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NON-CLINICAL REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

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Product: ELZONRIS (tagraxofusp)

Indication: Blastic plasmacytoid dendritic cell neoplasm (BPDCN)

Applicant: Stemline Therapeutics, Inc.

Review Division: Division of Hematology Oncology Toxicology (DHOT) for Division of Hematology Products (DHP)

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1 Executive Summary

1.1 Introduction

Tagraxofusp is a CD123-directed cytotoxin composed of recombinant human interleukin-3 (IL-3) and a truncated diphtheria toxin (DT) fusion protein that binds to CD123 (IL-3 receptor alpha, IL-3RA) expressing cells. The proposed clinical dose of 12 µg/kg/day is to be administered as an intravenous infusion over 15 minutes on Days 1 through 5 of each 21-day cycle. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of tagraxofusp for the treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN).

1.2 Brief Discussion of Nonclinical Findings

Tagraxofusp, also known by laboratory codes SL-401, DT388IL3, and Molecule 129, is a recombinant fusion protein of the DT Q388R variant with the DT-receptor binding domain replaced by human IL-3. Tagraxofusp was shown to bind human IL-3RA with a K_d of 470 nM, which is comparable to human IL-3 binding to IL-3RA (hIL-3 K_d=670 nM). Submitted and published data support the mechanism of action of tagraxofusp, which upon binding to IL-3RA, is internalized and increases ADP ribosylation of elongation factor-2 (EF-2). It is widely accepted that ADP-ribosylation of EF-2 by DT blocks the ability of EF-2 to facilitate protein synthesis.

Data from the Applicant with SL-401 or with an earlier formulation (DT388IL3) the Applicant has the right to cross-reference demonstrated anti-tumor activity (reduced cell proliferation, increased expression of apoptotic markers) in BPDCN cell lines, and in blasts from BPDCN, acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL) patients, with BPDCN as the most sensitive cell type. In in vivo mouse xenograft models of BPDCN, mice administered tagraxofusp intraperitoneally (IP) for 5 consecutive days at 100 µg/kg/day increased overall survival from 17 and 58 days, compared to controls. In an AML patient derived mouse xenograft (PDX) models, 50 µg/kg tagraxofusp administered every other day (3 doses/week) for 5 weeks increased overall survival of mice compared with those treated with the vehicle control. Tagraxofusp will be able to bind to cells documented as expressing CD123 (e.g., hematopoietic myeloid progenitors, normal myeloid derived cell types, some endothelial cells, and osteoblasts). However, given the high CD123 expression in malignant cells, tumor cells may function as a target sink to reduce undesirable activity in normal tissues. Evidence supports the cynomolgus monkey as a pharmacologically relevant species for toxicology studies.

A single-dose crossover pharmacokinetic study with 2-day washout period was conducted in cynomolgus monkeys to bridge the tagraxofusp frozen solution used in the GLP-compliant 3-month toxicity study to a (b) (4) formulation, yielding no major differences. In both the GLP-compliant 1-month (30 and 60 µg/kg/day for 5 days with a 3-week recovery, or 1 cycle) and 3-month (35 and 45 µg/kg/day for 5 days with 3-week off dosing periods, or 3 cycles) toxicity studies in cynomolgus monkeys, exposure increased generally dose proportionally with a half-life of 30 minutes to an hour.

Neutralizing anti-drug antibodies (ADA) developed in the 3-month study that affected exposure. The impact this had on the toxicities observed in the 3-month study is unknown. The exposure and toxicities at the 30 and 35 µg/kg/day doses in the 1- and 3-month studies, respectively, were overall comparable.

In both the 1-month (i.e., 1 cycle) and 3-month (i.e., 3 cycle) GLP-compliant toxicity studies in cynomolgus monkeys, tagraxofusp was administered as an intravenous slow bolus infusion over 5 minutes for 5 consecutive days per cycle. Doses of 30 and 60 µg/kg/day were administered in the 1-month study with a 5% dextrose vehicle control and doses of 35 and 45 µg/kg/day were administered in the 3-month study with a 0.9% NaCl vehicle control. The lethal doses were 60 µg/kg/day in the 1-month study and 45 µg/kg/day in the 3-month study, and mortality occurred on Days 6 or 9. The cause of death was due to severe necrosis of renal cortical tubules (kidneys) at 60 µg/kg/day in the 1-month study and was undetermined for the death at 45 µg/kg/day in the 3-month study. Mortality was associated with clinical signs of blood collection site redness, inappetence, cold to touch, decreased activity, hunched posture, dehydration, increased body temperature, and bruising/swelling of limbs. The main toxicities noted between the two studies are inappetence and body weight decreases; lack of adaptive response to decreased red cell mass (as evidenced by decreased reticulocytes relative to controls); prolonged coagulation times; increased basophils; inflammatory response (increased neutrophils, globulin, and fibrinogen; decreased albumin); liver toxicity (increases in ALT and AST, liver weights, hepatocellular vacuolation); kidney toxicity (increased BUN and creatinine, decreased phosphorus and calcium, proteinuria, tubular degeneration/necrosis); reduced thymus weights and lymphoid depletion; mineralization of the ovaries in females; minor skin toxicity (dry/scabbing (multiple areas), minimal to mild perivascular hemorrhage/necrosis at the injection site); and, degeneration/necrosis of the brain choroid plexus.

Findings were reversible over the 3-week recovery period in the 1-month study except for decreases body weight, decreases in leukocytes and neutrophils, increased globulin, decreased thymus weights and lymphoid depletion, and degeneration/necrosis of the brain choroid plexus, which become more severe over the recovery period. Increased lung weights during the recovery period were observed in the 1-month study and mild lung edema was observed in the female with early mortality in the 1-month study and in one low dose male in the 3-month study. Minimal to mild degeneration/necrosis of the rectus femoris muscle or mild axonal/myelin degeneration of the sciatic nerve were observed in the 1- and 3-month studies, respectively, at low incidence.

Toxicities related to tagraxofusp administration can overall be monitored, except for the degeneration/necrosis of the brain choroid plexus. In the 3-month study, clinical signs present in monkeys with degeneration/necrosis of the brain choroid plexus (10/12 monkeys) were hunched posture (100%), tremors (40%), decreased activity (40%), eyes partially or completely closed (20%), and hypersensitivity (10%). Since most tagraxofusp treated monkeys in the 3-month study exhibited degeneration/necrosis of the brain choroid plexus and there was no apparent relationship of the severity of the degeneration/necrosis and incidence of clinical signs, it is unclear if these clinical signs

are related to the microscopic findings in the brain choroid plexus or the general condition of the monkeys. They are noted since all, except for tremors in one low dose animal, are observed only in monkeys with degeneration/necrosis of the brain choroid plexus.

No genetic toxicology, carcinogenicity, or reproductive and developmental toxicology studies were needed to communicate risk in the package insert for tagraxofusp since it is a biologic, the only relevant animal species is the nonhuman primate, it will be administered to a patient population with severe and life-threatening disease (BPDCN) and, due to the development of neutralizing ADA, it is unclear whether an increase in dose would increase exposure enough to provide relevant animal:human safety margins based on dose or exposure in reproductive and developmental toxicity studies. Labeling will be overall consistent with other products with the comparable mechanism of action, LUMOXITI and ONTAK. In an alternate reproductive assessment, the Applicant provided references to published literature suggesting that exogenous IL-3 is embryotoxic. (b) (4)

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/Toxicology perspective, approval of ELZONRIS is recommended for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The recommendations to the Applicant's proposed labeling were discussed internally and communicated to the Applicant. Information in the nonclinical sections of the label reflect findings from studies reviewed within this document.

2 Drug Information

2.1 Drug

CAS Registry Number	2055491-00-2
Generic Name	Tagraxofusp
Code Name	SL-401 (2 nd generation), DT388IL3 (1 st generation), Molecule 129
Chemical 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)
Molecular Formula/ Molecular Weight	C ₂₅₅₃ H ₄₀₂₆ N ₆₉₂ O ₇₉₈ S ₁₆ / 57.695 kDa
Structure or Biochemical	Tagraxofusp, is a 524 amino acid recombinant fusion

Description	protein expressed in <i>Escherichia coli</i> from a hybrid gene comprised of a deoxyribonucleic acid (DNA) sequence of IL-3 contiguous with a truncated version of DT intentionally engineered without its receptor binding domain (i.e., IL-3 replaces the receptor binding domain). The tagraxofusp protein consists of an N-terminal methionine, followed by DT residues 1-388 ~ comprising the catalytic (C) and translocation (T) domains of DT. This truncated DT fragment (Q388R variant) is genetically fused at the C-terminus through a His-Met dipeptide linker to the N-terminus of the full 133-amino acid sequence of human IL-3. Protein Sequence of Tagraxofusp: <u>MGADDVVDSSKSFVMENFSSYHG</u>TKPGYVDSIQKGIQKPKSGTQ GNYDDDWKGFYSTDNKYDAAGYSVDNENPLSGKAGGVVKVTYP GLTKVLALKVDNAETIKKELGLSLTEPLMEQVGTEEFIKRFGDGA SRVVLSPFAEGSSSVEYINNWEQAKALSVELEINFETRGRGQD AMYEYMAQACAGNRVRRSVGSSLSCINLDWDVIRDKTKTKIESLK EHGPINKMSESPNKTVSEEKAKQYLEEFHQTALEHPELSELKTV TGTNPVFAGANYAAWAVNVAQVIDSETADNLEKTTAALSILPGIGS VMGIADGAVHHNTEEIVAQSIALSSLMVAQAIPLVGELVDIGFAAYN FVESIINLFQVVHNSYNRPAYSPGHKTRPHMAPMTQTTSLKTSWVN CSNMIDEIITHLKQPPLPLLDFFNNLNGEDQDILMENNLRPNLEAFNR AVKSLQNASAIESILKNLLPCLPLATAAPTRHPIHIKGDGWDNEFRRKL TFYLKTLENAQAQQTTLSLAIF <u>Underline</u>: N-terminal methionine; Bold: Diphtheria; <i>Italics</i>: His-Met linker; Normal: human IL-3
Pharmacologic class	CD123-directed cytotoxin

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 114513 was the original submission for second generation tagraxofusp (with manufacturing changes) by Stemline Therapeutics (SL-401). First-generation tagraxofusp was submitted under IND 11314 as Interleukin-3 Diphtheria Toxin Fusion Protein (Recombinant, *E. coli*) (a.k.a., DT388IL3) by Arthur Frankel, MD. Stemline Therapeutics has a Letter of Authorization to cross-reference data from IND 11314, including data provided as published references for DT388IL3.

