

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

761116Orig1s000

PRODUCT QUALITY REVIEW(S)

Recommendation: Approval

BLA/NDA Number: 761116
Review Number: 01
Review Date: 11/28/2018

Drug Name/Dosage Form	Elzonris (tagraxofusp) / Solution, Injection
Strength/Potency	1 mg/ mL
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	For the treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN)
Applicant/Sponsor	Stemline Therapeutics, Inc
US agent, if applicable	n/a

Product Overview

Elzonris (tagraxofusp) is a fusion protein consisting of a truncated form of Diphtheria Toxin (DT) fused to human Interleukin-3 (IL3) via a HIS-MET dipeptide linker. The truncated DT portion of Elzonris consists of the catalytic and translocation DT domains but lacks the receptor-binding DT domain. The IL3 portion of Elzonris directs the molecule to cells expressing the IL-3 receptor on the cell surface, including blastic plasmacytoid dendritic cell neoplastic (BPDCN) cells. Elzonris is internalized via receptor-mediated endocytosis into early endosomes and lysosomes. The DT portion of Elzonris translocates from the lysosome into the cytosol, where it inactivates elongation factor 2, thereby leading to irreversible inhibition of protein synthesis and induction of cell apoptosis. The tagraxofusp drug substance is produced in *E. coli* (b) (4). Elzonris drug product is a sterile, clear, colorless solution supplied in a single-use vial containing 1.0 mg of tagraxofusp formulated in: (b) (4) sodium chloride (75 mM), sorbitol (50. (b) (4)mg), (b) (4) and Water for Injection, USP (b) (4). The Elzonris solution has a pH of 7.5.

Quality Review Team:

Discipline	Reviewer	Branch/Division
RBPM	Rabiya Haider	OPQ/OPRO
Drug Substance	Rong Wang	OBP/DBRRIII
Drug Product	Baikuntha Aryal	OBP/DBRRIII
Immunogenicity	Davina Ligons	OBP/DBRRIII
Labeling	Vicky Hemphill-Borders	OBP
Facility	Steven Fong	OPF/DIA
Microbiology – DS	Maria Lopez-Barragan	OPF/DMA
Microbiology – DP	Monica Commerford	OPF/DMA
QAL Microbiology	Reyes Candau-Chacon	OPF/DMA
Application Team Lead	Steven Bowen	OBP/DBRRIII

Multidisciplinary Review Team:

Discipline	Reviewer	Office/Division
RPM	Kristopher Kolibab	OND/OHOP/DHP
Cross-disciplinary Team Lead	Donna Przepiorka	OND/OHOP/DHP
Medical Officer	Emily Jen	OND/OHOP/DHP
Pharm/Tox	Natalie Simpson	OND/OHOP/DHOT
Clinical Pharmacology	Liang Li	OTS/OCP/DPM
Statistics	Xin Gao	OB/DBV

1. **Names:**

- a. Proprietary Name: Elzonris
- b. Trade Name: Elzonris
- c. Non-Proprietary Name/USAN: Tagraxofusp
- d. CAS Name: Diphtheria toxin (Corynebacterium diphtheriae catalytic domain/transmembrane domain-containing fragment) fusion protein with peptide (synthetic 2-amino acid linker) fusion protein with interleukin 3 (human) 2055491-00-2
- e. Common Name: Tagraxofusp
- f. INN Name: Tagraxofusp
- g. Compendial Name: N/A
- h. OBP systematic name: RPROTFRAG P00588 (DTX_CORBE); RPROT P08700 (IL3_HUMAN) [SL401]
- i. Other names: SL-401, DT388IL3, Molecule 129

2. **Pharmacologic category:** Tagraxofusp is a novel cytotoxic biologic targeted therapy directed to the interleukin-3 (IL-3) receptor CD123.

Submissions Reviewed:

Submission:	Date Received:	Review Completed (yes or no)
STN 761116/SN0004 New BLA	06/21/2018	Yes
STN 761116/SN0006 Response to Product Quality IR	07/13/2018	Yes
STN 761116/SN0008 Response to Product Quality IR	08/07/2018	Yes
STN 761116/SN0014 Quality Information: Stability Update	08/27/2018	Yes
STN 761116/SN0015 Response to Product Quality IR	9/14/2018	Yes
STN 761116/SN0016 Response to Product Quality IR	9/17/2018	Yes
STN 761116/SN0017	9/28/2018	Yes

Response to Product Quality IR STN 761116/SN0019	10/19/2018	Yes
Response to Product Quality IR STN 761116/SN0021	10/31/2018	Yes
Response to Product Quality IR STN 761116/SN0024	11/05/2018	Yes
Response to Product Quality IR STN 761116/SN0027	11/09/2018	Yes
Response to Product Quality IR STN 761116/SN0028	11/15/2018	Yes
Response to Product Quality IR STN 761116/SN0030	11/27/2018	Yes
Response to Product Quality IR STN 761116/SN0031	11/30/2018	Yes

Quality Review Data Sheet

1. **Legal Basis for Submission:** 351(a)

2. **Related/Supporting Documents:**

A. DMFs:

DMF#	DMF Holder	Item Referenced	Letter of Cross-Reference	Comments (status)
(b) (4)	(b) (4)	(b) (4)	Yes	(b) (4) No review was needed as all the relevant information related to compatibility with the product was in the BLA
			Yes	(b) (4) No review was needed as all the relevant information related to compatibility with the product was in the BLA.
			Yes	(b) (4) No review was needed as all the relevant information related to

				compatibility with the product was in the BLA
(b) (4)	Yes	(b) (4)	(b) (4)	No review was needed as all the relevant information related to compatibility with the product was in the BLA
	Yes	(b) (4)	(b) (4)	No review was needed as all the relevant information related to compatibility with the product was in the BLA
	Yes			Type V DMF for the DS manufacturing facility. No review was necessary.
	Yes			Type V DMF for the DP manufacturing facility. No review was necessary.

B. Other documents: IND, Referenced Listed Drug (RLD), or sister application.

- IND 114513: Tagraxofusp for the treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN).

Executive Summary

I. Recommendations: **Approval**

A. Recommendation and Conclusion on Approvability:

Recommendation:

The Office of Biotechnology Products (OBP, OPQ, CDER), the Division of Microbiology Assessment (DMA, OPF, OPQ, CDER), and the Division of Inspectional Assessment (DIA, OPF, OPQ, CDER) recommend approval of STN 761116 for Elzonris manufactured by Stemline Therapeutics, Inc. The manufacturing data and information provided in the submission are sufficient to support a conclusion that the manufacturing process of Elzonris is well controlled and leads to a product that is pure and potent for the duration of the product shelf life. OPQ recommends that this product be approved for human use under conditions specified in the package insert.

B. Approval Action Letter Language:

- Manufacturing location:
 - Drug Substance: [REDACTED] (b) (4)
 - Drug Product: [REDACTED] (b) (4)
USA Fill size and dosage form: 1.0 mg/1.0 mL in a single-dose vial
- Dating period:
 - Drug Product: 36 months at [REDACTED] (b) (4)
 - Drug Substance: [REDACTED] (b) (4) months at [REDACTED] (b) (4)
 - Stability:
 - We have approved the stability protocols in your license application for the purpose of extending the expiration dating of your [REDACTED] (b) (4) drug product under 21 CFR 601.12.
- Exempt from lot release
 - Elzonris is exempted from lot release per Docket No 95-29960 because Elzonris is a recombinant protein.

C. Benefit/Risk Considerations:

Elzonris is a recombinant, cytotoxic, fusion protein that selectively targets and kills cells expressing the IL3 receptor on the cell surface. BPDCN is a rare hematologic malignancy characterized by the proliferation of plasmacytoid dendritic cells expressing high levels of IL3 receptor. Patients with BPDCN generally present with skin lesions with extracutaneous malignant cells in the bone marrow, blood, lymph nodes, spleen and other organs, and have a median survival of 8 to 14 months. Currently there is no standard of care for BPDCN. If approved, Elzonris may address an unmet medical need for patients with BPDCN. The product received breakthrough therapy designation on August 22, 2016 and this BLA received priority review.

Five post-marketing commitments (PMCs) were agreed upon with the sponsor to improve the robustness of the DS manufacturing processes. [REDACTED] (b) (4) PMC-1 is to conduct a study to investigate whether [REDACTED] (b) (4) can be removed. [REDACTED] (b) (4)

[REDACTED] The Sponsor did not perform an assessment of product quality after the DS and DP shipping process. PMC-2 and PMC-4 are to conduct method qualification studies with additional batches. Stability of DS and DP during shipping is supported by simulated shipping studies and long-term stability

data. PMC-3 will be to perform a (b) (4) shipping qualification study evaluating the impact of the shipping process on DS and DP product quality. PMC-5 is to monitor (b) (4) sterile filtration. Overall, these five PMCs will result in improvement and increased robustness of the manufacturing processes for Elzonris.

In conclusion, the data submitted in this application support the conclusion that the manufacture of Elzonris is well controlled and yields a product that is safe, pure and potent. From a product quality perspective, this product is approvable for human use.

D. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if approvable:

The following draft post marketing commitments have been proposed and accepted by the Sponsor:

1. *Perform a study to evaluate the impact of the removal (b) (4) (b) (4). If the data support removal of (b) (4), a plan for the removal of (b) (4) from the tagraxofusp manufacturing process will be provided. The plan should include an evaluation of consistency of the (b) (4) process and comparability of tagraxofusp manufactured with and without (b) (4). The final study report will be submitted in accordance with 21 CFR 601.12.*

Final Study Report: March 31, 2020

2. *Conduct (b) (4) bioburden and endotoxin test method qualification using two additional drug substance batches. The final qualification report will be submitted in accordance with 21 CFR 601.12.*

Final Study Report: September 30, 2019

3. *Perform a drug substance and drug product shipping study using the proposed commercial shipping lanes and modes of transportation to evaluate the impact of shipping on product quality. The study conditions should represent the worst case for tagraxofusp shipping and should evaluate product quality post-shipment using validated methods with pre-defined acceptance criteria. The shipping qualification report will be submitted in accordance with 21 CFR 601.12.*

Final Study Report: September 30, 2019

4. *Conduct (b) (4) bioburden test method qualification using one additional batch of tagraxofusp drug product.*

Final Study Report: March 31, 2019

5. *Implement (b) (4) sterile filtration of tagraxofusp drug product and (b) (4)*

Final Study Report: March 31, 2019

II. Summary of Quality Assessments:

Table 1 below is a summary of critical quality attributes and the associated control strategies for attributes that are intrinsic to the Drug Substance. Table 2 summarizes critical quality attributes that are relevant to the drug substance. For additional information, see the technical document on primary reviews of the Drug Substance Quality and Drug Product Quality by OBP/DBRR-III and the Drug Substance Microbiology and the Drug Product Microbiology by OPF/DMA.

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 1: Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy
Cytotoxicity	Efficacy	Intrinsic to molecule, impacted by heat, oxidation, high and low pH	(b) (4)
Identity	Safety and Efficacy	Intrinsic to molecule	
Identity /Primary sequence	Safety and Efficacy	Intrinsic to molecule	
Higher Order Structure	Efficacy	Intrinsic to molecule	
High Molecular Weight (HMW) species/Dimer/Aggregates	Efficacy and Safety/Immunogenicity	Manufacturing process and exposure to agitation, heat, oxidation, and low and high pH stress	
Fragmentation	Efficacy and Immunogenicity	Manufacturing process and exposure to heat, low and high pH stress	
Methylated species	Efficacy	Manufacturing process and exposure to oxidation	
Deamidated species	Efficacy and Safety	Manufacturing process and exposure to high temperature, high pH, and oxidation	

(b) (4)

Oxidized species	Efficacy and Safety	Manufacturing process and exposure to oxidation

B. Drug Substance Tagraxofusp Quality Summary

CQA Identification, Risk, and Lifecycle Knowledge Management

Table 2: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management.

