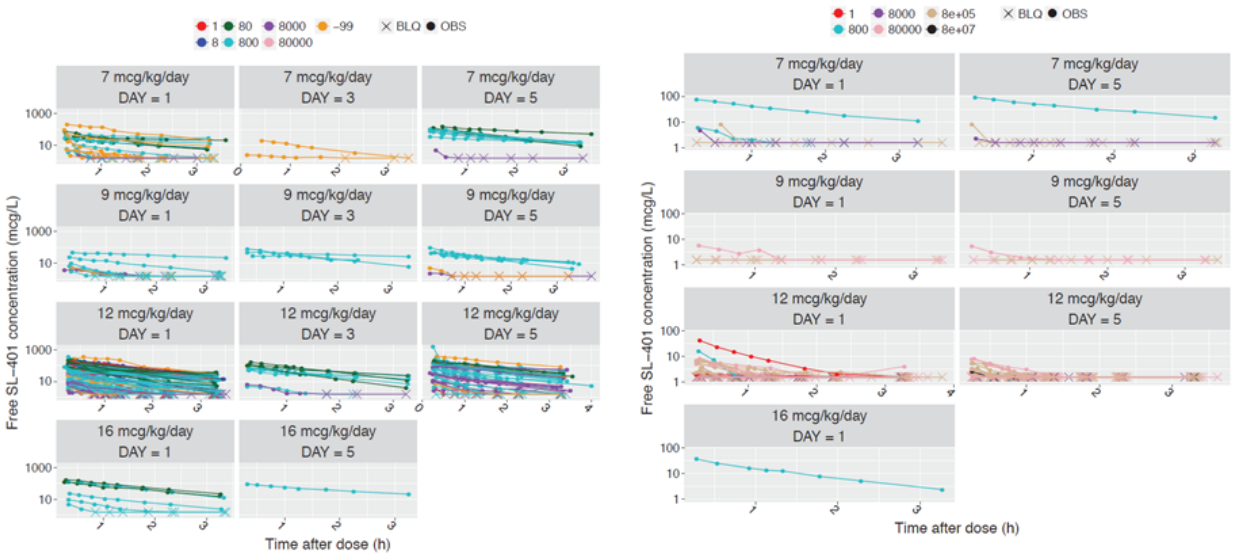


Figure 3 The observed PK profile of tagraxofusp by dose and day for Cycle 1 (Left) and Cycle 3 (Right)

Source: Applicant's population PK report, Page 65-66, Figure 13-14.

Patient demographics at screening are summarized in Table 15 and Table 16.

Table 15 Summary of categorical covariates by study

VARIABLE	COUNT114	%114	COUNT214	%214	COUNT314	%314	COUNT414	%414
Male	68	70.8	9	81.8	8	53.3	2	40
Female	28	29.2	2	18.2	7	46.7	3	60
Caucasian	86	89.6	10	90.9	11	73.3	5	100
Black	3	3.1	1	9.1	0	0.0	0	0
Asian	3	3.1	0	0.0	2	13.3	0	0
American Indian	1	1.0	0	0.0	0	0.0	0	0
Other	3	3.1	0	0.0	2	13.3	0	0
AML	49	51.0	11	100.0	0	0.0	0	0
BPDCN	47	49.0	0	0.0	0	0.0	0	0
Myeloma	0	0.0	0	0.0	0	0.0	5	100
HRMPN	0	0.0	0	0.0	15	100.0	0	0
Frozen solution	89	92.7	11	100.0	15	100.0	5	100
(b) (4)	7	7.3	0	0.0	0	0.0	0	0
ECOGBL 0	33	34.4	5	45.5	1	6.7	2	40
ECOGBL 1	60	62.5	5	45.5	12	80.0	3	60
ECOGBL 2	3	3.1	1	9.1	2	13.3	0	0

Source: Applicant's population PK report, Page 35, Table 6

Table 16 Summary of continuous covariates by study

STUDY	VARIABLE	MEAN	MEDIAN	STANDARD DEVIATION	MIN	MAX	N OBSERVATIONS
114	Age (years)	62.6	65.5	14.80	21.0	87.0	96
114	Baseline weight (kg)	83.7	82.2	17.10	56.9	162.0	96
114	Baseline albumin concentration (g/L)	35.7	36.0	2.95	29.0	43.0	96
114	Baseline EGFR (mL/min/1.73 m^2)	86.4	83.6	25.70	42.2	158.0	96
214	Age (years)	64.5	64.0	8.79	51.0	78.0	11
214	Baseline weight (kg)	84.7	85.2	13.70	56.7	108.0	11
214	Baseline albumin concentration (g/L)	36.2	37.0	3.16	31.0	42.0	11
214	Baseline EGFR (mL/min/1.73 m^2)	84.2	82.6	18.90	55.1	117.0	11
314	Age (years)	65.8	67.0	9.40	42.0	81.0	15
314	Baseline weight (kg)	72.8	72.3	15.20	46.7	92.8	15
314	Baseline albumin concentration (g/L)	37.8	39.0	4.41	30.0	44.0	15
314	Baseline EGFR (mL/min/1.73 m^2)	69.6	67.6	19.20	45.9	106.0	15
414	Age (years)	64.2	65.0	4.60	57.0	69.0	5
414	Baseline weight (kg)	85.1	76.4	22.00	69.4	123.0	5
414	Baseline albumin concentration (g/L)	34.2	34.0	4.15	30.0	41.0	5
414	Baseline EGFR (mL/min/1.73 m^2)	73.2	68.5	19.30	46.4	93.5	5

Source: Applicant's population PK report, Page 39, Table 10

A one-compartment model with first-order clearance was established to describe the PK of tagraxofusp. The C1D3-5 model was used as the reference model and the parameters (V/F and CL/F) of the other two models (C1D1 and C3-4 models) were fixed to final estimates from this reference model. The time-varying ADA titer, entered as categorical variable, showed statistically significant correlation on F1. The ADA=80 titer group was used as the reference, fixing F1 to 1, such that the effect of other titer levels on F1 was relative to this reference. The ADA = 80 group in the C1D3-5 data window was chosen as the reference since it had the most complete PK profiles (minimal BLQ presence). For a given titer level, F1 decreased for individuals at C1D1 or C3-4, relative to C1D3-5 (offset effect was incorporated in the model). The precision of estimates of ADA-related effects on F1 was poor; therefore, the model could not be used to make definitive conclusions about the effect of ADA titer on tagraxofusp exposure.

Body weight and renal function (eGFR) were identified as significant covariates (power model). Trends for increasing clearance with increasing weight and increasing clearance with increasing eGFR were observed but they were less influential than ADA titer on tagraxofusp exposure. Due to large uncertainty in covariate effect estimates, conclusive effects of baseline weight or renal function on exposure could not be drawn.

The goodness-of-fit plots for C1D3-5, C1D1 and C3-4 final models including titer, weight, and eGFR as covariates are presented in Figure 4 and Figure 5.