2.3 Drug Formulation

Tagraxofusp, injection (drug product) is a (b) (4), sterile (b) (4) dosage form (b) (4) containing an aqueous solution of 1.0 mg of tagraxofusp, 50.0 mg sorbitol, and 4.38 (b) (4) mg (75 mM) sodium chloride (NaCl) per 1 mL (b) (4) pH 7.5. The vial fill supports a 1.0 mL dose, plus a (b) (4)% volumetric overfill. The resulting target fill weight for vials, (b) (4) g (approximately (b) (4) mL; density is (b) (4) g/cm³), ensures a deliverable dose that meets or exceeds the label claim, accounting for losses to vial, needle, and syringe during dose preparation.

Table 1 Drug Product Quantitative Composition, 1.0 mL/Vial
(Excerpted from Module 3.2.P.1.)

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)	(b) (4)	(b) (4)	4.38		
Sorbitol, USP, EP			(b) (4)	50.0		
(b) (4)						
(b) (4)						
(b) (4)						
(b) (4)						
Water for injection, USP, EP	(b) (4)					
EP: European Pharmacopoeia; NF: National Formulary; q.s.: sufficient quantity; USP: United States Pharmacopoeia						
(b) (4)						

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical dose of 12 µg/kg/day is to be administered to BPDCN patients as an intravenous infusion over 15 minutes on Days 1 through 5 of each 21-day cycle.

2.7 Regulatory Background

Tagraxofusp was granted Breakthrough Therapy Designation on August 22, 2016 under IND 114513. The BLA 761116 was received from Stemline as a rolling submission between April and June of 2018, with the PDUFA goal date based on the date of the last submission date, June 21, 2018.

Information requests to clarify the mechanism of action (MOA) (specifically regarding the inhibition by tagraxofusp of EF-2 function) and the ownership rights of published data submitted to support the MOA were sent to the Applicant on July 31, 2018 and November 28, 2018, respectively. Responses were received on August 23, 2018 and November 29, 2018 and information from those responses have been incorporated into this review.

3 Studies Submitted

3.1 Studies Reviewed

Primary Pharmacology

Study#	Title	Module
2018-01	SL-401: Characterization of In Vitro Binding with Human and Mouse IL3RA	4.2.1.1.

Pharmacokinetics

Study #	Title	Module
Absorption		
2231-006	Biocomparability Study of SL-401 Following Intravenous Infusion Administration in Cynomolgus Monkeys	4.2.2.2.

Repeat Dose Toxicology

Study#	Title	Module
2231-007	SL-401: A 3-Month Intravenous Toxicity Study in Monkeys (GLP)(SEND)	4.2.3.2.
2231-002	SL-401: A 5-Day Intravenous Toxicity Study in Cynomolgus Monkeys with a 3-Week Recovery Period (GLP) (also referred to as 1-month study in this review)	4.2.3.2.

3.2 Studies Not Reviewed

See Appendix

3.3 Previous Reviews Referenced

Study 2231-002 was previously reviewed under IND 114513 and the content and format of the review of this study was slightly modified to fit this BLA review.

4 Pharmacology

Tagraxofusp functions by binding to cell surface IL-3 receptor alpha via its IL-3 domain. IL-3 binding has been shown to result in internalization of the IL-3-IL-3R complex.^{1,2} Once in the cytosol, the DT catalytic domain of tagraxofusp catalyzes the transfer of ADP-ribose from nicotinamide adenine dinucleotide (NAD) to a diphthamide residue on elongation factor 2 (EF-2), leading to inhibition of protein synthesis and ultimately apoptosis.

IL-3R is a heterodimeric receptor complex composed of 2 chains: a 70-kDa cytokine-specific alpha subunit (CD123, IL-3R α , or IL-3RA) and a 120-kDa common beta subunit (β c or CD131) that is shared among the interleukin IL-3, IL-5, and granulocyte macrophage colony-stimulating factor (GM-CSF) receptor systems. CD123 confers ligand specificity to the IL-3R and is important for IL-3 recognition and binding, whereas

¹ Gesner, T. G., R. A. Mufson, C. R. Norton, K. J. Turner, Y. C. Yang and S. C. Clark (1988). "Specific binding, internalization, and degradation of human recombinant interleukin-3 by cells of the acute myelogenous, leukemia line, KG-1." J Cell Physiol 136(3): 493-9.

² Rapoport, A. P., S. Luhowskyj, P. Doshi and J. F. DiPersio (1996). "Mutational analysis of the alpha subunit of the human interleukin-3 receptor." Blood 87(1): 112-22.

the βc enables high-affinity IL-3 binding and signal transduction.³ Studies using cell lines transfected with IL-3R components have demonstrated that IL-3 binds with low affinity (dissociation constant [Kd] $\approx$ 100 nM) to cells expressing CD123 alone, but when CD123 is co-expressed with βc , IL-3 binds with almost 3 orders of magnitude higher affinity (Kd $\approx$ 100 pM)^{3,4}.

CD123, the alpha subunit of the IL-3R, is expressed on both the tumor bulk and cancer stem cells (CSCs) of multiple hematologic malignancies, including BPDCN and AML, but has limited expression on normal tissue.

4.1 Primary Pharmacology

Target Binding, Species Relevance, and Specificity

Study title: SL-401: Characterization of In Vitro Binding with Human and Mouse IL3RA

Study No.: 2018-01
Report Date: January 17, 2018
Study report location: 4.2.1.1.
Conducting Laboratory: (b) (4)
GLP: No

Methods:

Human and mouse IL-3RAs were immobilized onto a standard CM5 sensor chip in a Biacore 2000 system. SL-401 and the native human (h) and mouse (m) IL-3s were tested for binding in a three-fold concentration series. SL-401 was tested using a 1:10 dilution of the provided stock (assumed to be 17.33 μ M) as the highest concentration. The highest concentration tested for hIL-3 and mIL-3 was 1 μ M. A three-fold dilution series of six concentrations total was prepared for each of these three analytes. The dilution series of each analyte was tested three times for binding to hIL-3RA and mIL-3RA. The responses were fit to a 1:1 interaction model to obtain binding parameters for these interactions.

Results:

SL-401 and hIL-3, but not mIL-3, bound in a concentration-dependent and reproducible manner to hIL-3R. Mouse IL-3 bound to mIL-3R, but not hIL-3R.

³ Kitamura, T., N. Sato, K. Arai and A. Miyajima (1991). "Expression cloning of the human IL-3 receptor cDNA reveals a shared beta subunit for the human IL-3 and GM-CSF receptors." *Cell* 66(6): 1165-74.

⁴ Broughton, S. E., T. R. Hercus, M. P. Hardy, B. J. McClure, T. L. Nero, M. Dottore, H. Huynh, H. Braley, E. F. Barry, W. L. Kan, U. Dhagat, P. Scotney, D. Hartman, S. J. Busfield, C. M. Owczarek, A. D. Nash, N. J. Wilson, M. W. Parker and A. F. Lopez (2014). "Dual mechanism of interleukin-3 receptor blockade by an anti-cancer antibody." *Cell Rep* 8(2): 410-9.

Table 2 Binding parameters for SL-401 to hIL-3R and mIL-3R Determined at 25°C

(Excerpted from the study report)

receptor	analyte	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (nM)
hIL-3RA	SL-401	3.62(8)e5	1.71(2)e-1	470(10)
	hIL-3	2.66(4)e5	1.79(2)e-1	670(10)
	mIL-3	----	----	NB
mIL-3RA	SL-401	----	----	NB
	hIL-3	----	----	NB
	mIL-3	5.21(3)e5	8.21(3)e-2	158(1)

NB = no binding observed

Numbers in parentheses are the errors in the last digits

In a publication the Applicant has the right to cross-reference, the K_d s was 1.5 nM for binding of unlabeled DT388IL3 to TF/Hras cells in a cold competition receptor binding assay with ^{125}I -labeled IL-3 variant.⁵

Cynomolgus monkey IL-3 and CD123 have the highest homology and identity to humans of the species tested. It is known that subcutaneous injection of recombinant human IL-3 induces proliferation of immature hematopoietic cells from the myeloid, megakaryocytic, and erythroid series at the injection site in cynomolgus monkeys presumed to be related to exaggerated pharmacology.⁶ Thus, evidence supports the cynomolgus monkey as a pharmacologically relevant species for toxicology studies.

Table 3 Homology and Identity of the Interleukin-3 and CD123 Amino Acid Sequence from the Indicated Species to Humans

(Tables 2 and 3 from Nonclinical Overview combined by the reviewer)

Species	Homology		Identity	
	hIL-3	hCD123	hIL-3	hCD123
Monkey (<i>Macaca fascicularis</i>)	85%	92%	81%	87%
Mouse (<i>Mus musculus</i>)	50%	65%	50%	49%
Dog (<i>Canis lupus familiaris</i>)	52%	63%	37%	44%
Rat (<i>Rattus norvegicus</i>)	54%	46%	30%	31%
Rabbit (<i>Oryctolagus cuniculus</i>)	NA	43%	NA	30%

Abbreviations: BLAST=Basic Local Alignment Search Tool; IL-3=interleukin-3

Note: BLAST conducted on 10 January 2018 (Madden 2002)

CD123 is known to be expressed in multiple normal cell types including hematopoietic myeloid vs. lymphoid progenitors,⁷ plasmacytoid and myeloid dendritic cells,⁸ normal

⁵ Urieto, J. O., T. Liu, J. H. Black, K. A. Cohen, P. D. Hall, M. C. Willingham, L. K. Pennell, D.E. Hogge, R. J. Kreitman and A. E. Frankel (2004). "Expression and purification of the recombinant diphtheria fusion toxin DT388IL3 for phase I clinical trials." *Protein Expr Purif* 33(1): 123-33.