CQA (type)	Risk	Origin	Control Strategy
Appearance	Safety	Controlled by the manufacturing process	(b) (4)
pH	Efficacy and Safety	Controlled by the manufacturing process	
Osmolality	Safety	Controlled by the manufacturing process	
Host Cell Proteins (process-related impurity)	Safety and Immunogenicity	Production cell line	
Host Cell DNA (process-related impurity)	Safety	Production cell line	
Free sulfhydryl	Efficacy	Disulfide bond breakage	
Residual (b) (4)	Safety and Immunogenicity	Process-related impurity (media component)	
Residual (b) (4)	Safety	Process-related impurity (media component)	
Residual (b) (4)	Safety	Process-related impurity (media component)	

Lysogenic bacteriophage (contaminant)	Safety	Lysogenic bacteriophage contamination would likely occur during cell bank production or during cell culture operations.
Leachables (Process-related impurity)	Safety	Process-related impurities potentially from contact materials during manufacture and the DS container closure system (CCS)
Bioburden	Safety, purity, and efficacy (degradation or modification of the product by contaminating microorganisms)	Raw materials and manufacturing process
Bacterial Endotoxin	Safety and purity	Raw materials and manufacturing process

- **Description:**
Tagraxofusp is a 524-amino acid Diphtheria Toxin-Interleukin-3 Fusion Protein. Tagraxofusp consists of an N-terminal methionine followed by diphtheria toxin (DT) catalytic (C) and translocation (T) domains. The DT fragment (Q388R variant) is joined at the C-terminus through a His-Met dipeptide linker (His390-Met391) to the N-terminus of the full 133-amino acid sequence of human interleukin 3 (IL-3). Tagraxofusp is expressed in *E. coli*.
- **Mechanism of Action (MoA):**
The IL-3 domain of tagraxofusp targets and binds to cells overexpressing the IL-3 receptor on cell surface, resulting in receptor-mediated endocytosis into early endosomes and lysosomes. The catalytic domain of DT translocates through the vesicle membrane and is released from the tagraxofusp molecule in the cytosol. The DT catalytic domain then catalyzes the transfer of adenosine diphosphate (ADP) ribose from nicotinamide adenine dinucleotide (NAD) to elongation factor 2, thereby leading to inhibition of protein synthesis and resulting in cell apoptosis.
- **Potency Assay:**

Relative potency of tagraxofusp DS and DP is determined by measuring its cytotoxic effect on TF-1-hRas cells which express IL-3 receptor on cell surface. The reference standard or test sample is serially diluted from 100 ng/mL to 0.009 ng/mL and added into cells in triplicate in 96-well plate and cultured at 37 °C for 48 hours. Resazurin is added into plate and the fluorescence is read at 544 nm (excitation) and 590 nm (emission) using a plate reader. This results in a dose dependent inhibition curve. The data is analyzed using a four-parameter curve fit. The relative potency value is determined by calculating the ratio of the IC50 of the test sample to the IC50 of the reference standard (RS). Each sample is tested in two independent runs and the mean of the two independent runs is reported as the relative potency value for that sample.

- Reference Materials:

(b) (4)

- Critical starting materials or intermediates:

(b) (4)

- Manufacturing process summary:

(b) (4)

- Container closure:

The tagraxofusp DS solution is stored

(b) (4)

(b) (4)

- Dating period and storage conditions:
DS ^{(b) (4)} months when stored at ^{(b) (4)}

C. Drug Product Elzonris Quality Summary:

Table 3 provides a summary of the identification, risk, and lifecycle knowledge management for drug product CQAs that derive from the drug product manufacturing process and general drug product attributes.

Table 3: Drug Product CQA Identification, Risk, and Lifecycle Management

CQA (type)	Risk	Origin	Control Strategy
Color and Degree of Opalescence	Safety and Efficacy	Formulation, Contamination, or Degradation	(b) (4)
Osmolality	Safety	Formulation	
pH	Safety and Efficacy	Formulation	
Particulate Matter	Safety / Immunogenicity	Formulation, Manufacturing process, container closure system, and on stability	
Extractable Volume	Efficacy / Dosing	Manufacturing process	
Leachables (process-related impurities)	Safety	Manufacturing equipment and CCS	
Bacterial endotoxin (contaminant)	Purity and Safety (immunogenicity)	Raw materials and bacterial contamination throughout the DP manufacturing process.	
Sterility	Safety, Purity, and Efficacy (degradation or modification of the product by contaminating microorganisms)	Contamination may be introduced throughout the DP manufacturing process or by container closure integrity failure.	

Container Closure Integrity Testing (CCIT)	Safety	Container closure breaches during storage.	(b) (4)

- Description and Strength:**
Elzonris (injection) is supplied in a single use 2 mL vial containing 1.0 mL of tagraxofusp at a strength of 1.0 mg/ 1.0 mL. Potency of Elzonris is determined with a cell-based cytotoxicity assay and expressed relative to a reference standard. The potency assay is the same as described in the drug substance section of this review.
- Summary of Product Design:**
Elzonris is supplied as a sterile, single-use, ready-to-use, preservative-free solution for intravenous injection in a vial. The drug product formulation consists of Elzonris (1.0 mg/mL) Elzonris, (b) (4) sodium chloride (75 mM), sorbitol (50 (b) (4) mg), (b) (4), (b) (4), and Water for Injection, USP. The extractable volume for Elzonris DP vials (b) (4)
- List of Excipients:**
Excipients include (b) (4) sodium chloride, sorbitol, (b) (4).
- Reference Materials:**
The primary and working reference standards used for Elzonris DP are the same as described for the drug substance.
- Manufacturing process summary:**
(b) (4)
- Container closure:**
The container closure system is a single-use, 2.0 mL (b) (4) glass vial (b) (4) with a 13mm gray (b) (4) rubber, (b) (4) stopper (b) (4)

(b) (4) and a 13mm flip-off seal (b) (4) Letters of Authorization for DMFs pertaining to the DP container closure system were provided.

- Dating period and storage conditions:
The dating period for Elzonris drug product is 36 months at (b) (4) C, protected from light.

D. Novel Approaches/Precedents:

E. Any Special Product Quality Labeling Recommendations:

Store in freezer between -25°C and -15°C (b) (4)°F and (b) (4)°F).

Protect from light by storing in the original package until time of use.

Thaw vials between 15°C and 25°C (59°F and 77°F) prior to preparation.

Do not refreeze the vial once thawed. Do not use beyond expiration date on container.

F. Establishment Information:

The Division of Inspectional Assessment recommends approval of the BLA. The manufacturing and testing facilities, their responsibilities in tagraxofusp production, and inspectional outcomes are summarized in the table below.

Overall Recommendation: APPROVE			
Drug Substance			
Responsibility	Site Information	DUNS/FEI Number	Facility Status
Drug substance manufacturing, release testing, and stability testing (pH, osmolality, appearance, (b) (4))	(b) (4)	(b) (4)	Approve
Drug substance release and stability testing (b) (4)			Approve
Master and working cell bank storage, drug substance storage			Approve
Master and working cell bank storage			Approve

Master and working cell bank manufacture and release; cell bank stability testing	(b) (4)	Approve
Sample receipt and storage for release and stability testing of drug substance		Approve
Drug substance storage		Approve

Drug Product			
Responsibility	Site Information	DUNS/FEI Number	Facility Status
Drug product formulation and fill, storage, analytical testing, drug product release testing (sterility only)	(b) (4)	(b) (4)	Approve
Drug product release and stability testing			Approve
Drug product release and stability testing			Approve
Drug product release and stability testing (identity, appearance, osmolality, particulates, container content, sterility, endotoxin, CCIT)			Approve
Drug product labeling, packaging, storage, and distribution			Approve
Sample receipt and storage for release and stability testing of drug substance			Approve

G. Facilities:

Tagraxofusp DS manufacturing is performed at (b) (4)
 (b) (4) A pre-license inspection (PLI) of (b) (4) was

conducted from [REDACTED] (b) (4) by OPF/DIA and OBP. A 4-item Form 483 was issued with a recommendation of Voluntary Action Indicated (VAI). The responses to the Form 483 observations were adequate.

Tagraxofusp DP manufacturing is performed at [REDACTED] (b) (4). A PLI was performed at [REDACTED] (b) (4) by OPF/DIA. A 2-item Form 483 was issued with a recommendation of VAI. The responses to the Form 483 observations were adequate.

H. Lifecycle Knowledge Management:

a. Drug Substance:

- i. Protocols approved:
 - annual stability protocol
 - requalification protocols for tagraxofusp PRS and WRS
 - qualification protocols for new tagraxofusp PRS and WRS
 - concurrent validation [REDACTED] (b) (4)
- ii. Outstanding review issues/residual risk:
 - See Post-Marketing Commitments in Section IB
- iii. Future inspection points to consider:
 - Follow up on 483 observations and CAPAs

b. Drug Product

- i. Protocols approved:
 - annual stability protocol
- ii. Outstanding review issues/residual risk:
 - See Post-Marketing Commitments in Section IB
- iii. Future inspection points to consider:
 - Follow up on 483 observations and CAPAs



Steven
Bowen

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Maria
Gutierrez Lugo

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Food and Drug Administration
Center for Drug Evaluation and Research
Office of Pharmaceutical Quality
Office of Process and Facilities
Division of Microbiology Assessment

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Date: 30 November 2018
To: Administrative File, **STN 761116/0**
From: Monica Commerford, Ph.D., Primary Reviewer, CDER/OPQ/OPF/DMA/BIV
Through: Reyes Candau-Chacon, Ph.D., Acting Quality Assessment Lead,
CDER/OPQ/OPF/DMA/BIV
Subject: New Biologics License Application
Applicant: Stemline Therapeutics, Inc.
US License: 2088
Product: ELZONRIS™ (tagraxofusp)
Dosage: 1.0 mg/mL solution for injection via intravenous infusion
Indication: Treatment of patients with blastic plasmacytoid dendritic cell neoplasm
Facilities:  (b) (4)

Receipt Date: 21 June 2018

Action date: 21 December 2018

Recommendation for Approvability: The drug product section of BLA 761116/0, as amended, was reviewed from a sterility assurance and microbiology product quality perspective and is recommended for approval. The following post-marketing commitments were identified and communicated to the sponsor:

1. To monitor  (b) (4) sterile filtration of tagraxofusp drug product by 1Q 2019.
 2. To qualify the  (b) (4) drug product bioburden method using one additional batch of tagraxofusp. Qualification data will be submitted by March 2019.
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Review Summary

Stemline Therapeutics, Inc. has submitted this new 351(a) Biologics License Application for ELZONRIS™ (tagraxofusp), a diphtheria toxin Interleukin-3 fusion protein. The drug substance is manufactured at (b) (4). The drug product is manufactured at (b) (4). This application contains CMC information in an eCTD format. The BLA was originally submitted on 21 June 2018. Module 2 contains a Quality Overall Summary. Module 3 contains sections describing manufacturing, characterization, control of drug product, stability, and drug product information. This review contains an assessment of the drug product manufacturing process of tagraxofusp from a sterility assurance and product quality microbiology perspective and may reference the Drug Master Files (DMFs) listed in the table below.

DMFs associated with BLA 761116/0

DMF	DMF Holder	Subject of DMF
(b) (4)		

Amendments associated with this review memo are also listed in the table below. FDA information requests are listed at the end of this review memo.

Amendments Reviewed for Drug Product Quality Microbiology

Document Type	eCTD Sequence	Date
Responses to filing information request	0006	13 July 2018
Responses to Information Request #1	0017	28 2018
Responses to Information Request #2	0021	31 October 2018
Responses to Information Request #2 update	0024	5 November 2018
Responses to Information Request #3	0027	9 November 2018
Responses to Information Request #4	0028	15 November 2018

Product Quality Microbiology Assessment: Drug Product

MODULE 1

Labeling review: Prescribing information

ELZONRIS is provided in (b) (4) glass vials containing (b) (4) tagraxofusp drug product (DP). This DP is a (b) (4) sterile (b) (4) for injection and is preservative-free. Vials are stored at (b) (4) and protected from light in the original packaging until use. Vials are thawed at room temperature before dose preparation (b) (4) and the DP is clear and colorless with few white to translucent particles. The recommended dose is 12 µg/kg/day once daily over the course of 15 minutes on days 1 – 5 of a 21-day cycle.

Preparation occurs in a two-step process. First, 10 mL of 100 µg/mL ELZONRIS is made by diluting thawed 1 mg/mL DP in 9 mL 0.9% Sodium Chloride Injection, USP (diluent) and mixing. Then, the required volume of ELZONRIS is calculated using the patient’s weight. A separate syringe is prepared with at least 3 mL 0.9% Sodium Chloride Injection, USP to be used as a saline flush after administration. Prepared ELZONRIS should be administered within 4 hours and may be left at room temperature. Any excess DP should be thrown away.