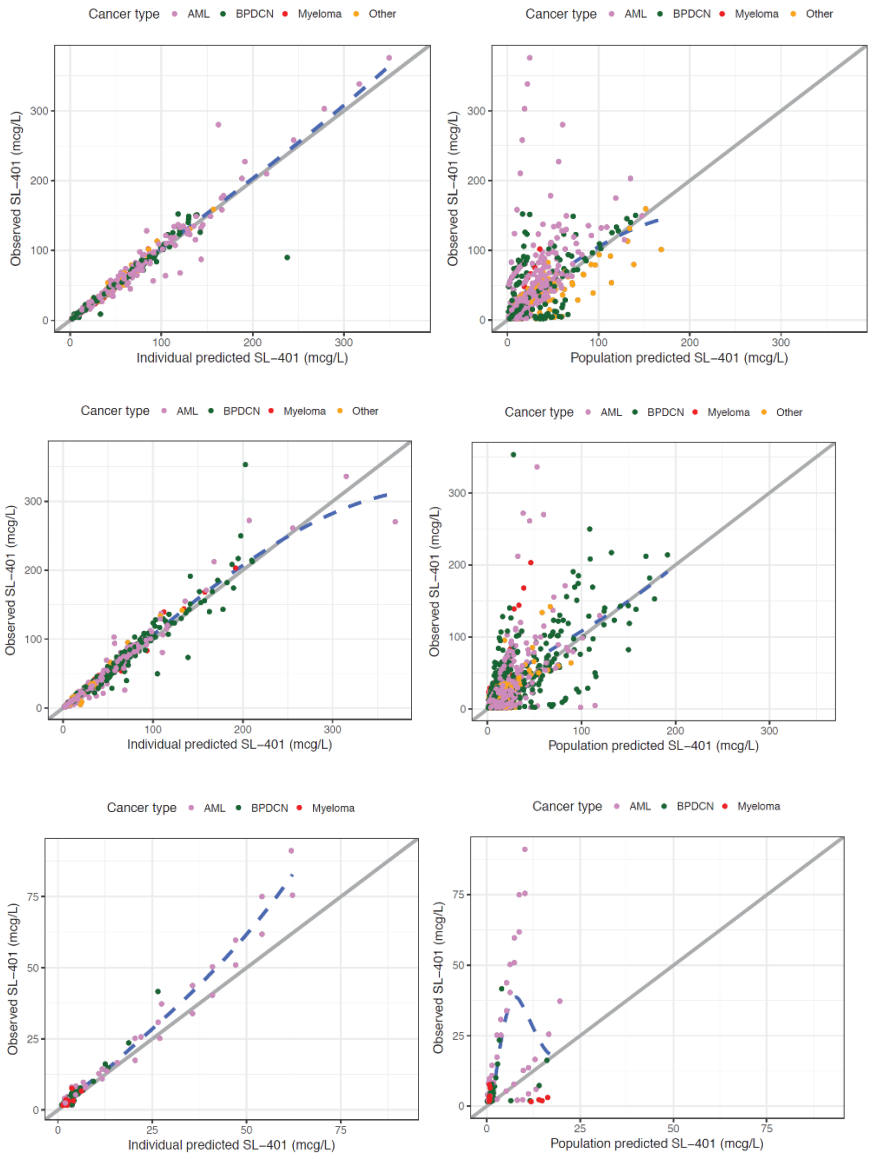
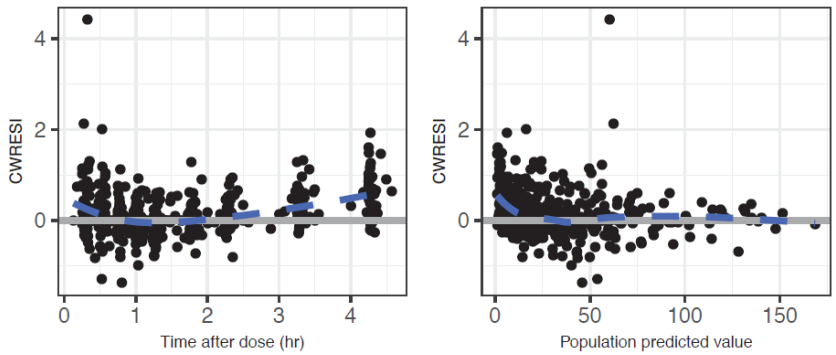


Figure 4 Observed tagraxofusp vs individual/population predictions forC1D3-5 (top two), C1D1 (middle two), and C3-4 (bottom two)

Source: Applicant's population PK reports, Pages 110, 112, 131, 133, 152, and 154, Figures 58, 60, 79, 81, 100, and 102



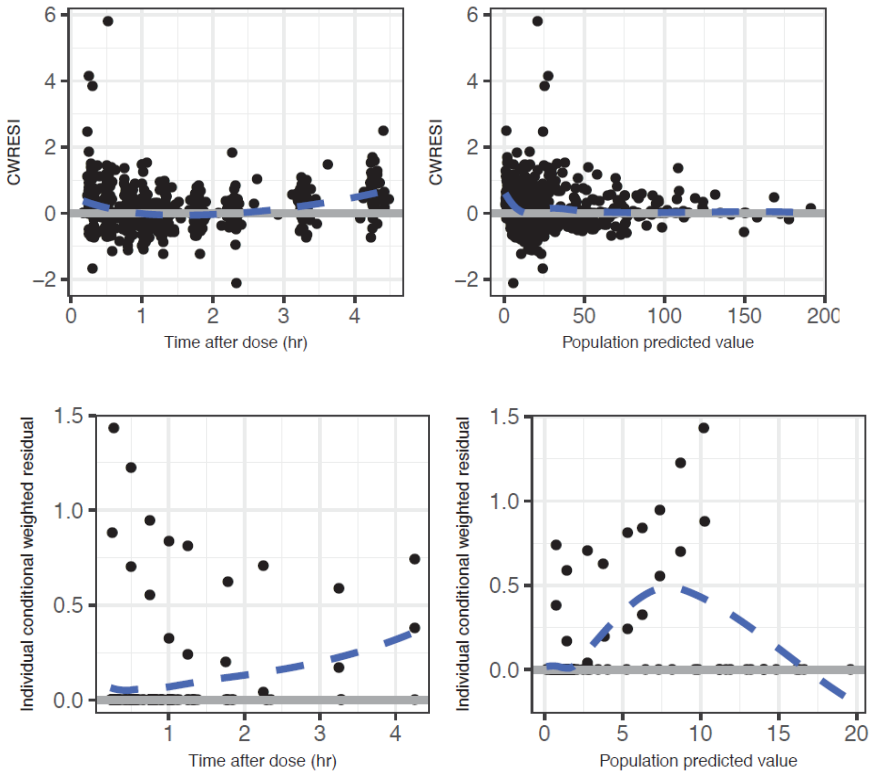


Figure 5 Residual plots forC1D3-5 (top two), C1D1 (middle two), and C3-4 (bottom two)

Source: Applicant's population PK reports, Pages 114, 135, and 156, Figures 62, 83, and 104

For C3-4 model, there was a strong population under-predictive bias because of the heavy presence of BLQ, influencing the population prediction. Thirty out of 58 subjects' data was entirely BLQ for this data window. While no specific time-dependent ADA mechanism could be included to describe this due to sparse ADA sampling.