⁶ Khan, K. N. M., A. A. Kats, M. M. Fouant, S. S. Snook, J. P. McKearn, C. L. Alden, and P. F. Smith (1996). "Recombinant human interleukin-3 induces extramedullary hematopoiesis at subcutaneous injection sites in cynomolgus monkeys." *Toxicologic Pathology* 24(4): 391-77.

⁷ Munoz, L., J. F. Nomdedeu, O. Lopez, M. J. Carnicer, M. Bellido, A. Aventin, S. Brunet and J. Sierra (2001). "Interleukin-3 receptor alpha chain (CD123) is widely expressed in hematologic malignancies." *Haematologica* 86(12): 1261-9.

⁸ Willmann, K. and J. F. Dunne (2000). "A flow cytometric immune function assay for human peripheral blood dendritic cells." *J Leukoc Biol* 67(4): 536-44.

myeloid derived cell types (basophils, monocytes, eosinophils),^{9,10,11} some endothelial cells,^{12,13} and osteoblasts.¹⁴ CD123 has been shown to be expressed malignant cells at high levels.^{15,16}

Mechanism of Action:

In a response to information request, the Applicant provided data showing that tagraxofusp treatment results in ADP-ribosylation of EF-2 in THP-1 cells (AML cell line) and that it does not occur in SL-401 resistant cell lines.



⁹ Han, X., J. L. Jorgensen, A. Brahmandam, E. Schlette, Y. O. Huh, Y. Shi, S. Awagu and W. Chen (2008). "Immunophenotypic study of basophils by multiparameter flow cytometry." *Arch Pathol Lab Med* 132(5): 813-9.

¹⁰ Elliott, M. J., M. A. Vadas, J. M. Eglinton, L. S. Park, L. B. To, L. G. Cleland, S. C. Clark and A. F. Lopez (1989). "Recombinant human interleukin-3 and granulocyte-macrophage colony stimulating factor show common biological effects and binding characteristics on human monocytes." *Blood* 74(7): 2349-59.

¹¹ Sun, Q., J. M. Woodcock, A. Rapoport, F. C. Stomski, E. I. Korpelainen, C. J. Bagley, G. J. Goodall, W. B. Smith, J. R. Gamble, M. A. Vadas and A. F. Lopez (1996). "Monoclonal antibody 7G3 recognizes the N-terminal domain of the human interleukin-3 (IL-3) receptor alpha-chain and functions as a specific IL-3 receptor antagonist." *Blood* 87(1): 83-92.

¹² Korpelainen, E. I., J. R. Gamble, W. B. Smith, M. Dottore, M. A. Vadas and A. F. Lopez (1995). "Interferon-gamma upregulates interleukin-3 (IL-3) receptor expression in human endothelial cells and synergizes with IL-3 in stimulating major histocompatibility complex class II expression and cytokine production." *Blood* 86(1): 176-82.

¹³ Tettamanti, S., V. Marin, I. Pizzitola, C. F. Magnani, G. M. Giordano Attianese, E. Cribioli, F. Maltese, S. Galimberti, A. F. Lopez, A. Biondi, D. Bonnet and E. Biagi (2013). "Targeting of acute myeloid leukaemia by cytokine-induced killer cells redirected with a novel CD123-specific chimeric antigen receptor." *Br J Haematol* 161(3): 389-401.

¹⁴ Barhanpurkar, A. P., N. Gupta, R. K. Srivastava, G. B. Tomar, S. P. Naik, S. R. Joshi, S. T. Pote, G. C. Mishra and M. R. Wani (2012). "IL-3 promotes osteoblast differentiation and bone formation in human mesenchymal stem cells." *Biochem Biophys Res Commun* 418(4): 669-75.

¹⁵ Garnache-Ottou, F., J. Feuillard, C. Ferrand, S. Biichle, F. Trimoreau, E. Seilles, V. Salaun, R. Garand, P. Lepelletier, M. Maynadie, E. Kuhlein, E. Deconinck, S. Daliphard, L. Chaperot, L. Beseggio, V. Foisseaude, E. Macintyre, M. C. Bene, P. Saas, M. C. Jacob, Goelams and G. study (2009). "Extended diagnostic criteria for plasmacytoid dendritic cell leukaemia." *Br J Haematol* 145(5): 624-36.

¹⁶ See footnote 7.

Tagraxofusp's catalytic domain (in SL-401 and DT388IL3) has the same amino acid sequence as the catalytic domain of wildtype DT. In 1968, Honjo et al. demonstrated ADP-ribosylation of EF-2 by DT blocks the ability of EF-2 to facilitate protein synthesis.¹⁷

(b) (4)

DT388IL3 or SL-401 has demonstrated tumor cell killing in multiple assays in published studies conducted by the Applicant or that the Applicant has the right to cross-reference (see Table 4).

Table 4 Summary of Published Anti-Tumor Activity of DT388IL3 and SL-401 and in BPDCN, AML, and MDS Cell Lines/Patient-Derived Blasts

Test/Methods In Vitro	Result
Flow cytometry Annexin-V/7-AAD staining of peripheral blood or bone marrow aspirates from 12 BPDCN patients or GEN2.2 and CAL-1 BPDCN cell lines or cells from patients with AML of ALL; a cocktail consisting of monoclonal antibodies to CD45, CD56, CD123, BDCA-2, CD4, and CD3 was used to gate BPDCN cells; cytotoxicity was evaluated with a MTT assay (SL-401) ²¹	IC ₅₀ values for Annexin-V/7-AAD staining or MTT assay as a readout of cell viability were in the femtomolar range (10 ⁻¹⁵ M, experimental concentration of 1 to 21 ng/mL) for BPDCN blasts following 18 hours of treatment; a CD123-negative Daudi cell line was unaffected. Cells from patients with AML or ALL were less sensitive to 365 pM SL-401 when compared to BPDCN cells from patients.
Flow cytometry with Annexin-V/PI or 7-AAD staining of AML (n=16) and MDS (n=6/7 CD123+) cells from patients (SL-401) ²²	SL-401 was cytotoxic in patient-derived AML blasts following 24 hours of treatment; after 48 hours the % live cells was even lower and expression of CD123 was maintained. There was no significant correlation between SL-401 sensitivity and CD123 receptor density. Blasts from high risk MDS patients expressing CD123 were also sensitive to

(b) (4)

¹⁷ Honjo, T., Y. Nishizuka and O. Hayaishi (1968). "Diphtheria toxin-dependent adenosine diphosphate ribosylation of aminoacyl transferase II and inhibition of protein synthesis." J Biol Chem 243(12): 3553-5

(b) (4)

²¹ Angelot-Delettre, F., A. Roggy, A. E. Frankel, B. Lamarthee, E. Seilles, S. Biichle, B. Royer, E. Deconinck, E. K. Rowinsky, C. Brooks, V. Bardet, B. Benet, H. Bennani, Z. Benseddik, A. Debliquis, D. Lusina, M. Roussel, F. Solly, M. Ticchioni, P. Saas and F. Garnache-Ottou (2015). "In vivo and in vitro sensitivity of blastic plasmacytoid dendritic cell neoplasm to SL-401, an interleukin-3 receptor targeted biologic agent." Haematologica 100(2): 223-30.

²² Mani, R., Goswami, S., Gopalakrishnan, B., Ramaswamy, R., Wasmuth, R., Tran, M., Mo, X., Gordon, A., Bucci, D., Lucas, D.M., Mims, A., Brooks, C., Dorrance, A., Walker, A., Blum, W., Byrd, J.C., Lozanski, G., Vasu, S., and Muthusamy, N. (2017). "The interleukin-3 receptor CD123 targeted SL-401 mediates potent cytotoxic activity against CD34+CD123+ cells from acute myeloid leukemia/myelodysplastic syndrome patients and healthy donors." Haematologica 103(8):1288-97.

	SL-401 mediated toxicity.
Test/Methods In Vivo	Result
GEN2.2 BPDCN xenograft model in six-week-old NOD-SCID IL2Rγ-null (NSG) irradiated mice, 2 µg/mouse/injection (100 µg/kg SL-401) x 5 daily doses IP (SL-401) ²³	Median overall survival of mice increased from 17 days in control (PBS) mice to 58 ± 2 days in SL-401 treated mice [p<0.001] (8 mice/group in 3 independent experiments)
AML patient-derived xenografts into NRG-SGM3 (NRGS) mice, pre-conditioned with busulfan (50 µg/kg SL-401, 3 doses IP per week for 5 weeks) (SL-401) ²⁴	SL-401 significantly increased overall survival [p=0.0069] of mice compared with those treated with the vehicle control (n=4/group, 2 different AML PDX models).
(b) (4)	

PI=propidium iodide; 7-AAD=7-aminoactinomycin

4.2 Secondary Pharmacology

No secondary pharmacology studies were submitted. However, in a non-GLP study, binding of DT388IL3 to normal human tissue was evaluated by immunohistochemistry performed on acetone-fixed frozen sections obtained from Biochain Institutes (Hayward, CA) and (b) (4)

positive control TF/Hras cells.²⁶

4.3 Safety Pharmacology

Safety pharmacology assessments (cardiovascular, respiratory, and neurobehavioral) were incorporated into the GLP-compliant repeat-dose general toxicology studies (Study Nos. 2231-002 and 2231-007) and are reviewed under Section 6.2, Repeat-Dose Toxicity.