Reviewer’s comment: Microbial challenge studies to ascertain the microbial growth-promoting properties of the tagraxofusp DP when diluted into 0.9% Sodium Chloride (and stored at room temperature) are not required because the DP is administered within 4 hours of preparation. The label was updated to indicate that a sterile 10 mL vial and syringe should be used to prepare the DP.

Satisfactory

MODULE 3.2.P DRUG PRODUCT

P.1 Description and Composition of the Drug Product (DP)

The tagraxofusp DP is supplied as a (b) (4) sterile (b) (4) for injection. The composition of the DP is provided in Table 1 (copied from the submission) below. The DP is formulated in 50 mg/mL sorbitol, 75 mM sodium chloride, and (b) (4) pH 7.5. The description of the container closure system (CCS) is provided in Section 3.2.P.7. Tagraxofusp is supplied in 2 mL/13 mm (b) (4) glass with a 13 mm gray (b) (4) rubber (b) (4) stopper, and 13 mm flip-off (b) (4) seals. The DP is stored and shipped at (b) (4)

Table 1: Drug Product Quantitative Composition, 1.0 mL/Vial

Material	Purpose	MG/Vial ^a	Weight % ^b	MG/mL
Tagraxofusp	Active ingredient	(b) (4)	(b) (4)	1.0
(b) (4)				
Sodium chloride, USP, EP			(b) (4)	4.38 (b) (4)
Sorbitol, USP, EP				50.0
(b) (4)				
Water for injection, USP, EP				(b) (4)

EP: European Pharmacopoeia; NF: National Formulary; n.s.: sufficient quantity; USP: United States Pharmacopoeia

a (b) (4)
b (b) (4)

The description is satisfactory.

PRODUCT QUALITY MICROBIOLOGY REVIEW AND EVALUATION

Primary Reviewer: Maria Jose Lopez Barragan, Ph.D.
Secondary Reviewer: Reyes Candau Chacon, Ph.D.

Date: 12/3/2018
BLA: 761116
Applicant: Stemline Therapeutics, Inc.
US License: 2088
Subject: Original Biologic License Application (Priority Review)
Facilities: (b) (4)

Product: ELZONRIS® (tagraxofusp)
Indication: Treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN)
Dosage: Sterile solution (1.0 mg/mL) in single-use vials for intravenous infusion
Action date: 2/21/2019

Recommendation for approvability: The drug substance section of BLA 761116, as amended, is recommended for approval from a microbial control and microbiology product quality perspective, with the following post-marketing commitment:

1. To conduct (b) (4) bioburden and endotoxin test method qualifications using two additional drug substance batches.

SUMMARY

Stemline Therapeutics, Inc. has submitted BLA 761116 to obtain approval of tagraxofusp, a recombinant fusion protein for the treatment of patients with BPDCN. BLA 761116 was submitted as a rolling BLA with the final BLA component (including Module 3) submitted on 6/21/2018. This review contains the assessment of tagraxofusp drug substance from a microbiology product quality perspective. Microbiology product quality and sterility assurance of ELZONRIS® drug product was reviewed by Dr. Monica Commerford.

Drug Substance Microbiology Quality Information Reviewed

Description	eCTD Sequence	Date
Original BLA (Module 3)	0004	6/21/2018

Amendment (response to information request)	0015	9/14/2018
Amendment (response to information request)	0028	11/15/2018
Amendment (response to information request)	0030	11/27/2018
Amendment (response to information request)	0033	12/3/2018

3.2.S DRUG SUBSTANCE

S.1 GENERAL INFORMATION

Tagraxofusp is a recombinant fusion protein comprised of human interleukin-3 (IL-3) and truncated diphtheria toxin (DT) that is directed to CD123, a key marker of blastic plasmacytoid dendritic cell neoplasm (BPDCN). Tagraxofusp is expressed in a recombinant *E. coli* strain. Tagraxofusp is also referred to as SL-401, DT388IL3, and Molecule 129.

The description is satisfactory

S.2 MANUFACTURE

(b) (4)

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Memorandum of Immunogenicity Review

BLA: 761116

Subject: Immunogenicity assays for SL-401 (tagraxofusp): binding and neutralizing assays for anti-SL-401 and IL-3 antibodies

Primary Reviewer: Davinna L. Ligons, Ph.D.
Secondary Reviewer: Steve Bowen, Ph.D.
Tertiary Reviewer: Susan Kirshner, Ph.D.

Product: SL-401

Sponsor: Stemline Therapeutics, Inc.
Indication: Treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BDCN)

RPM Kristopher Kolibab

Clinical Division: OHOP/Division of Hematology Products

Received Date: June 21, 2018
Action Due Date: February 21, 2019

Recommendation: The screening, confirmatory, titer and neutralizing assays used to assess immunogenicity of SL-401 (tagraxofusp) during the clinical program for BLA 761116 were appropriately validated for the intended purpose. The assays performed consistently during the testing of clinical samples. There are no major deficiencies with the immunogenicity testing strategy, and therefore this BLA should be approved from an immunogenicity perspective.

Review

Immunogenicity Assays

This review memorandum covers the immunogenicity assays used to evaluate anti-SL-401 and anti-IL-3 binding and neutralizing antibodies in clinical trials 0114, 0214, 0314, and 0414. The testing was conducted by (b) (4). Assays originally developed by (b) (4) have since been transferred (b) (4) (b) (4).

(b) (4)

Table 1: Summary of Immunogenicity Assays and Testing Laboratories for the SL-401 Clinical Studies per Question 1b

Antibody Assay	Study Number			
	0114	0214	0314	0414
Anti-SL-401 Antibody Test Method GCL-319, Validation Report 11-145	(b) (4)			
Anti-hIL-3 Antibody Test Method GCL-321, Validation Report 13-100				
Anti-SL-401 NAb Test Method GCL-315, Validation Report 13-060				
Anti-hIL-3 NAb TM-TUF-0003 v2.0 TUF-17-056-VR01	(b) (4) ¹	NA	NA	NA

¹ Anti-hIL-3 NAb testing of STML-401-0114 Stage 3 only submitted to support the BLA.

NA = Not Applicable (no data submitted in BLA)

SL-401 is a truncated diphtheria toxin (DT) interleukin-3 fusion protein expressed in *E. coli* cells. The human IL-3 component of SL-401 allows for binding to leukemia blasts and cancer stem cells that over-express the IL-3 receptor. Binding of IL-3 to its receptor leads to receptor mediated endocytosis leading to DT-induced cell death through protein synthesis inhibition following cleavage of DT from IL-3. The Sponsor evaluated antibody responses to SL-401 and human IL-3. The presence of anti-IL-3 neutralizing antibodies was only determined in Study 0114, as indicated in Table 1 above.

A tiered approach was used to evaluate binding anti-drug antibodies (ADA) to SL-401. The first tier was a bridging electrochemiluminescent (ECL) screening assay to detect anti-SL-401 antibodies. Because most individuals have been vaccinated for diphtheria toxin, it is expected that most patients will have pre-existing anti diphtheria toxin antibodies, and thus be positive at baseline in the screening assay. Due to the unavailability of negative serum samples, a typical screening cut point was not established and serum samples were instead screened for ADA using the confirmatory assay. The tier 2a confirmatory assay was developed using inhibition with unlabeled SL-401 to confirm anti-SL-401 positive serum samples. Tier 2b assays were developed to determine SL-401 domain specificity of the antibody response by inhibition with unlabeled IL-3 or DT. Tier 3 examines the titer of binding antibodies. A separate cell-based assay was used to determine whether anti-SL-401 antibodies were neutralizing.

High levels of anti-DT antibodies expected in patient serum may interfere with the detection of anti-IL-3 antibodies when using excessive inhibitory IL-3 in the tier 2b SL-401 confirmatory assay. Thus, to circumvent this issue, the Sponsor developed a separate

assay to detect anti-IL-3 binding antibodies and anti-IL3 titer. In addition, a separate cell-based assay was developed to detect neutralizing anti-IL-3 antibodies.

A total of four assays were developed to detect binding and neutralizing anti-SL-401 and IL-3 antibodies. Table 2 indicates the bioanalytical, method development, and validation reports associated with each immunogenicity assay.

Table 2: Immunogenicity Bioanalytical Methods for SL-401 Immunogenicity Assessments

Antibody Assay	CRO	Bioanalytical Method #	Method Development Report or Study #	Method Validation Report or Study #
Anti-SL-401 Antibody	(b) (4)	GCL-319	11-145	11-145
Anti-hIL-3 Antibody		GCL-321	13-100	13-100
Anti-SL-401 NAb		GCL-315	13-060	13-060
Anti-hIL-3 NAb		TM-TUF-0003 v2.0	TUF-17-055	TUF-17-056-VR01

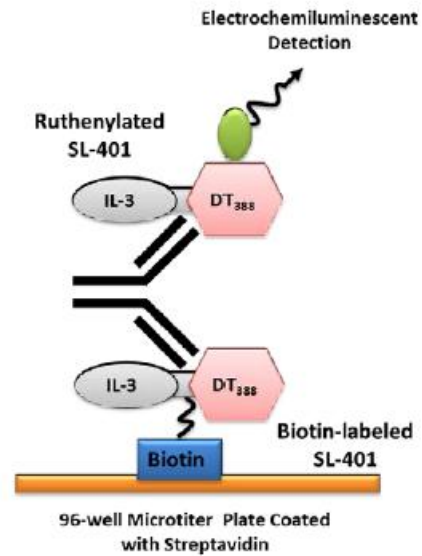
Abbreviations: anti-hIL-3 = anti-human interleukin-3; (b) (4); CRO = contract research organization; NAb = neutralizing antibodies.

Assay Review

- **GCL-319 Validation of a Bridging Immunoassay (IA-ECL) to Detect Antibodies to SL-401 in Human Serum (Validation report #11-145)**

Background

The assay is a bridging assay designed to detect antibodies to SL-401. The assay will also detect antibodies to the truncated DT and IL-3 components and neo-epitopes. The samples are incubated with biotin and ruthenium labeled SL-401 to form immune complexes followed by incubation on a streptavidin coated plate. The signal is detected after washing.

Figure 2: Bridging Assay to Detect SL-401 Antibodies

Abbreviations: DT = diphtheria toxin; IL-3 = interleukin-3.

This screening assay is expected to yield many positive samples because most individuals in the United States have been immunized with DT and have anti-DT antibodies. Thus, it would be difficult to reliably establish a screening cut point. To circumvent this issue, the confirmatory assay (in which the samples were inhibited with unlabeled SL-401) was used to screen for positive samples using the confirmatory cut point. DT and IL-3 are used as inhibitors in the confirmatory assay and to determine the domain specificity of the ADA. Samples are diluted with assay buffer with a minimum required dilution (MRD) of 1:8.

Positive and Negative Controls

The positive controls consist of previously screened positive pooled normal human serum samples that are categorized by ECL units into low, mid and high positive controls. The signal is likely generated by antibodies to DT. The negative control is assay buffer instead of normal human serum because serum samples without anti-DT antibodies are not available. The positive control for anti-SL401 antibodies was prepared from pooled serum samples with different levels of ECL signal. It should be noted that the low, mid, and high positive controls are not diluted to yield indicated levels of signal. Instead the serum samples are categorized based on the signal they yield. The criteria to determine the validity of a run have been established and described in the bioanalytical method report #GCL-319.

Normal human serum:

- Low: 2,000-4,000 ECL units
- Mid: 20,000 – 40,000 ECL units
- High: 220,000 – 280,000 ECL units

Positive control antibodies:

- Goat Anti-Diphtheria Toxin polyclonal antibody
- Affinity purified rat anti-IL-3 monoclonal antibody

Validation Principle	Acceptance Criteria	Validation Result
Screening Cut Point Determination	N/A	Due to the prevalence of human samples with antibodies against diphtheria toxin due to immunizations, no screening cut point was established. Confirmatory cut point was established with use of percent inhibition values. Out of 156 individual serum samples, 8 samples were removed from confirmatory cut point determination – 2 had high %CVs and 6 were analytical outliers. The data are displayed in Table 3 and the data analysis is displayed in Annex C .