The estimates of parameters for C1D3-5, C1D1, and C3-4 models are listed in Table 17, Table 18 and Table 19, respectively.

Table 17 Population PK model parameter estimates for C1D3-5 model

Parameter	Estimate	relative standard error
Volume of distribution (V/F, L)	5.2	0.93
Clearance (CL/F, L/h)	3.5	0.90
F1 ADA = -99 effect	0.22	0.95
F1 ADA = 1 effect	0.035	8.0
F1 ADA = 80 effect	1, FIX	NA
F1 ADA = 800 effect	0.47	0.86
F1 ADA = 8000 effect	0.16	0.92
F1 ADA = 80000 effect	0.12	4.1
V/F BL weight effect	1.5	0.78
CL/F BL weight effect	1.2	0.78
CL/F BL EGFR effect	0.21	1.9
Residual error	0.17	0.013
V/F inter-individual variance	1.2	0.18
V/F - CL/F covariance	1.2	0.22
CL/F inter-individual variance	1.6	0.19

Source: Applicant's population PK reports, Page 43, Table 17

Table 18 Population PK model parameter estimates for C1D1 model

Parameter	Estimate	Approximate relative standard error
Volume of distribution (V/F, L)	6.0, FIX	NA
Clearance (CL/F, L/h)	3.9, FIX	NA
C1D3-5 F1 ADA = -99 effect	0.21, FIX	NA
C1D3-5 F1 ADA = 1 effect	0.036, FIX	NA
C1D3-5 F1 ADA = 80 effect	1.0, FIX	NA
C1D3-5 F1 ADA = 800 effect	0.48, FIX	NA
C1D3-5 F1 ADA = 8000 effect	0.15, FIX	NA
C1D3-5 F1 ADA = 80000 effect	0.12, FIX	NA
C1D1 F1 ADA = -99 offset	2.9	0.54
C1D1 F1 ADA = 1 offset	40	0.39
C1D1 F1 ADA = 8 effect	1.9	0.082
C1D1 F1 ADA = 80 offset	0.60	0.083
C1D1 F1 ADA = 800 offset	0.64	0.12
C1D1 F1 ADA = 8000 offset	1.1	0.26
C1D1 F1 ADA = 80000 offset	1.4	0.24
Residual error	0.18	0.095
V/F inter-individual variance	0.90	0.091
V/F - CL/F covariance	1.0	0.11
CL/F inter-individual variance	1.8	0.12

Source: Applicant's population PK reports, Page 43, Table 18

Table 19 Population PK model parameter estimates for C3-4 model

Parameter	Estimate	Approximate relative standard error
Volume of distribution (V/F, L)	6.0, FIX	*NA*
Clearance (CL/F, L/h)	3.9, FIX	*NA*
C1D3-5 F1 ADA = 1 effect	0.036, FIX	*NA*
C1D3-5 F1 ADA = 80 effect	1.0, FIX	*NA*
C1D3-5 F1 ADA = 800 effect	0.48, FIX	*NA*
C1D3-5 F1 ADA = 8000 effect	0.15, FIX	*NA*
C1D3-5 F1 ADA = 80000 effect	0.12, FIX	*NA*
C3-4 F1 ADA = 1 offset	1.3	0.22
C3-4 F1 ADA = 800 offset	0.31	0.17
C3-4 F1 ADA = 8000 offset	0.063	0.30
C3-4 F1 ADA = 80000 offset	0.099	0.12
C3-4 F1 ADA = 800,000 effect	0.0094	0.09
C3-4 F1 ADA = 80,000,000 effect	0.0043	0.34
Residual error	0.42	0.02
V/F inter-individual variance	0.68	0.00
V/F - CL/F covariance	0.35	0.05
CL/F inter-individual variance	0.68	0.03

Source: Applicant's population PK reports, Page 44, Table 19

Figure 6 describes the effect that ADA has on F1. On average, F1 decreased with increasing ADA titer within a given time, since greater presence of ADA increased the immediate binding of tagraxofusp. For a given titer level, F1 showed a decreasing trend for individuals at C1D1 or C3-4, relative to C1D3-5.

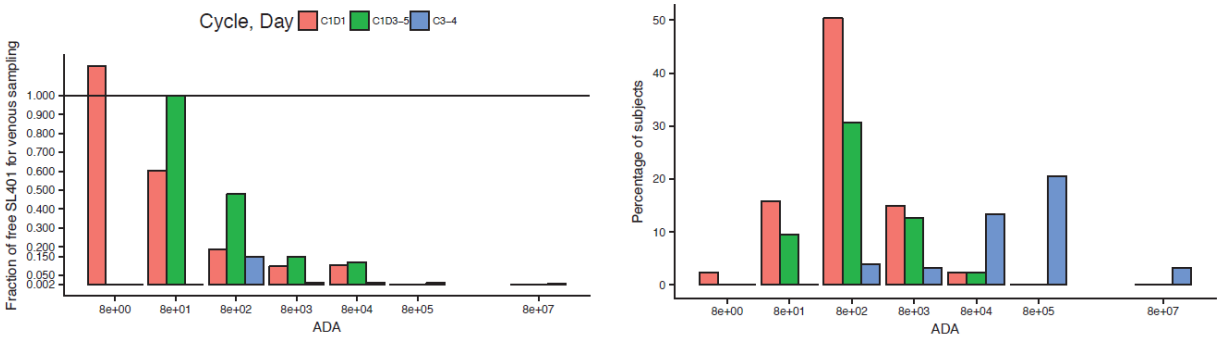


Figure 6 F1 vs. ADA titer for C1D3-5, C1D1, C3-4 (left) and percentage of total subjects (n = 127) in each titer group across data windows (right)

Source: Applicant's population PK reports, Page 174, Figure 122

The weight and renal function had relative small impact on AUC as shown in Figure 1.

Reviewer's comments: The Reviewer repeated the population PK model developed by Applicant. In general, the population PK model cannot precisely describe the PK data and large variability was derived with PK parameters. The Applicant added ADA as a covariate on F1 while it may be more reasonable to be a covariate on CL. However, due to the fact that immunogenicity samples were not collected at the same time with PK samples, the impact of ADA titer on PK was unable to be accurately characterized. Besides, the original population PK model did not consider hepatic impairment as a potential covariate. An IR letter was sent to Applicant on July 26, 2018, to request further assessment of effect of hepatic impairment on the PK of tagraxofusp. The Applicant replied on Aug 8, 2018 with a reasonable analysis (C1D1 and C1D3-5 pop PK models only) showing that hepatic impairment is not a significant covariate. In the analysis, patients with mild and moderate hepatic impairment were pooled together due to the small sample size (Table 20).