5 Pharmacokinetics/ADME/Toxicokinetics

Type of Study	Major Findings																			
Absorption <i>Biocomparability Study of SL-401 Following Intravenous Infusion Administration in Cynomolgus Monkeys/ # 2231-006</i>	<table><tr><th>Dose Level</th><th>Parameter</th><th>Day 1</th></tr><tr><td>30 µg/kg</td><td>AUC_{0-∞} (hr.ng/mL)</td><td>269</td></tr><tr><td rowspan="2">Frozen</td><td>Cmax (ng/mL)</td><td>440</td></tr><tr><td>T1/2 (min)</td><td>25</td></tr><tr><td>30 µg/kg</td><td>AUC_{0-∞} (hr.ng/mL)</td><td>285</td></tr><tr><td rowspan="2">(b) (4)</td><td>Cmax (ng/mL)</td><td>465</td></tr><tr><td>T1/2 (min)</td><td>25</td></tr></table> AUC_{0-∞}=area under the plasma concentration-time curve from time 0 extrapolated to infinity; Cmax=maximum concentration; single-dose crossover study (n=8 female monkeys, 48 hours separation between doses)	Dose Level	Parameter	Day 1	30 µg/kg	AUC _{0-∞} (hr.ng/mL)	269	Frozen	Cmax (ng/mL)	440	T1/2 (min)	25	30 µg/kg	AUC _{0-∞} (hr.ng/mL)	285	(b) (4)	Cmax (ng/mL)	465	T1/2 (min)	25
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²³ See footnote 21.

²⁴ See footnote 22

²⁶ See footnote 5.

Type of Study	Major Findings																																																														
TK data from general toxicology studies <i>SL-401: A 5-Day Intravenous Toxicity Study in Cynomolgus Monkeys with a 3-Week Recovery Period/ #2231-002</i>	<table><tr><th>Dose Level</th><th>Parameter</th><th>Day 1</th><th>Day 5</th></tr><tr><td rowspan="3">30 µg/kg</td><td>AUC_{0-∞} (h*ng/mL)</td><td>380</td><td>445</td></tr><tr><td>Cmax (ng/mL)</td><td>602</td><td>566</td></tr><tr><td>T1/2 (min)</td><td>26</td><td>31</td></tr><tr><td rowspan="3">60 µg/kg</td><td>AUC_{0-∞} (h.ng/mL)</td><td>747</td><td>1088</td></tr><tr><td>Cmax (ng/mL)</td><td>1170</td><td>1110</td></tr><tr><td>T1/2 (min)</td><td>26</td><td>36</td></tr></table> AUC_{0-∞}=area under the plasma concentration-time curve from time 0 extrapolated to infinity; Cmax=maximum concentration; male and female data are combined; AUC unit was in min.µg/mL but was converted to h.ng/mL for comparison purposes by the reviewer by multiplying value by 1000 and dividing by 60.	Dose Level	Parameter	Day 1	Day 5	30 µg/kg	AUC _{0-∞} (h*ng/mL)	380	445	Cmax (ng/mL)	602	566	T1/2 (min)	26	31	60 µg/kg	AUC _{0-∞} (h.ng/mL)	747	1088	Cmax (ng/mL)	1170	1110	T1/2 (min)	26	36																																						
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TK data from general toxicology studies <i>SL-401: A 3-Month Intravenous Toxicity Study in Monkeys/ #2231-007</i>	Table generated in Janus Nonclinical with minor reviewer modifications: <table><tr><th>Dose Level</th><th>Parameter</th><th>Sex</th><th>Day 1</th><th>Day 27</th><th>Day 54</th></tr><tr><td rowspan="6">35 µg/kg</td><td rowspan="2">AUC to Last Nonzero Conc (h*ng/mL)</td><td>Male</td><td>402</td><td>183</td><td>4</td></tr><tr><td>Female</td><td>250</td><td>125</td><td>3.29*</td></tr><tr><td rowspan="2">Max Conc (ng/mL)</td><td>Male</td><td>550</td><td>282</td><td>11</td></tr><tr><td>Female</td><td>535</td><td>221</td><td>6.58*</td></tr><tr><td rowspan="2">Half-Life Lambda z (h)</td><td>Male</td><td>1</td><td>0</td><td>0.292*</td></tr><tr><td>Female</td><td>1</td><td>1</td><td>0.395*</td></tr><tr><td rowspan="6">45 µg/kg</td><td rowspan="2">AUC to Last Nonzero Conc (h*ng/mL)</td><td>Male</td><td>486</td><td>437</td><td>15</td></tr><tr><td>Female</td><td>387</td><td>79*</td><td>4</td></tr><tr><td rowspan="2">Max Conc (ng/mL)</td><td>Male</td><td>655</td><td>573</td><td>20*</td></tr><tr><td>Female</td><td>675</td><td>84.7*</td><td>6</td></tr><tr><td rowspan="2">Half-Life Lambda z (h)</td><td>Male</td><td>1</td><td>1</td><td>1</td></tr><tr><td>Female</td><td>1</td><td>0.827*</td><td>1</td></tr></table> * Data obtained from only one animal; assessment Days 5 and 31 are excluded from the table since they did not add additional information. Total exposure during later cycles may not have been accurately estimated due to anti-drug antibody interference.	Dose Level	Parameter	Sex	Day 1	Day 27	Day 54	35 µg/kg	AUC to Last Nonzero Conc (h*ng/mL)	Male	402	183	4	Female	250	125	3.29*	Max Conc (ng/mL)	Male	550	282	11	Female	535	221	6.58*	Half-Life Lambda z (h)	Male	1	0	0.292*	Female	1	1	0.395*	45 µg/kg	AUC to Last Nonzero Conc (h*ng/mL)	Male	486	437	15	Female	387	79*	4	Max Conc (ng/mL)	Male	655	573	20*	Female	675	84.7*	6	Half-Life Lambda z (h)	Male	1	1	1	Female	1	0.827*	1
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6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicity studies were submitted.

6.2 Repeat-Dose Toxicity

(b) (4)

(b) (4)

Study title/ number: SL-401: A 5-Day Intravenous Toxicity Study in Cynomolgus Monkeys with a 3-Week Recovery Period/ 2231-002

Key Study Findings

- The highest dose of 60 µg/kg/day resulted in mortality caused by kidney toxicity in one female.
- A NOAEL was not identified.
- Body weight decreases and inappetence were observed.
- Predominant organs of toxicity with SL-401 were the kidney, liver, brain, lymphoid organs (thymus, spleen, lymph node), and skin (injection site)

Conducting laboratory and location:

(b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 30, and 60 µg/kg once daily for 5 consecutive days followed by a 3-week recovery period (refrigerated SL-401)

Route of administration: Intravenous (5 min slow bolus)

Formulation/Vehicle: 5% Dextrose

Species/Strain: Cynomolgus Monkey

Number/Sex/Group: Terminal: 3/sex/group
Recovery: 2/sex/group (control and high-dose groups only)

Age: ~2.5 to 4 years at receipt

Satellite groups/ unique design: Beginning on Day 5, animals were administered supplemental fluids (Lactated Ringer's Solution) via subcutaneous injection – 60 mL if dehydrated; 20 mL if blood collection volume limit was exceeded.

Deviation from study protocol affecting interpretation of results: None were considered by the study director to affect the integrity or interpretation of the study.

Observations and Results: changes from control

Parameters	Major findings
Mortality	60 µg/kg: One female was euthanized in extremis on Day 6 due to signs of moribundity caused by severe renal dysfunction. The cause of mortality was severe

(b) (4)

	necrosis of the renal cortical tubules (kidneys). The only clinical signs noted during the dosing phase were injection site and/or blood collection site redness during Days 1-5, inappetence on Days 2, 4 and 5, and cold to touch on Day 5. Clinical pathology data from Day 6 showed urea nitrogen and creatinine levels consistent with decreased glomerular filtration associated with renal injury. These findings correlated with severe tubular degeneration and necrosis, as noted microscopically. A clinical pathology assessment, taken prior to necropsy, showed much larger changes in inflammatory parameters and liver enzymes than in animals that survived until scheduled necropsy, as well as a decrease in platelets.																																																																																																																																																																																		
Clinical Signs	There were clinical signs of decreased appetite in 2 females at 60 µg/kg/day on Day 2 and in all animals at 30 and 60 µg/kg/day on Days 4 and 5. Decreased appetite persisted during the recovery period in animals at 60 µg/kg/day.																																																																																																																																																																																		
Body Weights	Body weight decreases and food consumption at both doses correlated with decreased appetite.																																																																																																																																																																																		
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	reticulocytes) were observed in controls only (up to 203% relative to pre-test values) was not observed in SL-401 treated animals. - Increases in neutrophils (up to +166% in HD males) on Day 3 were consistent with an inflammatory response, as further supported by the presence of Döhle bodies (noted microscopically on blood smears among individual animals administered 60 µg/kg/day) and increases in fibrinogen and globulin. - Despite considerable individual animal variability, there were trends in decreased lymphocytes, monocytes, eosinophils, and basophils that reached statistical significance on Day 3 only. - Generally, all findings were reversible except leukocytes and neutrophils were reduced 51 and 79%, respectively, when compared to pretest values in one HD female.						
Clinical Chemistry	Findings were observed as early as Day 3.						
Clinical Chemistry		Male			Female		
Dose (µg/kg)		0	30	60	0	30	60
Parameter	Day	mean	%Δ	%Δ	mean	%Δ	%Δ
AST (U/L)	3	120	91%	308%	110	4%	375%
	14	86	207%	779%	114	-1%	340%
ALT (U/L)	3	101	56%	60%	93	-31%	63%
	14	119	55%	150%	112	-17%	137%
BUN (mg/dL)	3	20	5%	32%	18	-6%	37%
	14	22	0%	97%	20	-8%	54%
	28	22	NM	9%	19	NM	51%
Creatinine (mg/dL)	3	0.7	36%	36%	0.6	25%	34%
	14	0.6	34%	129%	0.6	33%	67%
Total protein (g/dL)	14	6.3	0%	-4%	6.46	0%	-10%
Albumin (g/dL)	3	4.0	-4%	-11%	4.1	-2%	-16%
	14	3.8	-17%	-26%	4.0	-12%	-30%
	28	3.9	NM	-10%	4.2	NM	-12%
Globulin (g/dL)	3	2.6	18%	26%	2.7	10%	23%
	14	2.4	28%	30%	2.5	19%	22%
	28	2.7	NM	32%	3	NM	23%
A;G ratio (g/dL)	3	1.5	-20%	-29%	1.5	-11%	-31%
	14	1.6	-37%	-43%	1.6	-26%	-43%
	28	1.5	NM	-31%	1.4	NM	-29%
TRIG (mg/dL)	3	49	-29%	468%	48	6%	56%
	14	47	-42%	72%	47	-39%	9%
Chloride (mEq/L)	3	111	-6%	-5%	109	-3%	-1%
	14	109	-2%	-1%	109	-1%	2%
	28	110	NM	0%	109	NM	-4%
Calcium (mEq/L)	3	9.6	-1%	-5%	9.6	-3%	-6%
	14	9.4	-5%	-7%	9.7	-3%	-10%
	28	9.3	NM	4%	10.1	NM	-6%
PHOS (mEq/L)	3	5.6	-14%	0%	5.7	-16%	-25%