Validation Principle	Acceptance Criteria	Validation Result
Precision	intra- and inter-assay precision $\leq 30\%$	Intra-assay responses are precise with variability $\leq 19.4\%$ CV. Inter-assay responses met the acceptance criteria for the negative, low, and high controls with variability $\leq 29.5\%$ CV. The mid control level showed a 31.1% CV. The inter-assay %CV for the mid control is above the acceptable criteria of $\leq 30\%$. The precision data for the mid control will be accepted due to the qualitative nature of the method. Data are displayed in Table 4. The note-to-file justifying this decision is displayed in Annex D.
Confirmatory Cut Point % Inhibition (Specificity)	N/A	On each cut point plate, negative and positive control samples were assayed, both with and without inhibitor. All (48/48) assay buffer control samples gave % inhibition below the confirmatory cut point of 77.1%. All of the reported positive control samples (22/22 LPC, 22/22, MPC and 21/21 HPC) yielded % inhibition values that were above the confirmatory cut point. The % Inhibition for a sample must be at least 77.1% (confirmatory cut point for 0.1% false negative error rate) to be considered positive for anti-SL-401 antibodies. Table 5 and Annex C.
Batch Size	report value	A batch size of 2 plates is supported by validation data, specifically 2-plate batches were used during cut point analysis.
Tier 3	Signal titers to below the threshold level of 3X buffer background for the plate for each control.	Reference antibody controls demonstrate capacity for titer according to acceptance criteria. Buffer and serum are equivalent diluents for Tier 3. Diluents were considered comparable based on titer results within one dilution of each other. Table 6
Selectivity	$\geq 80\%$ of the test serum lots for each matrix type with a mean %Difference from the background subtracted spike control within $\pm 30\%$ at each level.	Sixty percent of the matrix lots evaluated were within the acceptance criteria when compared to % Recovery from Matrix Pool. The data are displayed in Table 7. It is difficult to assess the true selectivity of the assay with the presence of pre-existing antibodies in the matrix lots tested. Selectivity results are accepted; refer to Deviation DR-14-0113 for the justification.

Validation Principle	Acceptance Criteria	Validation Result
Ruggedness and Robustness	each assay must pass the acceptance criteria set post precision	The parameters/equipment used and control data are displayed in Table 8. Ruggedness was assessed during cut point/precision analysis. Additional robustness was explored within hemoglobin/Intralipid interference assays. The assay for the mid time point failed due to a processing error for one of the incubation times. However, the minimum and maximum times met the acceptance criteria and support the use of a plate incubation range of 30-40 minutes. Additionally, the data supports the use of a 115-120 minute sample incubation time; refer to Deviation DR-14-0097 for the justification.
Hemoglobin and Lipid Interference	report results	No interference by hemoglobin was observed since %Diff from the Ref was < 30% for the hemoglobin samples. The % Diff from Ref was -14.8% for the Low Control and -18.7% for the High Control. Table 9 No interference by lipid was observed since %Diff from the Ref was < 30% for the Intralipid samples. The % Diff from Ref was 12.1% for the Low Control and 16.4 % for the High Control. Table 10
Sensitivity	report results	The calculated sensitivity was determined to be 3.39 ng/mL (which is the mean of 3.65 ng/mL for assay 14MSD30953 and 3.12 ng/mL for assay 14MSD30962) based on the interpolation of the cut point for each assay on the response curve using the hyperbola algorithm regression. Table 11 and Figure 1

Validation Principle	Acceptance Criteria	Validation Result
Drug Tolerance	samples with zero antibody (negative) should remain below the cut point assess the drug tolerance based on expected therapeutic concentrations in samples	The assay buffer control consistently gave results below the cut point regardless of the amount of SL-401 added. The low QC level of anti-DT antibody consistently gave results above the cut point regardless of the amount of SL-401 added. At 500 ng/mL anti-IL-3 antibody, the drug tolerance was calculated to be 253.9 ng/mL SL-401 (the mean of 261.6 and 246.2 ng/mL for assays 14MSD31018 and 14MSD3019, respectively). Therefore, this method can tolerate SL-401 concentrations of 253.9 ng/mL without decreasing the signal to at or below confirmatory cut point levels for 500 ng/mL anti-IL-3-spiked human serum samples. This is acceptable as the SL-401 concentrations at sample collection are expected to be below this concentration. Table 12 and Figure 2
Freeze-Thaw Stability	within $\pm$ 25% of Difference from Reference value	All samples tested at 6 and 7 freeze/thaw cycles pass the acceptance criteria with a % Difference from Reference ranging from -16.2 to 17.0%. Stability is demonstrated for up to 7 cycles. Table 13
Short-Term Stability	within $\pm$ 25% of Difference from Reference value	All samples met the acceptance criteria with a % Difference from Reference ranging from -12.1 to 8.9%. Stability is demonstrated for up to 24 hours at 20 to 25 °C or 2 to 8 °C. Table 14
Domain Specificity/Tier 2b Inhibitor	anti-IL-3 positive controls should be inhibited $\geq$ the confirmatory cut point with the IL-3 inhibitor and $<$ the confirmatory cut point with the DT inhibitor. anti-DT positive controls should be inhibited $\geq$ the confirmatory cut point with the DT inhibitor and $<$ the confirmatory cut point with the IL-3 inhibitor.	With the confirmatory cut point set at 77.1% Inhibition, all positive controls met the acceptance criteria. The anti-IL-3 control sample inhibited with IL-3 demonstrates a % Inhibition $\geq$ 98.6 % and the anti-IL-3 control sample inhibited with DT demonstrates a % Inhibition $\leq$ 9.5%. The anti-DT control sample inhibited with DT demonstrates a % Inhibition of $\geq$ 91.3 % and the anti-DT inhibited with IL-3 demonstrates a % Inhibition $\leq$ 25.2%. This demonstrates that the domain specificity inhibitors (IL-3 and DT) selectively distinguish anti-IL-3 and anti-DT antibodies. Table 15

Reviewer comments:

Overall the validation of this assay is acceptable and meets FDA guidelines. The Agency agrees that setting a screening cut point based on negative serum is not practical because of the expected high percentage of samples with antibody reactive to the DT portion of the SL-401. It is appropriate to use the confirmatory assay to determine positive samples. Twenty-six individual normal human samples were used to calculate the confirmatory cut point and 10 μ g/mL of SL-401 is used as the inhibitor. If the sample is positive in the

confirmatory assay, 10.2 µg/mL of DT inhibitor and 2.6 µg/mL of IL-3 inhibitor are used to determine domain specificity of the response. The high level of inhibition with the SL-401, DT and IL-3 inhibitors also demonstrate specificity of the assay. The confirmatory assay cut point was determined by analyzing the distribution of % inhibition data across the validation samples. A lower-bound 99.9% confidence limit was used to establish the confirmatory cut point of 77.1% inhibition which is reasonable. In addition, a separate direct binding assay to anti-IL-3 was developed because of the concern that the anti-SL-40 detection and confirmatory assays would not be sensitive enough to detect anti-IL-3 antibodies. Along these lines, in the IR response dated 11/09/2018, the Sponsor provided a summary of the domain specificity test results for the clinical trial (Table 1 below).

Tier 2b domain specificity data are in the BLA listing [16.2.10.1](#) for clinical study 401-0114 and ISS listing [16.2.9.7.1](#) for the STML-401-0214, 401-0314 and 401-0414 clinical studies. The Sponsor proposes that inhibition with DT and IL-3 were intended to characterize the immune response and not to determine anti-IL-3 ADA responses. Overall, the incidence of anti-IL-3 antibody positive samples detected with the domain specificity assay is higher than what is reported with the anti-IL-3 binding assay. However, it is possible that the presence of anti-DT antibodies in the sample would mask the inhibition of IL-3 and reduce the ability to detect anti-IL-3 antibodies. The Agency agrees that the IL-3 characterization assay is not suitable to identify IL-3 domain specificity.

Table 1: Incidence of Anti-DT and Anti-IL-3 Antibody % Inhibition Using the Domain Specificity Assay

Sample Type	Anti-DT Antibody % Inhibition by Free DT	Anti-IL-3 Antibody % Inhibition by Free IL-3
Pre-treatment (baseline)		
n/N (%)	116/116 (100.0)	45/116 (38.8)
Median	98.28	6.32
Q1, Q3	97.17, 98.99	2.44, 11.67
Min, Max	13.30, 99.71	0.31, 99.70
Post-Treatment (any)		
n/N (%)	111/111 (100.0)	90/111 (81.1)
Median	95.30	8.30
Q1, Q3	84.20, 98.31	3.71, 17.50
Min, Max	0.30, 99.95	0.03, 97.70

The titer of the anti-SL-401 detection assay is determined by performing a 2-fold serial dilution of test samples with assay buffer. The MRD is factored in the reported titer. The titer of a sample is the dilution step above where the ECL value falls below the cut point of 3X greater than the mean ECL of the assay buffer samples on the plate. Table 6 in the validation report shows the selected titer for normal human serum levels low, mid, and high control levels. However, the dilutions indicated in the yellow highlights do not appear to meet the 3X buffer background cut point. In an IR response dated September 17, 2018 (sequence #0016), the Sponsor clarified that the yellow highlighted dilutions are

not the titers, but instead the first dilutions below the titer. The titer was correctly indicated as 1:16 (low PC), 1:256 (mid PC), and 1:2,048 (high PC) which is the dilution that is above the 3X buffer background cut point. In the IR response dated 11/09/2018, the Sponsor clarified that the 3X buffer background was selected because establishing a titer cut point based on a false positive rate would be impossible due to the high expectance of anti-DT antibodies. Because the titer is a relative measure, establishing the cut point as 3X assay buffer background should not interfere with the comparison of the titer between samples. The titer of the assay appears to be in the linear range based on the data provided in Table 6 of the validation report. The precision of the titer was not reported. However, the overall precision of the assay is acceptable, and the titers across several assays reported as the system suitability control during the testing of clinical samples are reasonable.

The sensitivity of the confirmatory assay is 3.39 ng/mL and was determined with the anti-IL-3 positive control antibody. The sensitivity of the assay is acceptable. Blood samples for immunogenicity are taken when SL-401 levels in the blood are negligible and thus, the drug tolerance of the assay is acceptable. The selectivity of the assay was determined with the anti-IL-3 antibody spiked into 10 pre-screened human serum samples. However, the acceptance criteria were not met for selectivity as 60% of the samples instead of 80% of the samples had recovery within 30% of the control. Because normal human samples likely have anti-DT antibodies, which may lead to a positive signal, the recovery may be higher in the assay. The low selectivity of the assay to detect anti-IL-3 antibodies is acceptable because of the expected interference of anti-DT antibodies. The acceptance criteria for precision were set to $\%CV \leq 30\%$. The negative control, low, and high positive controls passed the acceptance criteria; however, the precision for the mid-level positive control was 31.1%. Because the precision of the assay was determined with normal human samples which often have high variability, the precision of $\%CV \leq 30\%$ is acceptable.

Suitability controls used during the clinical trial are indicated in section 15 of the bioanalytical report GCL-19. This section outlines the controls that were used on each plate for each assay tier and acceptance criteria to accept clinical run results. The acceptance criteria for the system suitability controls are acceptable because they include parameters and criteria for the confirmatory and titer assays. IR response dated October 1, 2018 details the rejection rate for this assay as shown in the table below.

Table 2: Summary of Pass/Fail for Anti-SL-401 Antibodies by Method GCL-319

Parameter	Study 0114	Study 0214	Study 0314	Study 0414	Cumulative
Total assay runs (n)	467	21	72	12	572
Passed runs (n, %)	369 (79.0%)	19 (90.5%)	57 (79.2%)	10 (83.3%)	455 (79.5%)
Failed runs (n, %)	96 (20.6%)	2 (9.5%)	15 (20.8%)	2 (16.7%)	115 (20.1%)
Aborted runs (n, %)	2 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.3%)

Source: [13-189](#), [SC-15-136-008](#), [SC-15-136-012](#), [SC-17-136-022](#)

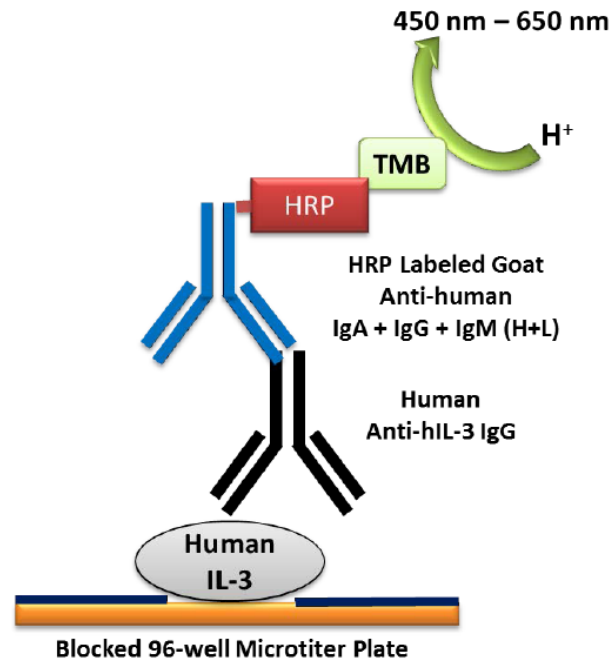
System suitability controls were only provided for plates where the system suitability controls meet the acceptance criteria and thus the assay was accepted. The low, medium and high positive controls had comparable signals within each level and signals had the following pattern HPC>MPC>LPC. The rejection rate of 20% is acceptable because clinical samples on plates that were rejected were re-assayed and only clinical samples on plates where the suitability controls were acceptable were used to assess immunogenicity.