Table 20 Number of subjects in population PK dataset by Cycle - Day and hepatic impairment category

DATA WINDOW	N TOTAL SUBJECTS	NORMAL	MILDB1	MILDB2	MODERATE	UNKNOWN
C1D1	127	111	8	6	1	1
C1D3-5	78	50	27	1	0	0
C3D1	27	21	6	0	0	0
C3D5	26	20	5	0	1	0
C4D1	3	2	1	0	0	0
C4D3	3	2	1	0	0	0

Source: Applicant's response to IR received on Aug 8, 2018

4.4.Exposure-Response Analyses for Efficacy

The exposure-response (ER) analysis was conducted in BPDCN subjects from Study 0114 for whom at least one individual predicted metric of exposure could be estimated based on the population PK model and who additionally had at least one Investigator Assessment of Clinical Response. A total of 45 subjects were included in this analysis with 2 subjects treated with 7 μg/kg/day and the remaining 43 were treated with 12 μg/kg/day.

Two primary efficacy endpoints were used: best overall response (BOR) and duration of response (DOR). BOR was defined as either complete response (CR) or complete response clinical (CRc). DOR was defined as the time difference, in days, between first assessment of CR or CRc and the earliest subsequent day on which the patient was assessed to no longer be in the CR or CRc state.

C_{max} and $AUC_{0\text{-}24hr}$ in Cycle 1, Day 1 were considered as PK metrics as more data were missing in Cycle 1, Day 3 or Day 5. Moreover, $AUC_{0\text{-}24hr}$ and Cmax were moderately correlated in Cycle 1, Days 1 (Figure 7), therefore, $AUC_{0\text{-}24hr}$ in Cycle 1, Day 1 was used as the primary PK parameter in the ER analysis.

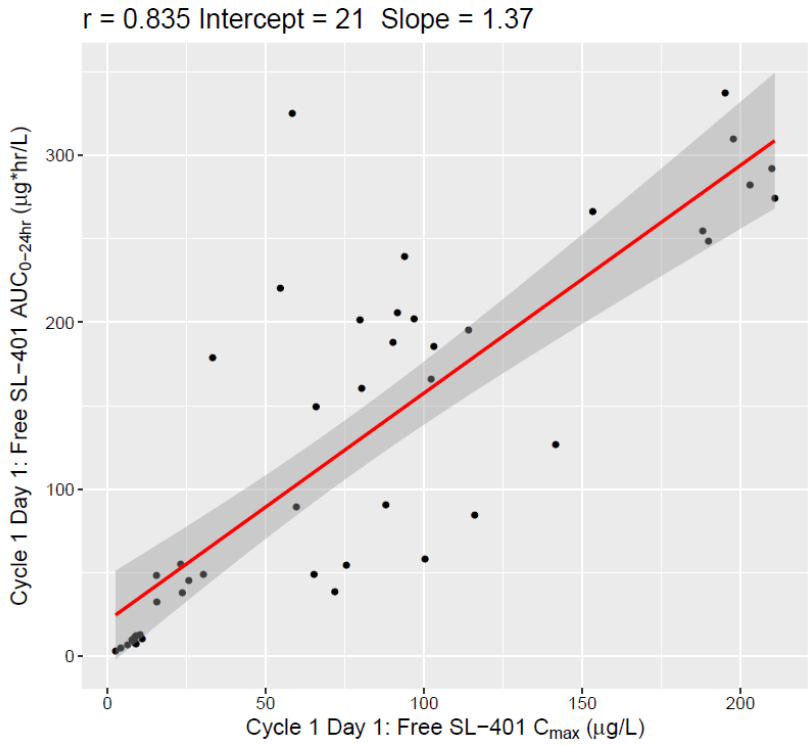


Figure 7 Cycle 1, Day 1 $AUC_{0\text{-}24hr}$ vs C_{max}: PK/PD analysis set, BPDCN population in study 0114

Source: Applicant's exposure-response analysis reports, Page 131, Figure 1

The following covariates were considered in the ER analysis: 1) presence of Cycle 1 drug-neutralizing antibodies; 2) presence of quantifiable tagraxofusp in Cycle 3; 3) dominant lesion type; 4) baseline skin involvement.

Based on exploratory graphical analysis, increasing incidence of a BOR of CR+CRc was associated with increasing C1D1 $AUC_{0\text{-}24hr}$ (Figure 8). Associations between BOR of CR+CRc and AUC were quantified via a multiple-predictor logistic regression model. C1D1 AUC was estimated to increase the odds of BOR of CR+CRc by approximately 3.70× per 100 (µg*h)/L. Presence of ADA neutralizing antibodies predose in Cycle 1 was associated with a 0.51× decrease in the odds and presence of quantifiable tagraxofusp in Cycle 3 was associated with a 0.24× decrease in the odds of a patient having a BOR of CR+CRc. These covariate effects are not statistically significant at the 0.05 level. Increasing extent of baseline skin involvement was associated with decreasing odds BOR of CR+CRc. Plaque-dominant lesion type was associated with the highest odds of a patient having a BOR of CR+CRc, tumor-dominant lesion type was associated with the lowest such odds, and patch- dominant lesion type was intermediate. However, none of these modeled relationships were found to be statistically significant at the 0.05 level.

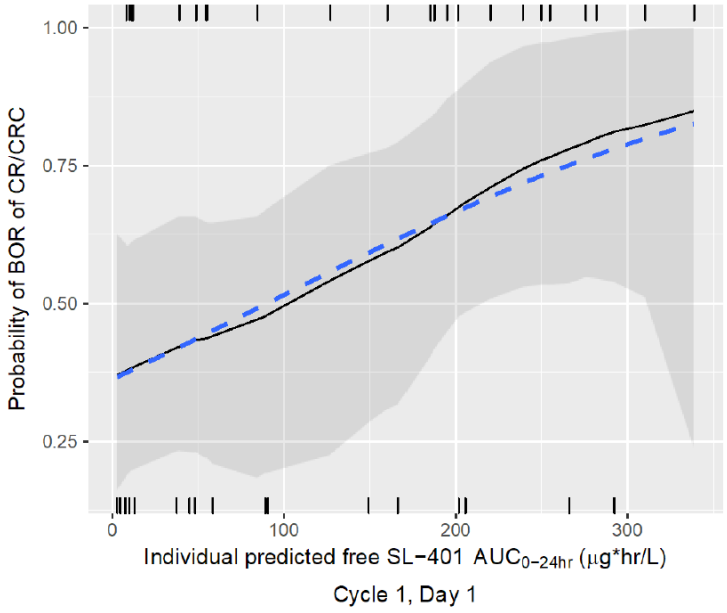


Figure 8 Model-Simulated and Observed Best Overall Response of CR+CRc by Cycle 1 Day 1 AUC

Dashed blue line represents a smooth of the original data. Solid black line (simulation median) and grey region (90% prediction interval) represent distribution of smooths fit to replicate data sets simulated from the estimated model.

Source: Applicant's exposure-response analysis report, Page 33, Figure 5

It is important to note that a weakness of the BOR E-R analyses is that BPDCN patient subtype: treatment naïve versus previously-treated, was not used as a covariate. It is conceivable that these 2 subtypes could have different baseline probabilities of response and/or E-R relationship.