		Clinical Chemistry		Male			Female																																																																																																																																																										
		Dose (µg/kg)		0	30	60	0	30	60																																																																																																																																																								
		Parameter	Day	mean	%Δ	%Δ	mean	%Δ	%Δ																																																																																																																																																								
		PHOS (mEq/L)	14	6.3	-34%	-41%	4.9	-22%	-34%																																																																																																																																																								
			28	6.0	NM	-25%	5.9	NM	-24%																																																																																																																																																								
	%Δ = percent change relative to concurrent control; AST = aspartate aminotransferase; ALT = alanine aminotransferase; BUN = urea nitrogen; TRIG = triglycerides; PHOS = phosphorus; bold = outside of range of %Δ on pretest day; NM = not measured.																																																																																																																																																																
Urinalysis	At terminal collection the magnitude of proteinuria (protein in urine; +300 mg/dL) was increased among individual animals administered 30 µg/kg/day (2 of 3 females) and 60 µg/kg/day (3 of 5 males; 4 of 4 females), relative to controls and pretest values. The presence of increased proteinuria was dose-dependent in females and was SL-401 related and adverse, and correlated with adverse microscopic changes in the kidney (renal tubular degeneration and necrosis and increased hyaline droplets). Decreased urine volume (up to -86%) with corresponding increases in urine specific gravity were noted in females administered 30 and 60 µg/kg/day, relative to pretest values.																																																																																																																																																																
Gross Pathology	HD males: tan discoloration of the kidneys correlated with tubular degeneration/ necrosis. HD males (recovery): small thymus correlated with generalized lymphoid depletion and was SL-401-related and/or associated with stress-related effects from generalized malaise. Swollen/thickened (HD only) or red/discolored saphenous veins (including controls) were observed at the injection site with microscopic correlates. HD female (moribund): red skin discoloration																																																																																																																																																																
Organ Weights	<table><tr><th colspan="3">Organ Weights-Terminal</th><th colspan="3">Male</th><th colspan="3">Female</th></tr><tr><th colspan="3">Dose (µg/kg)</th><th>0</th><th>30</th><th>60</th><th>0</th><th>30</th><th>60</th></tr><tr><th>Organ/Tissue</th><th>Test</th><th></th><th>mean</th><th>%Δ</th><th>%Δ</th><th>mean</th><th>%Δ</th><th>%Δ</th></tr><tr><td rowspan="3">Adrenals</td><td>Absolute (g)</td><td></td><td>0.434</td><td>37%</td><td>33%</td><td>0.482</td><td>18%</td><td>17%</td></tr><tr><td>Relative BW (%)</td><td></td><td>0.015</td><td>46%</td><td>47%</td><td>0.018</td><td>10%</td><td>35%</td></tr><tr><td>Rel Brain Wt (%)</td><td></td><td>0.006</td><td>52%</td><td>45%</td><td>0.008</td><td>17%</td><td>21%</td></tr><tr><td rowspan="3">Kidney</td><td>Absolute (g)</td><td></td><td>12.86</td><td>9%</td><td>20%</td><td>13.45</td><td>-</td><td>-</td></tr><tr><td>Relative BW (%)</td><td></td><td>0.442</td><td>11%</td><td>29%</td><td>0.501</td><td>-</td><td>-</td></tr><tr><td>Rel Brain Wt (%)</td><td></td><td>0.178</td><td>17%</td><td>30%</td><td>0.214</td><td>-</td><td>-</td></tr><tr><td rowspan="3">Liver</td><td>Absolute (g)</td><td></td><td>53.49</td><td>11%</td><td>34%</td><td>55.86</td><td>-</td><td>-</td></tr><tr><td>Relative BW (%)</td><td></td><td>1.836</td><td>14%</td><td>44%</td><td>2.091</td><td>-</td><td>-</td></tr><tr><td>Rel Brain Wt (%)</td><td></td><td>0.741</td><td>20%</td><td>44%</td><td>0.891</td><td>-</td><td>-</td></tr><tr><td rowspan="3">Spleen</td><td>Absolute (g)</td><td></td><td>2.179</td><td>70%</td><td>126%</td><td>3.662</td><td>-</td><td>-</td></tr><tr><td>Relative BW (%)</td><td></td><td>0.075</td><td>71%</td><td>137%</td><td>0.135</td><td>-</td><td>-</td></tr><tr><td>Rel Brain Wt (%)</td><td></td><td>0.030</td><td>81%</td><td>144%</td><td>0.578</td><td>-</td><td>-</td></tr><tr><td rowspan="3">Thymus</td><td>Absolute (g)</td><td></td><td>3.390</td><td>-</td><td>-</td><td>3.040</td><td>-19%</td><td>-63%</td></tr><tr><td>Relative BW (%)</td><td></td><td>0.116</td><td>-</td><td>-</td><td>0.114</td><td>-27%</td><td>-57%</td></tr><tr><td>Rel Brain Wt (%)</td><td></td><td>0.047</td><td>-</td><td>-</td><td>0.048</td><td>-22%</td><td>-62%</td></tr></table>									Organ Weights-Terminal			Male			Female			Dose (µg/kg)			0	30	60	0	30	60	Organ/Tissue	Test		mean	%Δ	%Δ	mean	%Δ	%Δ	Adrenals	Absolute (g)		0.434	37%	33%	0.482	18%	17%	Relative BW (%)		0.015	46%	47%	0.018	10%	35%	Rel Brain Wt (%)		0.006	52%	45%	0.008	17%	21%	Kidney	Absolute (g)		12.86	9%	20%	13.45	-	-	Relative BW (%)		0.442	11%	29%	0.501	-	-	Rel Brain Wt (%)		0.178	17%	30%	0.214	-	-	Liver	Absolute (g)		53.49	11%	34%	55.86	-	-	Relative BW (%)		1.836	14%	44%	2.091	-	-	Rel Brain Wt (%)		0.741	20%	44%	0.891	-	-	Spleen	Absolute (g)		2.179	70%	126%	3.662	-	-	Relative BW (%)		0.075	71%	137%	0.135	-	-	Rel Brain Wt (%)		0.030	81%	144%	0.578	-	-	Thymus	Absolute (g)		3.390	-	-	3.040	-19%	-63%	Relative BW (%)		0.116	-	-	0.114	-27%	-57%	Rel Brain Wt (%)		0.047	-	-	0.048	-22%	-62%
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	%Δ = percent change relative to concurrent control; "-" = no noteworthy changes																																																																																																																																																																