- **GCL-321: Validation of an ELISA for the Detection of Antibodies to Human IL-3 Human Serum (Validation report #13-100-VR)**

Background

The Sponsor developed an ELISA for the identification of anti-human IL-3 antibodies with a 3 tier approach including screening (Tier1), confirmatory (Tier2) and titer (Tier 3). Figure 4 below is a schematic for the ELISA method. Briefly, serum samples diluted with a 1:25 MRD are incubated on the IL-3 coated plate. Horseradish peroxidase (HRP) conjugated anti-human IgA, IgG, and IgM antibodies are used to detect human antibodies and HRP anti-rat antibody is used to detect the rat anti-IL-3 positive control antibody. The criteria to determine the acceptability of a run have been established and described in the bioanalytical method report #GCL-321.

Figure 4: Direct Binding Assay to Detect Anti-human Interleukin-3 Antibodies



Abbreviations: H = heavy; hIL-3 = human interleukin-3; HRP = horseradish peroxidase; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; IL-3 = interleukin-3; L = light; TMB = 3,3',5,5'-tetramethyl-benzidine.

Positive and Negative Controls

The negative control is pooled normal human serum. Various concentrations of rat anti-IL-3 antibody diluted in normal pooled human serum are used as the positive control.

Positive control low = 100 ng/mL

Positive control mid = 500 ng/mL

Positive control high = 1500 ng/mL

Validation Principle	Acceptance Criteria	Validation Result
Selectivity (Spike and Recovery)	≥ 80% of samples within ± 30% Difference from spike control	19 out of 20 (95%) of the matrix samples evaluated were within the acceptance criteria. Table 3 Note: some results for the unspiked samples are higher than the low QC due to the dual detection nature of the assay and the background differences between the two detection systems. The spiked samples were detected with the anti-rat HRP, whereas the unspiked samples were detected with the anti-human HRP.
Cut Point Determination and Normalization Factor	N/A	Out of 306 individual results, 56 results were removed from cut point determination – 20 demonstrated a high %CV, 26 were analytical outliers and 2 lots (10 values) were considered to be biological outliers. Table 4 The method will employ a floating cut point using a NF of 1.59. The titer cut point factor (tier 3) is 2.85. Annex C
% Inhibition (Specificity)	N/A	The % Inhibition for a sample must be ≥ 37.9% for the sample to be considered positive for anti-IL-3 antibodies. Table 5, Annex C
Precision	intra- and inter-assay precision ≤ 30% for floating cut points	The cumulative responses for the negative controls show an intra-assay variability of 20.2% and the inter-assay variability 33.3%. However, all individual runs demonstrated ≤ 30% CV. This reinforces the need for a floating cut point. Responses are precise for the positive controls with intra-assay variability ≤ 9.9% and inter-assay variability ≤ 13.1%. Table 6
Batch Size	report value	Three plates may be analyzed simultaneously since 3 plates were analyzed successfully during cut point analysis.

Validation Principle	Acceptance Criteria	Validation Result
Ruggedness and Robustness	each assay must pass the acceptance criteria set post precision	The table of parameters/equipment used is displayed in Table 7 . Ruggedness was assessed during cut point analysis. All assays passed the acceptance criteria. Note: Only one plate reader was used but since the plate readers are qualified for absorbance using universal test plates, it is reasonable to state that they are equivalent on that basis.
Hemolysis and Lipemia Interference	report results	Eighteen out of 20 hemolytic samples spiked with anti-IL-3 were within 30% Difference from the Control Spikes. Therefore, there is no apparent interference from hemolysis. Table 8 Eighteen out of 20 lipemic samples spiked with anti-IL-3 were within 30% Difference from the Control Spikes. Therefore, there is no apparent interference from lipemia. Table 9
Sensitivity	report results	The relative sensitivity was determined to be 68 ng/mL for assay 14PR60363 and 58 ng/mL for assay 14PR60377 based on the interpolation of the cut point for each assay on the response curve. Table 10 , Figure 1
Drug Tolerance	samples with zero antibody (negative) should remain below the cut point assess the drug tolerance based on expected therapeutic concentrations in samples	The negative sample gave results consistently below the cut point. At 100 ng/mL anti-IL-3 antibody, the drug tolerance was calculated to be <7.8 and 21.7 ng/mL. At 500 ng/mL anti-IL-3 antibody, the drug tolerance was calculated to be 228.5 and 238.6 ng/mL. This method can tolerate drug concentrations of <7.8 ng/mL without decreasing the signal to at or below cut point levels for 100 ng/mL anti-IL-3-spiked human serum samples. This level is acceptable as samples for immunogenicity testing will be drawn when the drug concentrations are negligible. The C _{max} is estimated at 1,000 ng/mL with a 30 minute half life. Table 11 , Figure 2
Freeze/Thaw Stability	within ± 25% Difference from Reference	Stable for up to 8 cycles. Table 12
Short-Term Stabilities	within ± 25% Difference from Reference	Stable for up to 24 hours at 15 – 30 °C or at 2 – 8 °C. Table 13
Validation Principle	Acceptance Criteria	Validation Result
Long-Term Frozen Stability (-10 to -35 °C)	within ± 30% Difference from Reference	Long-term stability assessment is ongoing and will be reported in an addendum to this report.
Long-Term Frozen Stability (-60 to -85 °C)	within ± 30% Difference from Reference	Long-term stability assessment is ongoing and will be reported in an addendum to this report.

Reviewer comments:

Overall the validation of this assay is acceptable and meets FDA guidelines. Fifty-one normal human serum samples were used to determine the screening, confirmatory and titer cut points. Using parametric analysis, the floating screening cut point normalization factor (NF) is 1.59 and based on a 5% false positive rate. Two methods can be used to determine a positive sample: 1) if the OD value is greater than 1.59 x the negative control on that plate or 2) if the log-transformed S/N value is greater than 1.59. With the low positive control anti-IL-3 antibody (100ng/mL), the false negative rate was determined to be 7.6% when using the NF of 1.59. Regarding the confirmatory assay, IL-3 is used as the inhibitor. Using parametric analysis, an inhibition of 32.8% is the cut point defined at a 1% false positive rate. Non-parametric analysis determined the cut point to be 37.9% with a 1% false positive rate. Outliers were removed from the analysis to determine the cut points. However, it is not clear whether the validation data were tested for normalization. It appears the parametric cut point of 1.59 and the non-parametric of 37.9% was used to determine positive clinical samples. Because the parametric and non-parametric cut points are very close, it is reasonable to use either cut point for the confirmatory assay. The titer assay has a normalization factor of 2.85 which is based on a 0.1% false positive rate. The reported titer factors in the MRD. The precision of the titer was not determined; however, the precision determined during serial dilution of the positive control antibody is < 13% for all dilution points.

The sensitivity of 58 – 68 ng/mL is acceptable and within FDA guidelines. The drug tolerance of the assay is reasonable because blood samples are collected when drug concentrations are at the lowest and the C_{max} of the drug is 1000 ng/mL with a half-life is 30 minutes. Thus, it is expected that drug levels will not interfere with the assay. The specificity of the assay was evaluated using the confirmatory assay approach of inhibition of the positive control with IL-3. The specificity of the assay is acceptable because all concentrations of the positive control antibody yielded a positive signal when inhibited with IL-3. The selectivity of the assay is acceptable and meets the acceptance criteria. However, it should be noted that a different detection antibodies were used for spiked and non-spiked samples. Specifically, an anti-rat HRP detection antibody was used for the spiked samples and an anti-human HRP detection antibody was used for non-spiked samples. As a result, there is a difference in the background levels and for some non-spiked samples the signal is higher than low positive spiked samples, according to Table 3 of the validation report. The Sponsor was requested to provide an explanation for using different detection antibodies when determining the selectivity of the assay. In an IR response dated September 17, 2018 (sequence #0016), the Sponsor clarifies that while the positive control antibodies are rat antibodies, the anti-human antibodies were used to determine variability in the background for this reagent when used with human serum samples. As an added control when assessing clinical samples, a few wells were coated with human IgG and detected with human specific reagents. Using both reagents in the selectivity assay does not appear to affect assay results and no other issue needs to be addressed. The precision of the assay is acceptable despite the slightly high %CV of 33.3% for inter-assay variability for the negative control. This is acceptable because the negative control normal human serum is expected to have high variability. The precision for the positive control antibody is %CV < 20% and meets FDA guidance.

Suitability controls used during the clinical trial are indicated in section 16 of the bioanalytical report GCL-321. This section outlines the controls that were used on each plate and acceptance criteria to accept clinical run results. IR response dated October 1, 2018 details the rejection rate for this assay as shown in the table below.

Table 4: Summary of Pass/Fail Rate for Anti-IL-3 Antibodies by Method GCL-321

Parameter	Study 0114	Study 0214	Study 0314	Study 0414	Cumulative
Total assay runs (n)	326	17	32	13	388
Passed runs (n, %)	261 (80.1%)	14 (82.4%)	31 (96.9%)	12 (92.3%)	318 (82.0%)
Failed runs (n, %)	63 (19.3%)	3 (17.6%)	1 (3.1%)	1 (7.7%)	68 (17.5%)
Aborted runs (n, %)	2 (0.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.5%)

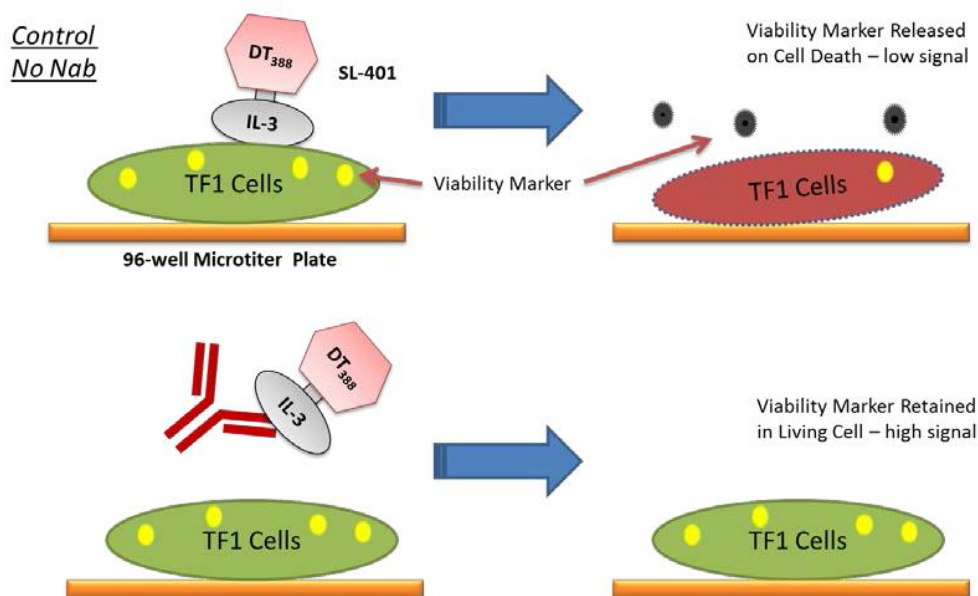
Source: SC-14-136-005, SC-15-136-009, SC-15-136-013, SC-17-136-023

Comparable signals were found within the low, medium and high positive controls which have lower and upper limits established and signals had the following pattern HPC>MPC>LPC in the absence of the inhibitor. In addition, the signal for the human IgG coated wells which serve as a positive control was comparable across plates. Overall, the parameters and acceptance criteria outlined in section 16 of the bioanalytical report GCL-319 are acceptable for the system suitability controls. The rejection rate of 19% is acceptable for the study 0114 because only clinical samples on plates where the suitability controls were acceptable were used to assess immunogenicity. Clinical samples on plates that were rejected were re-assayed.