For DOR, the following states were defined:

- State 1: Not CR or CRc and prior to discontinuation. All subjects were assumed to be in state 1 at baseline.
- State 2: CR or CRc.
- State 3: Discontinuation associated with Progressive Disease.
- State 4: Discontinuation or censoring not associated with Progressive Disease.

The numbers of observed transitions between the efficacy and censoring states are described in Table 21 which shows six observed transitions from state 2 and state 1.

Table 21 Numbers of observed state transitions

From	To			
	1	2	3	4
1	77	25	23	3
2	6	45	0	19

Source: Applicant's exposure-response analysis report, Page 124, Table 56

Among the 6 subjects with observed (non-censored) DOR, longer durations were associated with higher Cycle 1-Day 1 $AUC_{0\text{-}24hr}$ levels, and the one subject who had quantifiable tagraxofusp in Cycle 3 had the longest of the non-censored durations, as shown in Figure 9. None of the 6 subjects with non-censored DOR had detectable Cycle 1 drug neutralizing antibodies. No consistent ordering of dominant lesion types was observed with respect to non-censored DOR.

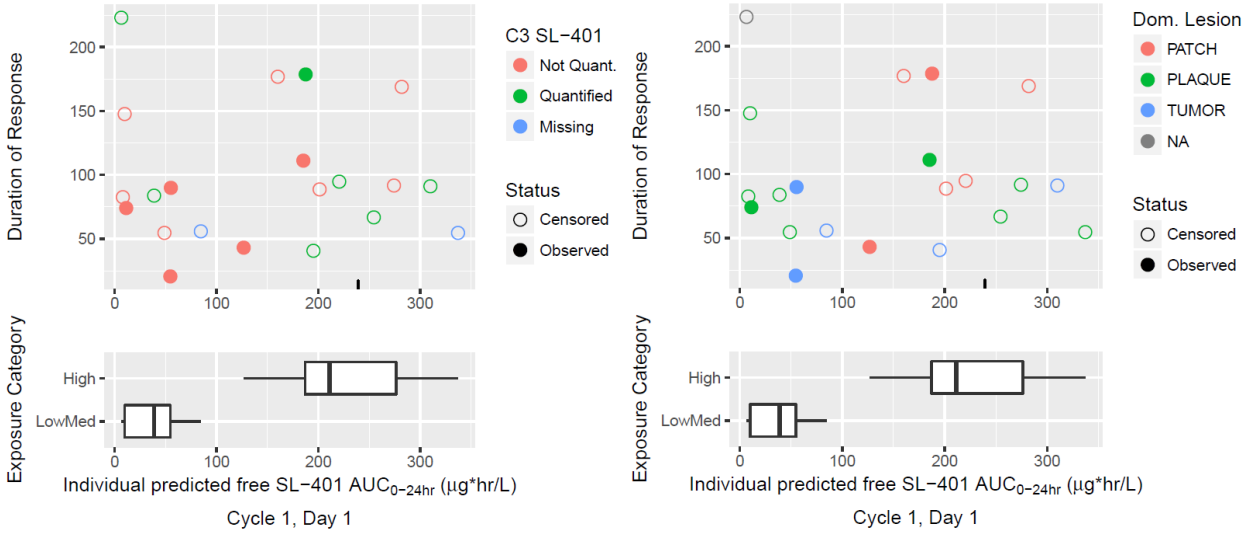


Figure 9 Observed and censored durations of response versus Cycle 1-Day 1 AUC0-24hr. Color is used to distinguish patients with and without quantifiable tagraxofusp in Cycle 3 (left) and dominant lesion types (right).

Only patients with a response of CR/CRc are represented.

Source: Applicant's exposure-response analysis report, Pages 258-259, Figures 128-129.

Reviewer's comments: The Reviewer conducted an independent analysis to quantify the relationship between AUC at Day 1 Cycle 1 and BOR (N=45). A borderline statistically significant exposure-response relationship was identified (Figure 10). Quantifiable PK in Cycle 3 and presence of neutralizing ADA in Cycle 1 were not statistically associated with BOR.

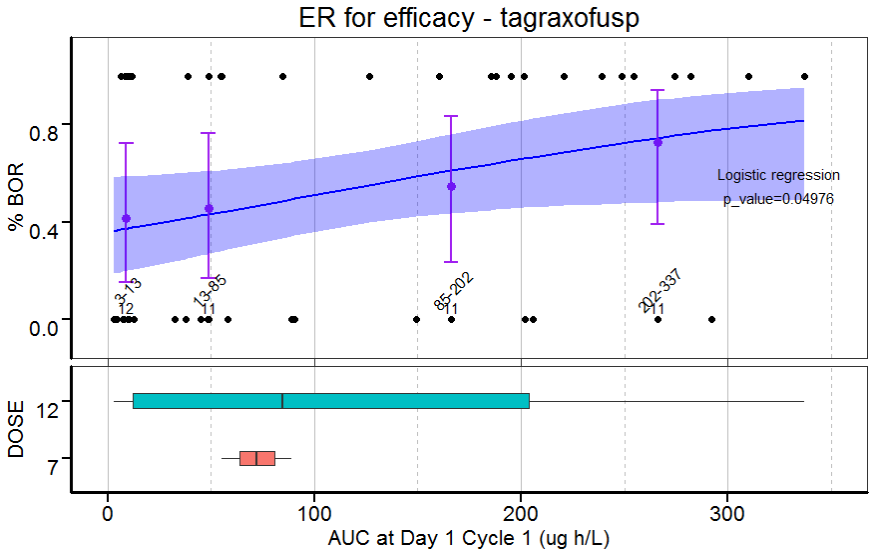


Figure 10 Exposure-response analysis for BOR

Source: Reviewer's independent analysis.

The Applicant did not consider the patient subtype: treatment-naïve and previous-treated, as a covariate on BOR. The Reviewer conducted multivariate logistic analysis for BOR using AUC at Day 1 Cycle 1 and patient subtype. Both AUC at Day 1 Cycle 1 and patient subtype were significant covariates (p<0.05). The exposure-response analysis by patient subtype are presented in Figure 11.