	<table><tr><th colspan="2">Organ Weights-Recovery</th><th colspan="2">Male</th><th colspan="2">Female</th></tr><tr><th colspan="2">Dose (µg/kg)</th><th>0</th><th>60</th><th>0</th><th>60</th></tr><tr><th>Organ/ Tissue</th><th>Test</th><th>mean</th><th>%Δ</th><th>mean</th><th>%Δ</th></tr><tr><td rowspan="3">Lung</td><td>Absolute (g)</td><td>16.27</td><td>34%</td><td>16.60</td><td>21%</td></tr><tr><td>Relative BW (%)</td><td>0.521</td><td>87%</td><td>0.552</td><td>52%</td></tr><tr><td>Rel Brain Wt (%)</td><td>0.244</td><td>41%</td><td>0.247</td><td>28%</td></tr><tr><td rowspan="3">Thymus</td><td>Absolute (g)</td><td>4.408</td><td>-87%</td><td>3.977</td><td>-70%</td></tr><tr><td>Relative BW (%)</td><td>0.142</td><td>-82%</td><td>0.132</td><td>-62%</td></tr><tr><td>Rel Brain Wt (%)</td><td>0.067</td><td>-86%</td><td>0.058</td><td>-68%</td></tr></table> %Δ = percent change relative to concurrent control	Organ Weights-Recovery		Male		Female		Dose (µg/kg)		0	60	0	60	Organ/ Tissue	Test	mean	%Δ	mean	%Δ	Lung	Absolute (g)	16.27	34%	16.60	21%	Relative BW (%)	0.521	87%	0.552	52%	Rel Brain Wt (%)	0.244	41%	0.247	28%	Thymus	Absolute (g)	4.408	-87%	3.977	-70%	Relative BW (%)	0.142	-82%	0.132	-62%	Rel Brain Wt (%)	0.067	-86%	0.058	-68%																																																																																																																																																																													
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Histopathology Adequate battery: Yes	<table><tr><th colspan="3">Terminal/Recovery Microscopic Findings</th><th colspan="3">Male</th><th colspan="3">Female</th></tr><tr><th colspan="3">Dose (µg/kg)</th><th>0</th><th>30</th><th>60</th><th>0</th><th>30</th><th>60</th></tr><tr><th>Organ/ Tissue</th><th>Finding</th><th>Severity</th><th></th><th></th><th></th><th></th><th></th><th></th></tr><tr><td rowspan="3">BRAIN</td><td></td><td>#Exam</td><td>3/2</td><td>3/0</td><td>3/2</td><td>3/2</td><td>3/0</td><td>4/1</td></tr><tr><td rowspan="2">infiltration, mixed leukocyte</td><td>MIN</td><td></td><td>1/--</td><td></td><td></td><td>2/--</td><td>3/0</td></tr><tr><td>MILD</td><td></td><td>1/--</td><td>1/0</td><td></td><td>0/--</td><td>1/0</td></tr><tr><td rowspan="6">BRAIN, CHOROID PLEXUS</td><td></td><td>#Exam</td><td>3/2</td><td>3/0</td><td>3/2</td><td>3/2</td><td>3/0</td><td>4/1</td></tr><tr><td>hemorrhage</td><td>MIN</td><td></td><td></td><td></td><td></td><td></td><td>1/0</td></tr><tr><td rowspan="4">Inflammation/ degeneration/ necrosis</td><td>MIN</td><td></td><td>1/--</td><td>1/0</td><td></td><td>1/--</td><td></td></tr><tr><td>MILD</td><td></td><td>2/--</td><td>1/0</td><td></td><td>1/--</td><td>2/0</td></tr><tr><td>MOD</td><td></td><td></td><td>1/2</td><td></td><td></td><td>2/0</td></tr><tr><td>SEV</td><td></td><td></td><td></td><td></td><td></td><td>0/1</td></tr><tr><td rowspan="5">KIDNEY</td><td></td><td>#Exam</td><td>3/2</td><td>3/0</td><td>3/2</td><td>3/2</td><td>3/0</td><td>4/1</td></tr><tr><td rowspan="4">degeneration/ necrosis, tubular</td><td>MIN</td><td></td><td></td><td></td><td></td><td>1/--</td><td>2/0</td></tr><tr><td>MILD</td><td></td><td></td><td>1/0</td><td></td><td>1/--</td><td>1/0</td></tr><tr><td>MOD</td><td></td><td></td><td>2/0</td><td></td><td></td><td></td></tr><tr><td>SEV</td><td></td><td></td><td></td><td></td><td></td><td>1/0</td></tr><tr><td></td><td>hyaline, droplets, increased</td><td>MILD</td><td></td><td></td><td>1/0</td><td></td><td></td><td></td></tr><tr><td rowspan="5">LIVER</td><td></td><td>#Exam</td><td>3/2</td><td>3/0</td><td>3/2</td><td>3/2</td><td>3/0</td><td>4/1</td></tr><tr><td>necrosis, hepatocytes, cenrilobular</td><td>MIN</td><td></td><td></td><td>1/0</td><td></td><td></td><td></td></tr><tr><td>vacuolation, cenrilobular</td><td>MILD</td><td></td><td></td><td>1/0</td><td></td><td></td><td></td></tr><tr><td>vacuolation, diffuse</td><td>MILD</td><td></td><td></td><td>2/0</td><td></td><td></td><td></td></tr><tr><td>vacuolation, periportal</td><td>MIN</td><td></td><td></td><td></td><td></td><td></td><td>1/0</td></tr><tr><td rowspan="5">THYMUS</td><td></td><td>#Exam</td><td>3/2</td><td>3/0</td><td>3/2</td><td>3/2</td><td>3/0</td><td>4/1</td></tr><tr><td rowspan="4">depletion, lymphoid, generalized</td><td>MIN</td><td></td><td></td><td>1/0</td><td></td><td>1/--</td><td>1/0</td></tr><tr><td>MILD</td><td></td><td></td><td>1/1</td><td></td><td>1/--</td><td>2/1</td></tr><tr><td>MOD</td><td></td><td></td><td>1/0</td><td></td><td></td><td>1/0</td></tr><tr><td>SEV</td><td></td><td></td><td>1/1</td><td></td><td></td><td></td></tr></table>	Terminal/Recovery Microscopic Findings			Male			Female			Dose (µg/kg)			0	30	60	0	30	60	Organ/ Tissue	Finding	Severity							BRAIN		#Exam	3/2	3/0	3/2	3/2	3/0	4/1	infiltration, mixed leukocyte	MIN		1/--			2/--	3/0	MILD		1/--	1/0		0/--	1/0	BRAIN, CHOROID PLEXUS		#Exam	3/2	3/0	3/2	3/2	3/0	4/1	hemorrhage	MIN						1/0	Inflammation/ degeneration/ necrosis	MIN		1/--	1/0		1/--		MILD		2/--	1/0		1/--	2/0	MOD			1/2			2/0	SEV						0/1	KIDNEY		#Exam	3/2	3/0	3/2	3/2	3/0	4/1	degeneration/ necrosis, tubular	MIN					1/--	2/0	MILD			1/0		1/--	1/0	MOD			2/0				SEV						1/0		hyaline, droplets, increased	MILD			1/0				LIVER		#Exam	3/2	3/0	3/2	3/2	3/0	4/1	necrosis, hepatocytes, cenrilobular	MIN			1/0				vacuolation, cenrilobular	MILD			1/0				vacuolation, diffuse	MILD			2/0				vacuolation, periportal	MIN						1/0	THYMUS		#Exam	3/2	3/0	3/2	3/2	3/0	4/1	depletion, lymphoid, generalized	MIN			1/0		1/--	1/0	MILD			1/1		1/--	2/1	MOD			1/0			1/0	SEV			1/1			
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THYMUS		#Exam	3/2	3/0	3/2	3/2	3/0	4/1																																																																																																																																																																																																																								
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Terminal/Recovery Microscopic Findings			Male			Female		
Dose (µg/kg)			0	30	60	0	30	60
Organ/ Tissue	Finding	Severity						
SKELETAL MUSCLE, RECTUS FEMORIS		#Exam	3/2	3/0	3/2	3/2	3/0	4/1
	degeneration/ necrosis, myofiber	MIN		1/--			1/--	2/0
		MILD			1/0			
	infiltration, mononuclear cell	MIN		1/--	1/0	1/0		2/0
SPLEEN		#Exam	3/2	3/0	3/2	3/2	3/0	4/1
	vacuolation, histiocytic/ parenchymal cells	MOD			1/0			

MIN=minimal; MOD=moderate; SEV=severe; #Exam=number of animals examined/group; "--" = no recovery animals were examined

- Perivascular hemorrhage of the injection site and erosion/ulcers were noted at higher incidence and severity in SL-401-treated monkeys compared to controls.
- Mild lung edema was observed in the HD female with early mortality.
- Mild generalized lymphoid depletion of the mandibular lymph node was observed in 1 HD male.
- Mineralization of the ovaries was observed in 1, 2, and 4 control, LD, and HD females (including the female with early mortality), respectively.
- The following were noted with adverse findings in the choroid plexus.
 - o Infiltrates of lymphocytes, macrophages, and an occasional neutrophil within the interstitial area of the choroid plexus.
 - o Areas of epithelial cells lining the choroid plexus were degenerate or necrotic.
 - o Free leukocytes and cellular debris were noted to be present within brain ventricles of some monkeys (including the female with early mortality).
 - o Lymphocyte, macrophage, and occasionally neutrophil infiltrates were observed within the perivascular space of blood vessels (perivascular cuffing) adjacent to the choroid plexus.
 - o In recovery animals the choroid plexus contained fewer leukocytes but there was an increased presence of fibrous stroma. Occasional macrophages contained small amounts of light brown granular pigment consistent with hemosiderin (special stains were not done to confirm the presence of iron). Epithelial cells lining the choroid plexus were focally attenuated or appeared disorganized.

LD: low dose; HD: high dose; minus sign: indicates reduction in parameters compared to control.

Study title/ number: SL-401: A 3-Month Intravenous Toxicity Study in Monkeys/ 2231-007

Key Study Findings

- The highest dose of 45 µg/kg/day resulted in mortality of unknown cause in a female. Clinical signs included decreased activity, hunched posture, inappetence, dehydration, increased body temperature, and bruising/swelling of limbs. There were no severe microscopic findings in this female.
- Toxicities were overall consistent with the 1-month study, except monkeys in the 3-month study displayed more clinical signs (hypersensitivity with suppressed erythropoiesis, hunched posture, tremors).
- Neutralizing ADA was observed with more than 1 cycle of dosing, with low exposure to SL-401 detected at the end of the study. While SL-401 is

pharmacologically active at low serum levels, the impact this low exposure would have on the study results would, in theory, be less toxicity.

Conducting laboratory and location:

(b) (4)

GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 35, and 45 µg/kg once daily for 5 consecutive days, separated by a 3-week off dosing period (15 doses total; Day 58 is the last dose) (frozen SL-401)

Route of administration: Intravenous (5 min slow bolus)

Formulation/Vehicle: 0.9% Sodium Chloride for Injection, USP

Species/Strain: Cynomolgus Monkey

Number/Sex/Group: Terminal: 3/sex/group

Age: ~2 to 4 years at receipt

Satellite groups/ unique design: No

Deviation from study protocol affecting interpretation of results: Analysis, by the UV method, revealed average percentages of the nominal concentration of 50 µg/mL SL-401 of 89%, 86%, and 79% for Days 1, 27, and 54, respectively. This is likely a result of the instability and potential precipitation of the samples after a freeze/thaw cycle. This was not considered a deviation by the study director but is noted.

This study was submitted in SEND format and data analysis (except for ECG data) was conducted using Janus Nonclinical, an application in development by the FDA Office of Computational Sciences to store, view, analyze, and export data in SEND format. Summary tables were generated in Janus Nonclinical with minor reviewer modifications.