- **GCL-315: Validation of a Cell Proliferation Assay for the Detection of Neutralizing Antibodies to SL-401 in Human Serum (Validation report #13-060-VR)**

Background

A cell-based assay was developed to assess neutralizing antibodies to SL-401. Human IL-3 receptor expressing erythroleukemic cells (TF-1 hRas) are plated and undergo proliferation. After adding SL-401 the cells undergo cell death and reduced proliferation via DT activity of the drug following uptake mediated by binding of the IL-3 portion of the drug to the IL-3 receptor. Samples diluted to 1:100 MRD or positive control anti-IL-3 antibody are pre-incubated with SL-401 to allow for sufficient neutralization. The final concentration of SL-401 in the assay is 30 ng/mL. Samples are then placed on cells and the proliferation of the cell is measured following 3 days of proliferation. The viability of the cells is measured with a commercially available solution called Resazurin. If either portion of the drug, DT or IL-3 is neutralized, the cells will proliferate and have more viable cells.

Figure 3: Cell-Based Method for the Detection of SL-401 Neutralizing Antibodies

Abbreviations: DT = diphtheria toxin; IL-3 = interleukin-3; Nab = neutralizing antibodies.

The percent of neutralization is calculated as:

$$\% \text{ Neutralization} = \left[\frac{(\text{Mean Sample Response} - \text{Mean Drug Response})}{(\text{Mean Media Response} - \text{Mean Drug Response})} \right] * 100$$

where: Drug response = NQC + SL-401; Media Response = NQC - SL-401

The design of the assay does not specifically identify anti-IL-3 neutralizing antibodies because anti-DT neutralizing antibodies may give a similar reduction in proliferation. To address this issue, the Agency asked the Sponsor to develop an assay that can specifically identify anti-IL-3 neutralizing antibodies (see review for assay #TUF-17-056-VR01).

Positive and Negative Control

Human monoclonal anti-IL-3 antibody diluted in pooled normal human serum is used as the positive control antibody. The human serum samples are pre-screened to not contain pre-existing antibodies. The negative base pool control is prescreened pooled normal human serum.

Low quality control = 10 µg/mL

High quality control = 50 µg/mL

System Suitability

To ensure that the cells adequately respond to SL-401 compared to media only, the following method acceptance criteria for cell proliferation was established: NC + Media ≥ HPC > LPC > NC + SL-401.

Validation Principle	Acceptance Criteria	Validation Result
Selectivity and MRD Confirmation	≤ 30% CV between replicates for each sample ≥ 66% of test lots in assessment with an average % Difference from spike control must be within ± 30% at each spike level for selectivity to pass. Note: This criterion does not include samples with potential pre-existing antibodies that might interfere with the assessment. As stated in the plan, these samples yield less than 2-fold difference between stimulated and un-stimulated response.	Three out of 12 samples were removed from the selectivity assessment because the samples had less than 2-fold difference between stimulated and un-stimulated response. Eight out of the 9 remaining (89%) positive spikes met the acceptance criteria. This parameter passes. Table 3
Precision	≤ 30% CV for Inter- and Intra-assay mean and ≤ 30 %CV for each Precision sample.	Outliers were detected in the results. Representative box and whisker plots identifying those outliers are displayed in Annex C. Responses for negative control + drug (raw responses) are precise with %CV = 10.6% for intra-assay, 11.3% for inter-assay, and ≤ 15.6% for individual samples. Table 4 Responses for negative control + media (raw responses) are precise with %CV = 7.7% for intra-assay, 10.9% for inter-assay, and ≤ 12.2% for individual samples. Table 4 Responses for positive controls (raw responses) are precise with %CV = 10.3 and 8.3% for intra-assay, 11.6 and 9.3% for inter-assay, and ≤ 15.4% for individual samples. Table 5 The Note-to-File documenting the acceptable control performance ranges can be found in Annex D.

Validation Principle	Acceptance Criteria	Validation Result
Cut Point Determination	report value	Out of 306 individual samples, 96 individual serum samples were removed from cut point determination; 1 result was removed for high %CV between replicate results, 21 were identified as analytical outliers and 14 lots (74 values) were considered biological outliers. Table 6 The method will employ a fixed cut point of 8.5% Neutralization at a 1% false positive rate. Annex E
Sensitivity	report value	The sensitivity was determined to be 2.85 µg/mL, The cut point intersects the response curve at 28.5 ng/mL. Multiplying that result by the MRD of 1:100 yields 2.85 µg/mL. Table 7, Figure 1
Robustness and Ruggedness	assay must pass the acceptance criteria set post precision assessment	Cell assay plates can be incubated from 68 hours to 77 hours. With this range of incubation times, all samples demonstrate a ≤ 13.3% Difference from Mid. TF-1 hRas cells may be used through passage 26. All samples test representing passage 9 through 26 demonstrate a ≤ 13.3% Difference from Mid. Table 8
Drug Tolerance	report value	The drug tolerance is > 1000 ng/mL, based on the fact that PC sample results remained above the cut point at all concentrations of SL-401 tested. Note: the NC sample results remained below the cut point. Table 9, Figure 2

Validation Principle	Acceptance Criteria	Validation Result
Freeze/Thaw Stability	$\pm 30\%$ Difference from Reference for each freeze/thaw for each concentration	For 6X F/T samples, assay 14PR90782 was an acceptable assay but the F/T samples did not meet the acceptance criteria. Samples were re-tested in assay 14PR90801 in which the samples met the acceptance criteria. For 7X F/T samples, assay 14PR90783 was an acceptable assay but the F/T samples did not meet the acceptance criteria. Samples were re-tested in assay 14PR90802 in which the samples met the acceptance criteria. For 8X F/T samples, assay 14PR90784 failed. The samples were re-tested in assay 14PR90803 and all samples met the acceptance criteria. Samples are considered stable for up to 8 cycles because all samples demonstrate a % Difference from Reference range of -25.5 to -0.6%. Table 10
Short-Term Stability	$\pm 30\%$ Difference from Reference for each condition for each concentration	All controls met the acceptance criteria under both conditions. Samples are stable for up to 24 hours at 2 – 8 °C and at 15 – 30 °C. Table 11
Long-Term Stability	$\pm 30\%$ Difference from Reference for each time point for each concentration	Is ongoing and will be reported in an addendum to this report.

Reviewer comments:

Overall, the validation of the assay is acceptable and demonstrates that the assay is able to identify neutralizing SL-401 antibodies. It is possible that patient samples will have neutralizing antibodies to DT due to diphtheria toxin vaccination and these antibodies will likely cross-react with SL-401. There is a 2X reduction in proliferation when SL-401 is added to the cells compared to media conditions only. This 2X difference was used to identify samples with pre-existing antibodies. Individual lots of human serum were screened to identify samples containing pre-existing antibodies. In order for a sample to be valid, the difference between the media control and SL-401 stimulation must be 2X. If the difference is less than 2X, the sample would be considered to contain pre-existing antibodies. Invalid serum samples containing pre-existing antibodies are not used to create the negative base pool. The method to identify samples with pre-existing neutralizing antibodies is acceptable. Of note, the cell line has a mutation that allows for its growth independent of GM-CSF.

Using 51 normal human serum samples, a fixed cut point of 8.5% has been established based on a 1% false positive rate. Any sample with a percent neutralization greater than 8.5% is considered positive. The cut point is acceptable because it meets FDA guidelines. An IR was sent to the Sponsor requesting clarification on whether pre-existing anti-DT NAbs present in normal serum samples interfere with assay results and determining the cut point. The Sponsor proposed that the assay was designed to detect antibodies that block the internalization of the drug by the IL-3 receptor and IL-3 portion of the drug and

that pre-existing anti-DT NABs do not interfere with binding of the IL-3 portion of the drug to the receptor. The Agency proposes that while anti-DT antibodies may prevent cytotoxicity as would anti-IL-3 NAB antibodies, the presence of anti-DT NAB antibodies may not block the IL-3 portion of the drug from interacting with the IL-3 receptor expressed on the cells. Thus, these data support that the presence of anti-IL-3 and anti-DT NABs are not distinguishable by this assay. While it is possible that pre-existing anti-DT antibodies present in the serum used to determine the cut point did interfere with assay results, the very conservative cut point of 8.5% neutralization (the blockade of SL-401 induced cellular lethality) will likely allow for detection of NABs whether they are anti-IL-3 or anti-DT antibodies.

The sensitivity of the assay is 2.85 µg/mL. In the presence of the LQC and HQC, the assay is sensitive with drug levels up to 1000 ng/mL. Because the assay is a cell-based assay, the sensitivity of the assay is acceptable. Additionally, the assay is able to detect neutralizing SL-401 antibodies in over 80% of the total patient population which further supports the sensitivity and drug tolerance of the assay. The precision of the assay is acceptable because the %CV for the negative and positive controls is less than 20%. The specificity of the assay was not directly determined. However, an increase in proliferation following the addition of SL-401 would likely result from inhibition or neutralization of SL-401. The selectivity of the assay is acceptable because it demonstrates that the matrix of the assay does not interfere with the neutralizing antibody.

Section 16 of the bioanalytical report GCL-315 contains the suitability controls used during the clinical trial and acceptance criteria to accept clinical run results. IR response dated October 1, 2018 details the rejection rate for this assay as shown in the table below.

Table 3: Summary of Pass/Fail Rate for SL-401 NAB by Method GCL-315

Parameter	Study 0114	Study 0214	Study 0314	Study 0414	Cumulative
Total assay runs (n)	62	2	7	2	73
Passed runs (n, %)	45 (72.6%)	2 (100.0%)	7 (100.0%)	2 (100.0%)	56 (76.7%)
Failed runs (n, %)	11 (17.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	11 (15.1%)
Aborted runs (n, %)	6 (9.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (8.2%)

Source: [SC-14-136-003](#), [SC-15-136-010](#), [SC-15-136-014](#), [SC-17-136-021](#)

Ranges for percent neutralizing were established for the low and high positive controls. The variability in the percent neutralization across plates is 32.9 %CV and 24.0% CV for the low and high positive control, respectively. The percentage of neutralization had the following pattern NC + Media ≥ HPC > LPC > NC + SL-401 for the anti-IL-3 control antibody. The acceptance criteria for system suitability controls are acceptable. The rejection rate of 18% is acceptable for the study 0114 because clinical samples on plates that were rejected were re-assayed.

- **TUF-17-086: VALIDATION OF A CELL BASED ASSAY FOR THE DETECTION OF ANTI-INTERLEUKIN-3 (IL-3) NEUTRALIZING ANTIBODIES IN HUMAN SERUM**

Screening anti-IL-3 neutralizing antibody assay

The human derived erythroblast-like cell line, TF-1, relies on GM-CSF or IL-3 for proliferation and growth and are maintained with recombinant human GM-CSF during routine culture. In the neutralizing assay, the cells proliferate in response to 1 ng/mL IL-3 cells. After washing cells to remove GM-CSF, and incubated in growth media for approximately 24 hours to allow the cells to reach the same growth phase of the cell cycle. Samples and controls are diluted in a 1:20 MRD and incubated with recombinant human IL-3 (rhIL-3) for approximately 30 minutes. Samples and controls are added to the TF-1 cells (10000 cells/well) for approximately 48 hours to allow for cell proliferation. Proliferation is measured by the amount of ATP present in the cell. The ATP is measured using the CellTiter Glo reagent and signal is measured on a luminometer to generate a luminescent signal (relative light units (RLU)). The RLU signal is directly proportional to the number of cells in the well. The RLU for the serum samples and positive controls is normalized to the drug control (DC) which is IL-3 stimulation only.

$$\text{Normalized RLU} = \frac{\text{Mean RLU DC}}{\text{Mean RLU Sample}}$$

In the absence of anti-IL-3 neutralizing antibodies, cells proliferate and have a high luminescent signal in which the normalization ratio will be below the cut point. In the presence of anti-IL-3 neutralizing antibodies, cells have reduced proliferation due to the neutralization of IL-3 activity and thus, generate a lower signal corresponding to a normalization ratio above the cut point. As a control, cells are incubated without IL-3 to establish the signal corresponding to the lack of IL-3 mediated cell proliferation.

Screening cut point:

[copied from bioanalytical report TM-TUF-0003]

Samples with mean DC/Sample ≥ 1.3 will be reported as IL-3 Nab positive and will be tested in the Specificity Assay.