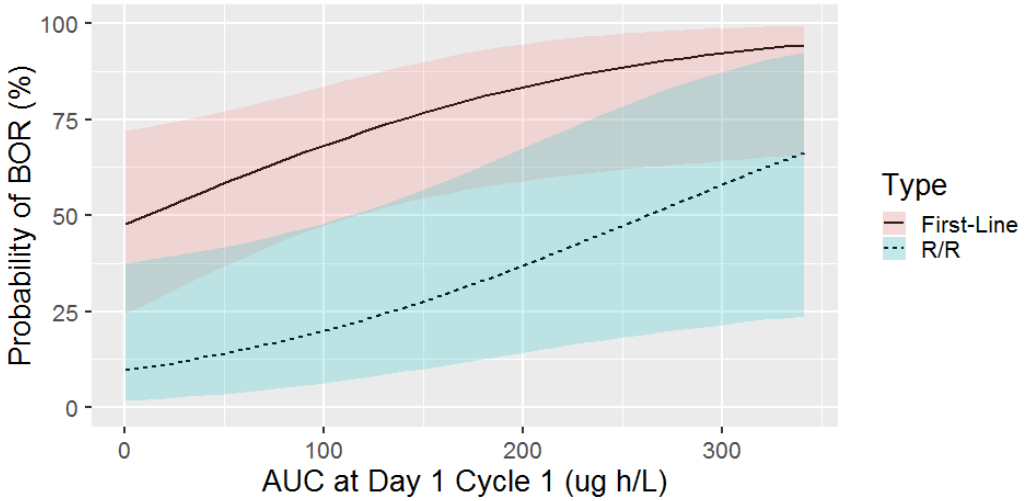


Figure 11 Multivariate exposure-response analysis for BOR

Source: Reviewer's independent analysis.

The exposure-response analysis for progression-free survival (PFS) is shown in Figure 12 (N=47). No statistically significant exposure-response relationship for PFS was identified.

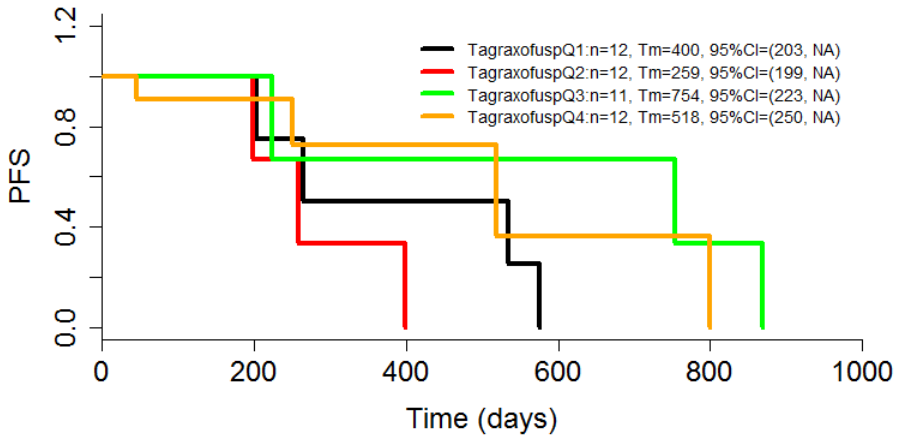


Figure 12 Exposure-response analysis for PFS

Source: Reviewer's independent analysis.

4.5.Exposure-Response Analyses for Safety

The exposure-response analysis for safety was conducted in all subjects from Studies 0114, 0214, and 0314 for whom at least one individual predicted metric of exposure could be estimated based on the population PK model. A total of 122 subjects were included in the analysis consisting of 96 subjects from study 0114, 11 subjects from 214 and 15 subjects from 314. The following AE endpoints were included in the analysis: 1) hypoalbuminemia, 2) probability of capillary leak syndrome (CLS), and 3) elevated transaminases. Tagraxofusp AUC for 24 hours following the C1D1 dose was used as the exposure matric. Covariates evaluated for exposure-response for safety included:

- Disease condition (BPDCN, AML, and MPN).
- Baseline albumin (only for hypoalbuminemia).
- Baseline AST and ALT (only for transaminase elevations).
- Presence or absence of quantifiable tagraxofusp levels in Cycles 3 and 4.
- Presence or absence of neutralizing ADA in Cycle 1.

Based on the exploratory graphical analyses, all 3 AEs exhibited both increasing incidence and increasing severity in association with increasing C1D1 $AUC_{0\text{-}24hr}$ as shown in Figure 13, Figure 14 and Figure 15.

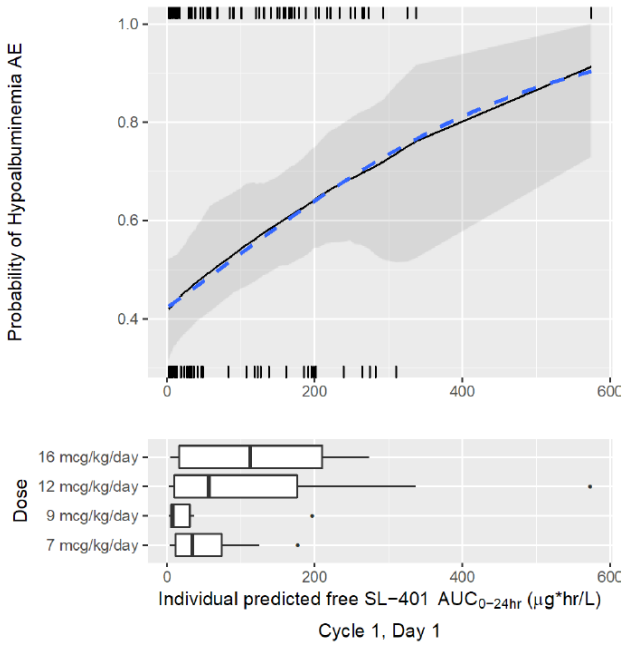


Figure 13 Simulation-Based Predicted Probability of Hypoalbuminemia Versus Cycle 1 Day 1 AUC

Dashed blue line represents a smooth of the original data. Solid black line (simulation median) and grey region (90% prediction interval) represent distribution of smooths fit to replicate data sets simulated from the estimated model.

Source: Applicant's summary of clinical pharmacology report, Page 44, Figure 11.

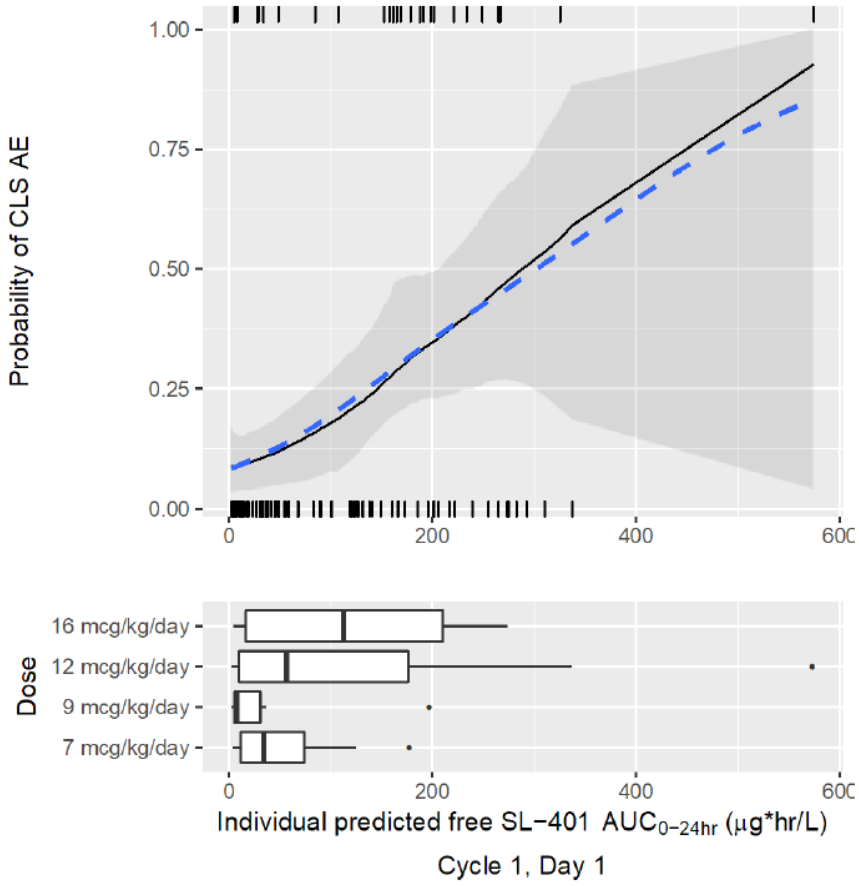


Figure 14 Simulation-Based Predicted Probability of Capillary Leak Syndrome Versus Cycle 1 Day 1 AUC