Observations and Results: changes from control

Parameters	Major findings						
Mortality	45 µg/kg: One female was euthanized in extremis on Day 9. This female had decreased activity on Day 5; hunched posture on Days 2 to 8, and inappetence Days 3 to 5. In addition to the clinical signs listed below, there was moderate dehydration, increased body temperature, bruising of the abdomen and hindlimbs, and swelling of the right inguinal area, hind limbs and dosing sites. Cause of death was undetermined.						
Clinical Signs	Number of Observations/Number of Animals with Clinical Sign (Routine):						
	Clinical Sign	Male			Female		
	Dose (µg/kg)	0	35	45	0	35	45
	Hypersensitive (Days 10-68)						52/1
	Decreased activity					1/1	4/3*
	Posture hunched		13/2	20/3		6/2	23/3*
	Thin		35/1	22/1		72/2	

Parameters	Major findings																																																																																																																														
	<table><tr><th>Clinical Sign</th><th colspan="3">Male</th><th colspan="3">Female</th></tr><tr><th>Dose (µg/kg)</th><th>0</th><th>35</th><th>45</th><th>0</th><th>35</th><th>45</th></tr><tr><td>Trembling†</td><td></td><td></td><td></td><td></td><td>1/1</td><td>2/2</td></tr><tr><td>Tremors†</td><td></td><td></td><td>2/2</td><td></td><td>1/1</td><td>4/2</td></tr><tr><td>Eyelids closed</td><td></td><td></td><td></td><td></td><td>2/1</td><td>1/1</td></tr><tr><td>Emesis</td><td></td><td></td><td></td><td></td><td></td><td>1/1</td></tr><tr><td>Inappetence†</td><td></td><td>3/3</td><td>6/3</td><td></td><td></td><td>17/3*</td></tr><tr><td>Loss of skin elasticity†</td><td></td><td>1/1</td><td>3/3</td><td>2/2</td><td>2/2</td><td>3/2</td></tr><tr><td>Right forelimb function impaired</td><td></td><td></td><td>1/1</td><td></td><td></td><td></td></tr><tr><td>Nodule:</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td> Inguinal region/right</td><td></td><td>16/1</td><td>2/1</td><td></td><td>6/1</td><td>14/2</td></tr><tr><td> Inguinal region/left</td><td></td><td>11/2</td><td>12/2</td><td></td><td></td><td>1/1</td></tr><tr><td> Injection Site 2</td><td></td><td></td><td>8/2</td><td></td><td></td><td></td></tr><tr><td>Skin discolored:</td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td> Injection Site 3</td><td></td><td>3/1</td><td>7/1</td><td></td><td>4/2</td><td>10/3*</td></tr><tr><td> Injection Site 4</td><td></td><td>2/1</td><td>4/1</td><td></td><td></td><td>3/1*</td></tr><tr><td>Feces changes: discolored, watery, soft</td><td colspan="6">All groups</td></tr><tr><td>Dry skin and scabbed areas on the face, limbs, inguinal area, and injection site</td><td colspan="6">All groups, but more prevalent in SL-401 treated animals than controls (sometimes w/wo dose-response)</td></tr></table> * denotes female with early death had finding; † denotes that unscheduled or veterinary observations were included in the incidence tally; blank cells denote absence of observation. Note: the animal that was hypersensitive to the touch had suppressed erythropoiesis. • Most test article-related routine clinical signs were observed between 2- and 4-hours postdose unless otherwise noted.	Clinical Sign	Male			Female			Dose (µg/kg)	0	35	45	0	35	45	Trembling†					1/1	2/2	Tremors†			2/2		1/1	4/2	Eyelids closed					2/1	1/1	Emesis						1/1	Inappetence†		3/3	6/3			17/3*	Loss of skin elasticity†		1/1	3/3	2/2	2/2	3/2	Right forelimb function impaired			1/1				Nodule:							Inguinal region/right		16/1	2/1		6/1	14/2	Inguinal region/left		11/2	12/2			1/1	Injection Site 2			8/2				Skin discolored:							Injection Site 3		3/1	7/1		4/2	10/3*	Injection Site 4		2/1	4/1			3/1*	Feces changes: discolored, watery, soft	All groups						Dry skin and scabbed areas on the face, limbs, inguinal area, and injection site	All groups, but more prevalent in SL-401 treated animals than controls (sometimes w/wo dose-response)					
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Body Weights	Body weight changes over the course of the 3-month study: Males Body Weight (kg) Day Females Body Weight (kg) Day Figure Legend: Shaded gray bars show intermittent 5-day dosing periods in the study; downward arrows point to the first observed and largest decrease relative to pretest (the percent change relative to pretest for a group is listed above the arrows). Control male and female decreases in body weight did not exceed 1%. Low-Dose = 35 µg/kg; High-Dose = 45 µg/kg. (Figure was generated by the reviewer from data exported from Janus Nonclinical)																																																																																																																														
Ophthalmoscopy	HD female: Discoloration of both eyes was observed on Day 56, but it was not considered by the ophthalmologist to be SL-401-related.																																																																																																																														
ECG	There were no consistent trends suggesting a test article-related effect. However, measurements were taken (Days 29 and 56) after the development of ADA and decline in SL-401 exposure.																																																																																																																														
Hematology	In animal with mortality at 45 µg/kg: The female euthanized early in extremis on Day 9 also displayed on Day 7 the changes noted below in the group data.																																																																																																																														

Parameters	Major findings							
	Group data:							
	Hematology		Male			Female		
	Dose (µg/kg)		0	35	45	0	35	45
	Parameter	Day	mean	%Δ	%Δ	mean	%Δ	%Δ
	Basophils (10^9/L)	7	0.03	133%	66%	0.23	214%	129%
	Erythrocytes (10^12/L)	7	4.91	-6%	0.7%	5.08	-13%	-9%
		34	4.94	-12%	-5.6%	5.12	-12%	-1.6%
	Hemoglobin (g/dL)	7	12	-3%	-4%	12	-12%	-12%
		34	12	-11%	-12%	12	-10%	-6%
	Large Unstained Cells (10^9/L)	7	0.05	500%	550%	0.083	212%	392%
		34	0.07	67%	200%	0.097	86%	-64%
	Reticulocytes (10^9/L)	7	194	-64%r	-70%r	268	-56%r	-84.5%r
		34	198	-35%r	-60.5%r	206.5	-24%r	-70%r
		68	89	-26%r	-32.5%r	110	-4.5%r	-56%r
	Platelets (10^9/L)	7	445	6%	-25%	521	3%	-33%
	APTT (sec)	7	20	18%	20%	20	11%	19%
	Fibrinogen (mg/dL)	7	304	95%	87%	328	52.6%	51%
34		268	50%	43%	289	13%	16%	
68		250	-8%	-11%	249	-19%	-30%	
%Δ = percent change relative to concurrent control; “r” indicates suppressed adaptive red cell mass response since reticulocyte levels increased on Days 7, 34, and 68, but mean levels in SL-401 treated monkeys did not; bold = outside of range of %Δ on pretest days - Suppressed hematopoiesis correlated with alterations in the myeloid:erythroid ratio and decreased polychromasia were noted microscopically in the bone marrow in a few animals. - Inflammatory response (increased fibrinogen and globulin concentrations, decreased albumin concentrations, and prolonged APTT).								
Clinical Chemistry	In animal with mortality at 45 µg/kg: The female euthanized early in extremis on Day 9 also displayed the changes noted below on Day 7 in the group data.							
	Group data:							
	Clinical Chemistry		Male			Female		
	Dose (µg/kg)		0	35	45	0	35	45
	Parameter	Day	mean	%Δ	%Δ	mean	%Δ	%Δ
	ALT (U/L)	7	70	155%	92%	75	171%	171%
		34	92	30%	28%	80	44%	26%
	AST (U/L)	7	39	405%	385%	37	377%	368%
		34	36	92%	243%	30	188%	89%
	Globulin (g/dL)	7	2.73	32%	26%	2.97	12%	16%
34		2.87	36%	27%	3.10	23%	35.5%	
68		2.83	22%	18%	3.23	0%	5%	
Albumin (g/dL)	7	4.13	-16%	-16%	4.00	-17%	-28%	
	34	4.13	-8%	-17%	4.03	-7%	-13%	
Albumin/Globulin ratio	7	1.53	-39%	-35%	1.40	-29%	-40.5%	
	34	1.47	-34%	-36%	1.30	-23%	-35%	