Confirmatory specificity assay

To demonstrate that the reduction in proliferation is due to neutralization of IL-3, the Sponsor developed a specificity assay. In the specificity assay, 0.1 ng/mL GM-CSF is used to induce proliferation instead of IL-3. If the positive signal in the screening assay is due to anti-IL-3 neutralizing antibodies, the signal in the specificity assay should be negative. However, if there is a non-specific factor inhibiting proliferation in the serum that generates a positive signal in the screening assay, it should also generate a positive signal in the specificity assay. Thus, the specificity assay is designed to confirm the presence of anti-IL-3 neutralizing antibodies identified in the screening assay. Only samples that test positive in the screening

anti-IL-3 NAb assay are tested in the specificity assay. Of note, there is no positive control antibody used in the specificity assay.

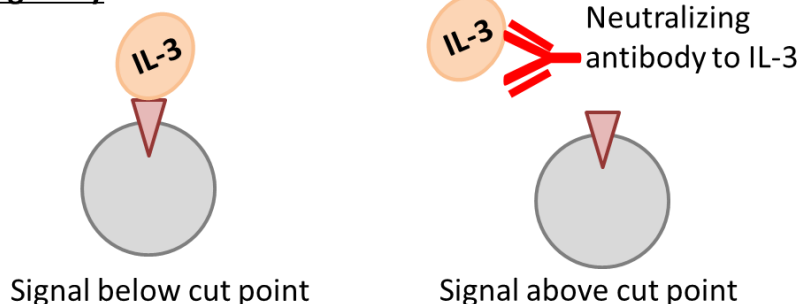
Specificity assay cut point:

[copied from bioanalytical report TM-TUF-0003]

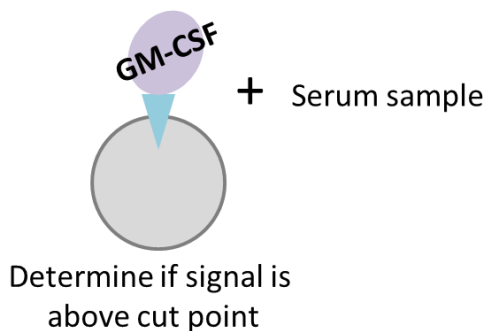
- Samples with mean DC/Sample ≥ 1.13 will be reported as IL-3 non-specific.
- Samples with mean DC/Sample < 1.13 will be reported as IL-3 specific.

The figure below is a schematic of the anti-IL-3 neutralizing antibody assay and the specificity assay.

Screening assay



Confirmatory specificity assay



Positive and Negative Controls for Screening Assay

A rat anti-human-IL-3 monoclonal antibody diluted in 5% pooled human serum is used as the positive control antibody.

High Positive Control (HPC): 20 $\mu\text{g/mL}$
Mid Positive Control (MPC): 2.0 $\mu\text{g/mL}$
Low Positive Control (LPC): 0.2 $\mu\text{g/mL}$
Negative Control (NC): 0 ng/mL
Drug Control (DC): 0 ng/mL + Growth Factor

VALIDATION PARAMETER	CRITERIA	RESULTS
Minimum Required Dilution (MRD)	Assay MRD is 1:20	MRD established in method development
Screening Assay Precision (%CV)	Intra and Inter-assay precision $\leq 20\%$ (Control ratios)	Inter-assay: DC/HPC: 10.9% DC/MPC: 10.9% DC/LPC: 8.9% DC/NC: 11.8% Intra-assay: DC/HPC: 1.3% DC/MPC: 1.9% DC/LPC: 4.0% DC/NC: 7.0%
Plate Acceptance Criteria (Screening Assay Control Acceptance Limits)	Report Results	Mean DC/Mean NC ratio ≥ 6.39 Mean DC/Mean HPC ratio ≥ 4.04 Mean DC/Mean MPC ratio ≥ 2.46 Mean DC/Mean LPC ratio ≥ 1.40
Screening Assay Cut Point	Report Results	Ratio Cut Point: 1.30
Specificity Assay Precision (%CV)	Intra and Inter-assay precision $\leq 20\%$ (Control ratios)	Inter-assay: DC/NC: 4.5%
Plate Acceptance Criteria (Specificity Assay Control Acceptance Limits)	Report Results	Mean DC/Mean NC ratio ≥ 8.07
Specificity Assay Cut Point	Report Results	Ratio Cut Point: 1.13
Sensitivity	Report Results	Assay sensitivity: 0.130 $\mu\text{g/mL}$
Selectivity (normal serum pool)	Unspiked: At least 80% (8 of 10) of samples must be negative in the screening assay. Spiked: 8 out of 10 spiked samples must be positive at the LPC level	Unspiked: 10/10 Negative LPC Spiked: 10/10 Positive

VALIDATION PARAMETER	CRITERIA	RESULTS
Drug Tolerance	Report Results	HPC tolerates >1000 ng/mL of drug MPC tolerates >1000 ng/mL of drug LPC tolerates 100 ng/mL of drug NC tolerates >1000 ng/mL of drug
IL-3 Interference	Report Results	HPC tolerates >100 ng/mL of IL-3 MPC tolerates >100 ng/mL of IL-3 LPC tolerates 10 ng/mL of IL-3 NC tolerates >100 ng/mL L of IL-3
Freeze-Thaw Stability (-70°C)	% difference for at least two (2) of the three (3) FT stability sample replicates must be within $\pm 30.0\%$ of the nominal value	HPC and LPC: for 5 FT cycles
Short-Term (ST) Stability (Ambient/Bench-Top)	% difference for at least two (2) of the three (3) ST stability sample replicates must be within $\pm 30.0\%$ of the nominal value	HPC and LPC: 23 hours 35 minutes @ ambient

Reviewer comments:

Overall, the validation of the assay is acceptable and demonstrates that the assay can identify neutralizing anti-IL-3 antibodies. Fifty-one normal human serum samples were used to determine the screening and specificity assay cut points of 1.30 and 1.13 normalization ratios, respectively. The screening cut point is based on a 1% false positive rate and meets FDA guidance for neutralizing assays. A 0.1% false positive rate is used for the confirmatory specificity assay and is acceptable because a higher cut point for the specificity assay allows for more samples to be considered IL-3 NAb positive if the sample is negative in the specificity assay. Therefore, would prevent the underrepresentation of IL-3 NAb antibodies.

In the IR response dated 11/09/2018, the Sponsor provided where in the BLA application the clinical test results for the confirmatory specificity assay are located. For study STML-401-0114 stage 3 patients, the results for the anti-IL-3 neutralizing screening assay and the confirmatory specificity assay is provided in tabular format in the BLA in listing 16.2.10.4. The data indicates that none of the samples positive in the screening assay were positive in the confirmatory assay. Thus, all screened anti-IL-3 NAb positive samples were considered positive for anti-IL-3 neutralizing activity.

The Sponsor tested various concentrations of anti-IL-3 antibodies and non-specific antibodies to TNF- α (Infliximab) and diphtheria toxin in the screening and specificity assays. As shown in the table below, only anti-IL-3 antibodies yield a positive signal in the screening assay and a corresponding negative response in the specificity assay. These data demonstrate that the screening assay in combination with the specificity assay can specifically identify neutralization of IL-3 antibodies.

Control	Conc. [µg/mL]	Screening Response	Specificity Response
Anti-IL-3	20	6.72	1.06
Anti-IL-3	0.2	1.87	1.06
Anti-IL-3	0	1.00	1.00
Anti-Infliximab	80.00	64.91	15.06
Anti-Infliximab	20.00	1.51	1.62
Anti-Infliximab	0.20	1.07	1.10
Anti-DT	80.00	1.17	1.21
Anti-DT	20.00	1.09	1.12
Anti-DT	0.20	1.13	1.05

The sensitivity of the assay is 130 ng/mL. The drug tolerance of the assay is acceptable because the serum samples are collected when the drug concentration in the serum is negligible. The C_{max} of the drug is 1000 ng/mL and the half-life is 30 minutes. Based on this information, the concentration of the drug in the serum should be below the drug tolerance level of 100 ng/mL of drug in the presence of 200 ng/mL of anti-IL-3 positive control within 2 hours of the C_{max} levels. Thus, it is expected that serum drug levels will not interfere with assay results because samples for immunogenicity are taken pre-dose. The selectivity of the assay is acceptable and demonstrates that the matrix of the assay does not interfere with assay results. The design of the confirmatory specificity assay is appropriate to determine the specificity of the signal generated in the anti-IL-3 neutralizing assay because it tests serum samples in an assay that generates proliferation by GM-CSF and not IL-3. The precision of the screening and confirmatory specificity assay is acceptable because the %CV is less than 20%.

Suitability controls used during the clinical trial are indicated in section 12.4 of the bioanalytical report TM-TUF-0003. This section outlines the controls that were used on each plate and acceptance criteria to accept clinical run results. In the IR response dated October 1, 2018, the Sponsor reports that a total of 5 plates were ran and included the screening and confirmatory specificity assays. No plates were rejected or aborted. Comparable signals were found within the low, and high positive controls across all plates for the screening assay. The high and low IL-3 positive control antibodies generated similar signals as expected because GM-CSF is used to induce proliferation and not IL-3 in the confirmatory specificity assay.

Clinical Results

The indication for SL-401 is blastic plasmacytoid dendritic cell neoplasm (BPDCN). The design of the clinical trial for first-line (FL) and relapsed/refractory (R/R) BPDCN, and acute myeloid leukemia (AML) is described in Table 1. Stage 3 is the pivotal trial for FL BPDCN and consisted of 13 patients. For study 401-00144, the drug is administered for 15 minutes intravenously for five consecutive days of a 21-day cycle. There was a total of

six cycles. The immunogenicity samples were collected at pre-infusion, day 15, and day 21 of each treatment cycle.

Table 1: Summary of SL-401 Clinical Study Designs

Study Identifier	Study Phase	Type of Study	Diagnosis of Patients	Test Product(s); Dosage Regimen; Route of Administration	Number of Patients	Duration of Study
STML-401-0114	1/2	Nonrandomized, open-label, multistage, dose escalation and expansion study	Patients with BPDCN or AML	SL-401 Stage 1: 23 patients (9 BPDCN and 14 AML) were treated with SL-401 infusion at doses 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. Stage 2: 58 patients (23 BPDCN and 35 AML) were treated at 12 µg/kg/day, the MTD, at which multiple DLTs were not observed during Stage 1. Stage 3: 13 patients with first-line BPDCN were treated at 12 µg/kg/day and contribute as the pivotal cohort. Stage 4: 2 patients with BPDCN were enrolled as of 17 March 2017. For all stages, a cycle of therapy is 21 days. SL-401 is administered as a 15-minute IV infusion for the first 5 consecutive days of a 21-day cycle. SL-401 is supplied as SL-401 Injection frozen solution (1 mg/mL) for Stages 1, 2, and 3. (b) (4) (SL-401 for Injection, (b) (4) at 1 mg/mL) was supplied for dosing in selected patients with AML in Stage 2 and is planned for treatment of patients with BPDCN enrolled in Stage 4 after Protocol Amendment 9 became effective (after 17 March 2017).	Stage 1: 23 patients Stage 2: 58 patients Stage 3: 13 patients Stage 4 (ongoing): up to 26 patients Total study enrollment cap of 120 patients 96 patients were enrolled at the enrollment cutoff of 17 March 2017	~60 months

Additional clinical conditions including AML, advanced high- risk myeloproliferative neoplasm (MPN), and relapsed/refractory (R/R) multiple myeloma (MM) were examined and the design of these clinical studies are described in the Table below.