Dashed blue line represents a smooth of the original data. Solid black line (simulation median) and grey region (90% prediction interval) represent distribution of smooths fit to replicate data sets simulated from the estimated model.

Source: Applicant's summary of clinical pharmacology report, Page 45, Figure 12.

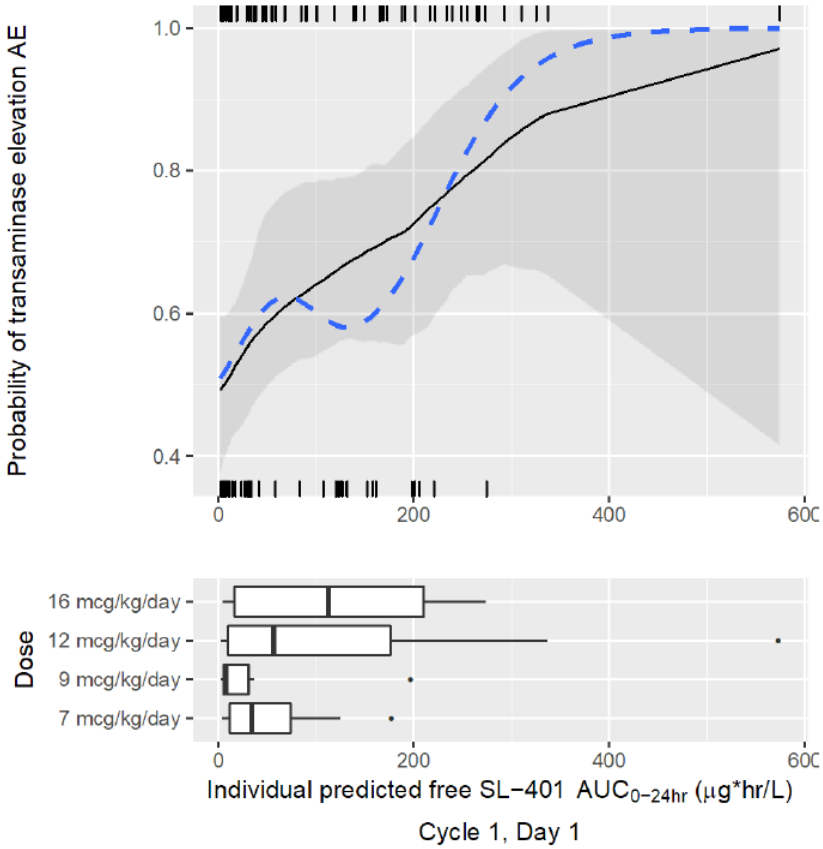


Figure 15 Simulation-Based Predicted Probability of Transaminase Elevation Versus Cycle 1 Day 1 AUC

Dashed blue line represents a smooth of the original data. Solid black line (simulation median) and grey region (90% prediction interval) represent distribution of smooths fit to replicate data sets simulated from the estimated model.

Source: Applicant's summary of clinical pharmacology report, Page 46, Figure 13.

Based on the multiple-predictor logistic regression models, the estimated effects of C1D1 AUC, expressed as odds ratios per 100 (µg*h)/L are shown in Table 22.

Table 22 Estimated effects of Cycle 1 Day 1 AUC for Adverse Events

Adverse Event	Odds Ratio Per 100 µg•h/L Free Tagraxofusp AUC	95% CI
Hypoalbuminemia AE	2.09	(0.62, 4.57)
CLS AE	1.95	(1.26, 3.16)
Transaminase elevation	1.39	(0.84, 2.44)

Source: Applicant's summary of clinical pharmacology report, Page 43, Table 13.

Quantifiable tagraxofusp in Cycle 3/4 was generally associated with increased risk of safety endpoints. Of the 122 subjects in the ER analysis for safety, 30 (24.5 %) had quantifiable tagraxofusp in Cycle 3. Presence

of drug-neutralizing antibodies in Cycle 1 was associated with decreased risk for safety endpoints except CLS, for which the covariate was not found to be predictive at all. Of the 122 subjects in the ER analysis for safety, 23 (18.9%) were positive for Cycle 1 drug-neutralizing antibodies. No covariate was significant at the 0.05 level.

Reviewer's comments: The Reviewer conducted an independent analysis to quantify the relationship between AUC at Day 1 Cycle 1 and hypoalbuminemia, CLS, and elevated transaminases. Statistically significant exposure-response relationships were identified for all the safety endpoints (Figure 16, Figure 17, and Figure 18). Quantifiable PK in Cycle 3 and presence of neutralizing ADA in Cycle 1 were not available for all the subjects that 70 subjects did not have data (neither quantifiable nor not) for PK in Cycle 3 and 16 subjects did not have data (neither presence nor not) for neutralizing antibody in Cycle 1.

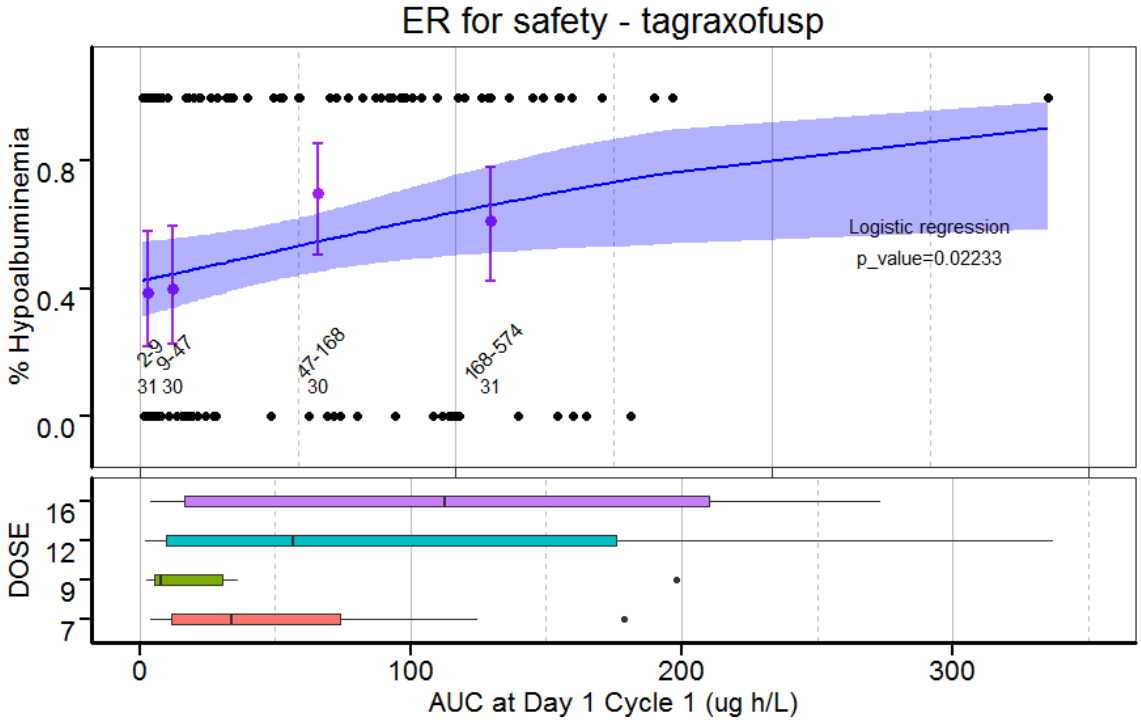


Figure 16 Exposure-response analysis for hypoalbuminemia

Source: Reviewer's independent analysis

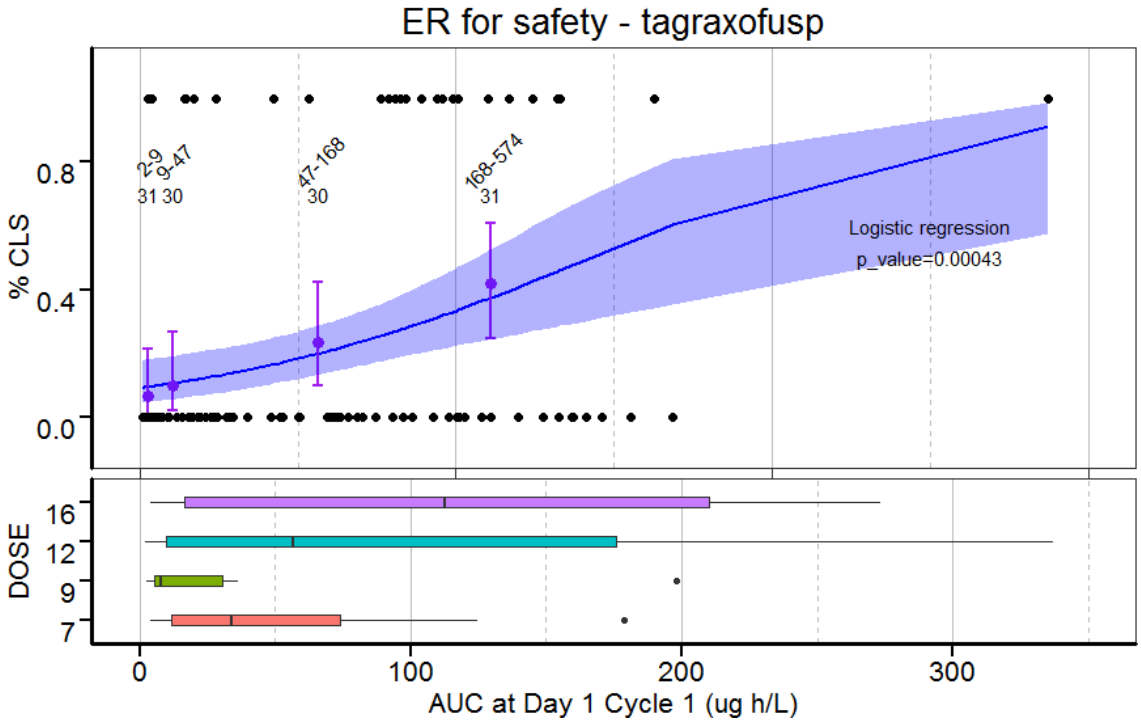


Figure 17 Exposure-response analysis for CLS

Source: Reviewer's independent analysis

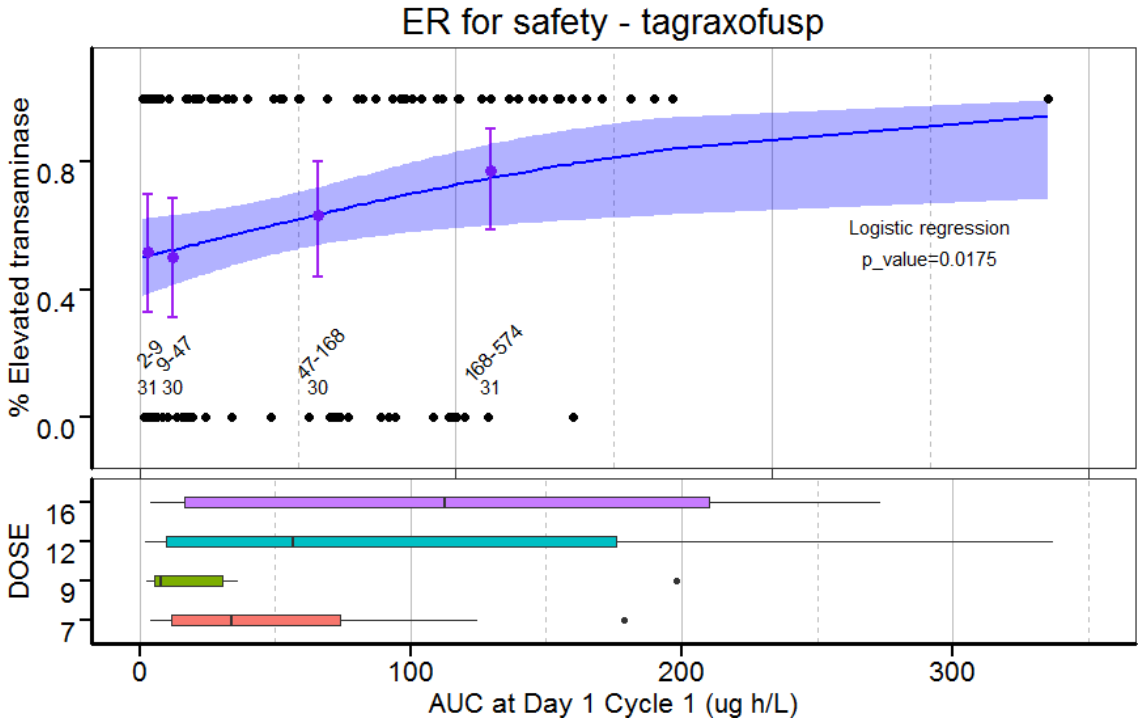


Figure 18 Exposure-response analysis for elevated transaminase

Source: Reviewer's independent analysis

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