Study Identifier	Study Phase	Type of Study	Diagnosis of Patients	Test Product(s); Dosage Regimen; Route of Administration	Number of Patients	Duration of Study
STML-401-0214	1/2	Nonrandomized, open-label, 2-stage dose escalation study	Patients with adverse risk AML in first or second CR, with or without evidence of MRD in first CR	SL-401 Stage 1: 9 patients were treated with SL-401 at doses of 7, 9, or 12 µg/kg/day for 5 consecutive days every 28 days. Stage 2: Up to 20 additional patients with evidence of MRD as determined locally will be treated at the MTD at which multiple DLTs are not observed so that at least 15 patients with evidence of MRD as determined centrally are evaluable for safety and response at this dose. SL-401 is supplied as SL-401 Injection frozen solution (1 mg/mL) and administered as a 15-minute IV infusion.	Stage 1: 9 patients Stage 2 (ongoing): up to 20 patients 14 patients were enrolled as of the enrollment cutoff of 07 May 2017	~36 months
STML-401-0314	1/2	Nonrandomized, open-label, 2 stage dose escalation study	Patients with advanced, high-risk MPNs	SL-401 Stage 1: 9 patients were treated with SL-401 at doses of 7, 9, or 12 µg/kg/day for 3 consecutive days every 21 days in Cycles 1-4, every 28 days in Cycles 5-7, and every 42 days in Cycle 8 and beyond. Stage 2: Up to 72 additional patients with MF, CMML, SM, or PED (18 each) will be treated at the MTD at which multiple DLTs are not observed, or at a lower dose provided there is preliminary evidence in Stage 1. SL-401 is supplied as SL-401 Injection frozen solution (1 mg/mL) and administered as a 15-minute IV infusion.	Stage 1: 9 patients Stage 2 (ongoing): up to 72 patients 15 patients were enrolled as of the enrollment cutoff of 07 May 2017	~24 months

Study Identifier	Study Phase	Type of Study	Diagnosis of Patients	Test Product(s); Dosage Regimen; Route of Administration	Number of Patients	Duration of Study
STML-401-0414	1/2	Nonrandomized, open-label, 2-stage dose escalation study	Relapsed or R/R/ MM	SL-401; POM/DEX Stage 1: 5 patients were treated with SL-401 at 7 µg/kg/day for 5 consecutive days every 28 days. After a run-in cycle of single-agent SL-401, standard doses of POM/DEX were added in Cycles 1 through 6. Stage 2: At least 14 additional patients (for a total of 20 patients) will be treated with SL-401 at the MTD at which multiple DLTs are not observed (identified in Stage 1) and standard doses of POM/DEX. SL-401 is supplied as SL-401 Injection frozen solution (1 mg/mL) and administered as a 15-minute IV infusion. Commercial supplies of POM are used and administered orally at 4 mg on Days 1-21 of each cycle. Commercial supplies of DEX are used and administered orally at 40 mg on Days 1, 8, 15, and 22 of each cycle.	Stage 1: 5 patients Stage 2 (ongoing): up to 14 additional patients 5 patients were enrolled as of the enrollment cutoff of 07 May 2017	~24 months

Abbreviations: AML = acute myeloid leukemia; BPDCN = blastic plasmacytoid dendritic cell neoplasm; CMML = chronic myelomonocytic leukemia; CR = complete response; DEX = dexamethasone; DLT = dose-limiting toxicity; IV = intravenous; MF = myelofibrosis; MM = multiple myeloma; MPN = myeloproliferative neoplasm; MRD = minimal residual disease; MTD = maximum tolerated dose; PED = primary eosinophilic disorder; POM = pomalidomide; R/R = relapsed/refractory; SM = systemic mastocytosis.

Table 12 shows the ADA response of patients with evaluable immune responses. As expected, most of the patients (81/85) were ADA positive at baseline and the few patients who were not positive at baseline had treatment-induced responses.

Table 12: Treatment-boosted and Treatment-induced Anti-drug Antibody Incidence in Study 0114 Patients by Disease and Overall (Evaluable Immunogenicity Population)

	FL BPDCN (N=31)	R/R BPDCN (N=14)	AML (N=40)	All Patients (N=85)
Treatment-boosted ADA Incidence				
Baseline ADA-positive patients, N	28	13	40	81
ADA-positive patients with ≥ 100 -fold increase in titer after drug administration, n (%)	26 (92.9)	10 (76.9)	19 (47.5)	55 (67.9)
Peak positive titer and range (IQR)	80,000,000 (800,000, 800,000)	80,000,000 (800, 440,000)	80,000,000 (80,000, 800,000)	80,000,000 (80,000, 800,000)
Maximum fold increase in titer ^a	100,000	100,000	100,000	100,000
Timing of maximum fold increase in titer	Cycle 2/Day 15	Cycle 1/Day 15	Cycle 2/Day 15	Cycle 2/Day 15
Treatment-induced ADA Incidence				
Baseline ADA-negative patients, N	3	1	0	4
ADA-negative patients at baseline to positive after drug administration, n (%)	3 (100)	1 (100)	N/A	4 (100)
Peak positive titer and range (IQR)	80,000 (80,000, 80,000)	80,000 (80,000, 80,000)	N/A	80,000 (80,000, 80,000)
Timing of peak positive titer	Cycle 2 Day 15	Cycle 2 Day 15	N/A	Cycle 2 Day 15
Overall ADA and NAb Incidence After Treatment				
Treatment-boosted + treatment-induced ADA-positive patients, n/N (%)	29/31 (93.5)	11/14 (78.6)	19/40 (47.5)	59/85 (69.4)
Total ADA-positive after treatment, n/N (%)	31/31 (100)	14/14 (100)	40/40 (100)	85/85 (100)
Total NAb-positive after treatment, n/N (%)	26/27 (96.3)	11/13 (84.6)	30/40 (75)	67/80 (83.8)

Abbreviations: ADA = anti-drug antibody; AML = acute myeloid leukemia; BPDCN = blastic plasmacytoid dendritic cell neoplasm; FL = first-line; IQR = interquartile range; N/A = not applicable; NAb = neutralizing antibody; R/R = relapsed/refractory.

^a Maximum fold increase in titer = ratio of peak titer after administration to baseline median titer.

Source: Study 0114 [Table 14.3.5.15.2](#), [Table 14.3.5.16A](#), [Table 14.3.5.17A](#), and [Table 14.3.5.18A](#).

Reviewer comments:

The treatment boosted anti-drug antibodies are defined as > 100-fold increase in the titer. However, according FDA guidance the treatment boosted responses should be determined as the titer that is two dilution steps greater than the pre-treatment titer, when two-fold dilutions are used. In response to the IR response dated 11/09/2018, the Sponsor stated that the titer for this assay was performed with 10-fold dilutions instead of 2-fold dilutions. A 2-fold increase using a 10-fold dilution scheme would be 100-fold increase in the titer. A 2-fold dilution scheme was used during validation of the assay to

determine the titer. While, it is ideal to use a 2-fold dilution when determining the titer, a 10-fold dilution is acceptable in this case, because patients samples are expected to have high titers of anti-DT antibodies.

Anti-IL-3 immune responses for study 0114 are shown in Table 15. All but one patient in the R/R BPDCN were negative for anti-IL-3 antibodies at baseline. However, following treatment, 63.1% of the baseline negative patients contained anti-IL-3 antibodies.

Neutralizing antibody (NAb) responses to IL-3 were only determined for the pivotal trial containing the 13 first-line BPDCN patients. Of those patients, 12 had reportable results and 11 of were negative at baseline for anti-IL-3 NAb. However, following treatment all 11 BPDCN patients were anti-IL-3 NAb positive. Table 17 summaries the immunogenicity ADA response to SL-401 and anti-IL-3 antibody response for the pivotal trial.

Table 15: Treatment-boosted and Treatment-induced Anti-human Interleukin-3 Antibody Incidence in Study 0114 Patients by Disease and Overall (Evaluable Immunogenicity Population)

	FL BPDCN (N=32)	R/R BPDCN (N=15)	AML (N=49)	All Patients (N=96)
Evaluable Population, N	31	14	40	85
Treatment-boosted AIA Incidence				
Baseline AIA positive patients, N	0	1	0	1
AIA positive patients with $\geq$ 4-fold increase in titer after drug administration, n (%)	0	1 (100) ^a	0	1 (100) ^a
Peak positive titer and range (IQR)	N/A	3200 (3200, 3200)	N/A	3200 (3200, 3200)
Maximum fold increase in titer ^b	N/A	128	N/A	128
Timing of maximum fold increase in titer (cycle/day)	N/A	Cycle 3 Day 15	N/A	Cycle 3 Day 15
Treatment-induced AIA Incidence				
Baseline AIA negative patients, N	31	13	40	84
AIA negative at baseline to positive after drug administration, n (%)	28 (90.3)	10 (76.9)	15 (37.5)	53 (63.1)
AIA negative at baseline patients with $\geq$ 4-fold increase in titer after drug administration, n (%)	28 (90.3)	10 (76.9)	15 (37.5)	53 (63.1)
Peak positive titer and range (IQR)	51,200 (1600, 12,800)	51,200 (1600, 51,200)	819,200 (1, 25,600)	819,200 (1, 6400)
Maximum fold increase in titer ^b	51,200	51,200	819,200	819,200
Timing of maximum fold increase in titer (cycle/day)	Cycle 3 Day 15	Cycle 3 Day 15	Cycle 2 Day 21	Cycle 2 Day 21
Overall AIA Incidence After Treatment				
Treatment-boosted + treatment-induced AIA positive patients, n/N (%)	28/31 (90.3)	11/14 (78.6)	15/40 (37.5)	54/85 (63.5)
Total AIA positive after treatment, n/N (%)	29/31 (93.5)	11/14 (78.6)	15/40 (37.5)	55/85 (64.7)

Abbreviations: AIA = anti-human interleukin-3 antibody; AML = acute myeloid leukemia; BPDCN = blastic plasmacytoid dendritic cell neoplasm; FL = first-line; IQR = interquartile range; NA = not applicable; R/R = relapsed/refractory.

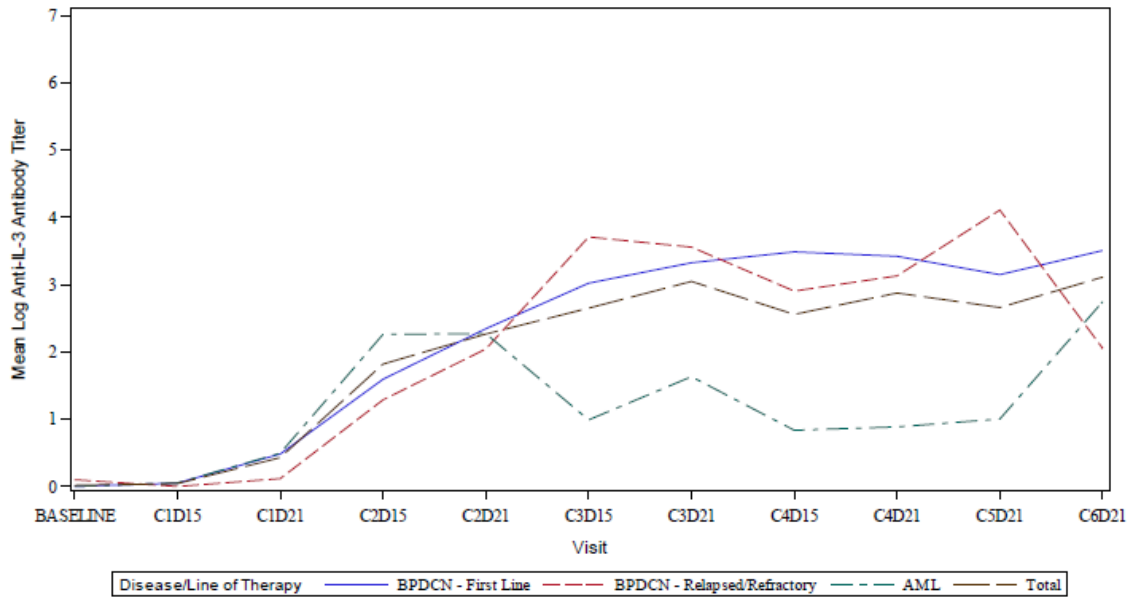
^a Titer of 1 patient with positive AIA antibody at baseline was 25.

^b Fold increase in titer = ratio of peak titer after administration to baseline titer.

Source: Study 0114 Table 14.3.5.15.1, Table 14.3.5.15.2, Table 14.3.5.15B, Table 14.3.5.16B, Table 14.3.5.17B, and Table 14.3.5.18B.

Anti-IL-3 antibody titer is highest in the BPDCN patients (Figure 6, below) and correlates with drug dosage (Figure 8 in Integrated Summary of Immunogenicity).

Figure 6 Mean Logarithmic Anti-human Interleukin-3 Antibody Titer in Study 0114 by Visit and Indication (Evaluable Immunogenicity Population)



Reviewer comments:

Overall, the immunogenicity of SL-401 is quite high, possibly due to the existence of pre-existing anti-DT antibodies in most patients. Moreover, 63% of all the patients developed anti-IL-3 antibodies and most are neutralizing the in the BPDCN patients. Refer to the clinical pharmacology review for BLA 761116 for details regarding the correlation and impact of anti-drug and anti-IL-3 antibodies on clinical efficacy and safety.



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