Parameters	Major findings																																																																																																																											
	Clinical Chemistry		Male			Female																																																																																																																						
	Dose (µg/kg)		0	35	45	0	35	45																																																																																																																				
	Parameter	Day	mean	%Δ	%Δ	mean	%Δ	%Δ																																																																																																																				
	Phosphorus (mg/dL)	7	6.87	-37%	-51.5%	6.13	-2%	-56%																																																																																																																				
	Calcium	7	9.63	-2%	-1%	10	-2%	-18%																																																																																																																				
	%Δ = percent change relative to concurrent control; ALT=Alanine Aminotransferase; AST= Aspartate Aminotransferase; bold = outside of range of %Δ on pretest days																																																																																																																											
Decreased phosphorus concentrations in males at 35 and 45 µg/kg and females at 45 µg/kg, primarily following Cycle 1 may have been secondary to inappetence.																																																																																																																												
Urinalysis	Protein level were elevated up to 100 mg/dL on Day 7 in SL-401 treated animals, including female with early mortality.																																																																																																																											
Gross Pathology	Unremarkable																																																																																																																											
Organ Weights	<table><tr><td colspan="2">Organ Weights</td><td colspan="3">Male</td><td colspan="3">Female</td></tr><tr><td colspan="2">Dose (µg/kg)</td><td>0</td><td>35</td><td>45</td><td>0</td><td>35</td><td>45</td></tr><tr><td>Organ/ Tissue</td><td>Test</td><td>mean</td><td>%Δ</td><td>%Δ</td><td>mean</td><td>%Δ</td><td>%Δ</td></tr><tr><td rowspan="3">GLAND, PITUITARY</td><td>Weight (g)</td><td>0.06</td><td>-27%</td><td>-26%</td><td>0.05</td><td>-23%</td><td>-43%</td></tr><tr><td>Organ to Body Weight Ratio (%)</td><td>0.00</td><td>-21.5%</td><td>-16%</td><td>0.00</td><td>-23%</td><td>-49%</td></tr><tr><td>Organ to Brain Weight Ratio (%)</td><td>0.09</td><td>-31.5%</td><td>-28%</td><td>0.07</td><td>-18%</td><td>-48%</td></tr><tr><td rowspan="3">THYMUS</td><td>Weight (g)</td><td>3.11</td><td>-21.5%</td><td>-42%</td><td>2.04</td><td>-14%</td><td>-10%</td></tr><tr><td>Organ to Body Weight Ratio (%)</td><td>0.09</td><td>-18%</td><td>-35%</td><td>0.07</td><td>-11%</td><td>-14%</td></tr><tr><td>Organ to Brain Weight Ratio (%)</td><td>4.54</td><td>-29%</td><td>-43%</td><td>3.17</td><td>-7%</td><td>-14%</td></tr><tr><td rowspan="3">TESTIS</td><td>Weight (g)</td><td>2.8</td><td>-43%</td><td>-43%</td><td>NA</td><td>NA</td><td>NA</td></tr><tr><td>Organ to Body Weight Ratio (%)</td><td>4.1</td><td>-38%</td><td>-35%</td><td>NA</td><td>NA</td><td>NA</td></tr><tr><td>Organ to Brain Weight Ratio (%)</td><td>0.08</td><td>-46%</td><td>-44%</td><td>NA</td><td>NA</td><td>NA</td></tr></table>								Organ Weights		Male			Female			Dose (µg/kg)		0	35	45	0	35	45	Organ/ Tissue	Test	mean	%Δ	%Δ	mean	%Δ	%Δ	GLAND, PITUITARY	Weight (g)	0.06	-27%	-26%	0.05	-23%	-43%	Organ to Body Weight Ratio (%)	0.00	-21.5%	-16%	0.00	-23%	-49%	Organ to Brain Weight Ratio (%)	0.09	-31.5%	-28%	0.07	-18%	-48%	THYMUS	Weight (g)	3.11	-21.5%	-42%	2.04	-14%	-10%	Organ to Body Weight Ratio (%)	0.09	-18%	-35%	0.07	-11%	-14%	Organ to Brain Weight Ratio (%)	4.54	-29%	-43%	3.17	-7%	-14%	TESTIS	Weight (g)	2.8	-43%	-43%	NA	NA	NA	Organ to Body Weight Ratio (%)	4.1	-38%	-35%	NA	NA	NA	Organ to Brain Weight Ratio (%)	0.08	-46%	-44%	NA	NA	NA																										
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Histopathology Adequate battery: Yes	<table><tr><td colspan="3">Terminal Microscopic Findings</td><td colspan="3">Male</td><td colspan="3">Female</td></tr><tr><td colspan="3">Dose (µg/kg)</td><td>0</td><td>35</td><td>45</td><td>0</td><td>35</td><td>45</td><td>45 (US)</td></tr><tr><td>Organ/ Tissue</td><td>Finding</td><td>Sev</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="9">BRAIN, CHOROID PLEXUS</td><td></td><td>#Ex</td><td>3</td><td>3</td><td>3</td><td>3</td><td>3</td><td>2</td><td>1</td></tr><tr><td>adhesion</td><td>MIN</td><td></td><td>1</td><td>1</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="3">degeneration/ necrosis</td><td>MIN</td><td></td><td></td><td>1</td><td></td><td>2</td><td></td><td>1</td></tr><tr><td>MILD</td><td></td><td>1</td><td></td><td></td><td></td><td>2</td><td></td></tr><tr><td>MOD</td><td></td><td>1</td><td>2</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="2">fibrosis</td><td>MIN</td><td></td><td>2</td><td>1</td><td></td><td></td><td>1</td><td></td></tr><tr><td>MILD</td><td></td><td></td><td>1</td><td></td><td></td><td></td><td></td></tr><tr><td rowspan="2">infiltration/ inflammation mononuclear cell</td><td>MIN</td><td></td><td>2</td><td>2</td><td></td><td>2</td><td>1</td><td></td></tr><tr><td>MILD</td><td></td><td></td><td></td><td></td><td></td><td>1</td><td></td></tr><tr><td></td><td>MOD</td><td></td><td></td><td></td><td></td><td></td><td></td><td>1</td></tr></table>								Terminal Microscopic Findings			Male			Female			Dose (µg/kg)			0	35	45	0	35	45	45 (US)	Organ/ Tissue	Finding	Sev								BRAIN, CHOROID PLEXUS		#Ex	3	3	3	3	3	2	1	adhesion	MIN		1	1					degeneration/ necrosis	MIN			1		2		1	MILD		1				2		MOD		1	2					fibrosis	MIN		2	1			1		MILD			1					infiltration/ inflammation mononuclear cell	MIN		2	2		2	1		MILD						1			MOD							1
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	Terminal Microscopic Findings			Male			Female		
	Dose (µg/kg)			0	35	45	0	35	45 (US)
	Organ/ Tissue	Finding	Sev						
BRAIN, CHOROID PLEXUS	pigment	#Ex	3	3	3	3	3	2	1
		MIN		1	1			1	
		MILD		1					
BONE MARROW, STERNUM	myeloid/ erythroid ratio, increased nodule, lymphoid	#Ex	3	3	3	3	3	2	1
		MILD							1
		MIN		1			1	1	
EYE	infiltration, mononuclear cell	#Ex	3	3	3	3	3	2	1
		MIN					2	1	
		MILD							
GLAND, ADRENAL	pigment, unilateral vacuolation, increased	#Ex	3	3	3	3	3	2	1
		MIN		1	1				
		MILD							1
SITE, INJECTION	hemorrhage necrosis, perivascular	#Ex	3	3	3	3	3	2	1
		MIN		2	1		2		1
		MILD			1				
KIDNEY	degeneration/ necrosis, tubular	#Ex	3	3	3	3	3	2	1
		MIN							1
		MILD							
LARGE INTESTINE, RECTUM	inflammation, subacute/ chronic	#Ex	3	3	3	3	3	2	1
		MIN						1	
		MILD							
LUNG	edema inflammation, perivascular/ vascular	#Ex	3	3	3	3	3	2	1
		MIN	1					1	
		MILD		1					
MUSCLE, RECTUS FEMORIS	fatty infiltration	#Ex	3	3	3	3	3	2	1
		MIN			1				
		MILD							
OVARY	immature mature mineralization	#Ex	0	0	0	3	3	2	1
		Total						1	1
		Total				3	3	1	
		MIN						2	1
		MILD					1		

Parameters	Major findings								
	Terminal Microscopic Findings			Male			Female		
	Dose (µg/kg)			0	35	45	0	35	45 (US)
	Organ/ Tissue	Finding	Sev						
	SMALL INTESTINE, DUODENUM		#Ex	3	3	3	3	3	2
		infiltration, mononuclear cell	MIN		2	2	2	1	1
			MILD			1			
	SPLEEN		#Ex	3	3	3	3	3	2
		pigment	MIN						1
	THYMUS		#Ex	3	3	3	3	3	2
		depletion, lymphoid, generalized	MIN			1		1	
			MILD			1		1	1
			MOD						1
	TRACHEA		#Ex	3	3	3	3	3	2
		infiltration, mononuclear cell	MIN	1	1	3		2	

US=unscheduled; Sev=Severity; #Ex=number of animals examined/group; MIN=minimal; MOD=moderate;

- Mild axonal/myelin degeneration of the sciatic nerve was observed in one LD male and minimal focal fibrosis and vacuolation of the liver was observed each in different LD males. They are noted because due to loss of exposure and the fact that some test article-related findings may not displaying a dose-response.

LD: low dose; HD: high dose; minus sign: indicates reduction in parameters compared to control.

7 Genetic Toxicology

No genetic toxicology studies were submitted.

8 Carcinogenicity

No carcinogenicity studies were submitted.

9 Reproductive and Developmental Toxicology

No reproductive and development toxicology studies were conducted for tagraxofusp. Based on the cytotoxic mechanism of action of tagraxofusp, it is expected to cause maternal and embryo-fetal toxicity when administered to a pregnant woman.

Since tagraxofusp is a biologic, the only relevant animal model is the nonhuman primate, it will be administered to a patient population with severe and life-threatening disease (BPDCN), and neutralizing ADA may limit identification of doses to provide relevant animal:human safety margins in reproductive and developmental toxicology studies, a literature assessment was provided by the Applicant, (b) (4)

In the literature assessment, the Applicant noted that specific spatial and temporal expression of IL-3 and CD123 in the embryo and fetus is unknown.

Mice lacking the βc subunit of IL-3R showed lung abnormalities, including lymphocytic infiltration and some areas of alveolar proteinosis, as well as a reduced number of eosinophils. $\beta c^{-/-}$ IL-3 $^{-/-}$ double knockout mice have a similar phenotype.²⁹ IL-3-deficient mice are also phenotypically normal with compromised hypersensitivity reactions, increased numbers of tissue mast cells, and enhanced basophil production.^{30,31} Assessment of 8 patients with pulmonary alveolar proteinosis demonstrated a βc deficiency at the genetic level in 4 of 8 patients and no apparent hematopoietic abnormalities, which is consistent with findings in knockout mice.³² Another study also suggests an embryotoxic effect of exogenous recombinant IL-3 in rodents.³³ In addition, it has been shown that drugs targeting the IL-3R may affect the development of fetal hematopoietic stem cells, because blocking IL-3 with an antibody in an explant culture before transplantation in mice or transplanting IL-3 $^{-/-}$ cells resulted in hematopoietic stem cells that were functionally normal but significantly decreased in number.³⁴ Together, these data suggest possible embryotoxic effects on fetal hematopoiesis and embryo-fetal development through exposure to exogenous IL-3 or blockade of IL-3 signaling.

Labeling will be overall consistent with LUMOXITI and ONTAK.

10 Special Toxicology Studies

None were submitted.

11 Integrated Summary and Safety Evaluation

Tagraxofusp is a CD123-directed cytotoxin indicated for the treatment of patients with BPDCN. Nonclinical pharmacology, pharmacokinetic, and toxicology studies have been submitted to support the approval of tagraxofusp for this indication.

Pharmacology

Tagraxofusp, also known by laboratory codes SL-401, DT388IL3, and Molecule 129, is a recombinant fusion protein of the DT Q388R variant with the DT-receptor binding domain replaced by IL-3. SL-401 was shown to bind human IL-3RA, using surface plasmon resonance (SPR), with a K_d of 470 nM. SL-401 and human IL-3 binding to IL-3RA were comparable (hIL-3 K_d =670 nM). SL-401 did not bind to mouse IL-3RA, which based on a BLAST search had 65% DNA sequence homology and 49% identity to human IL-3RA. It is inferred that tagraxofusp does not bind to dog, rat, and rabbit CD123 since these species DNA sequence homology and identity are lower than in the mouse with no measurable binding. Cynomolgus monkey IL-3 and CD123 have the

²⁹ Nishinakamura, R., A. Miyajima, P. J. Mee, V. L. Tybulewicz and R. Murray (1996). "Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions." *Blood* 88(7): 2458-64.

³⁰ Lantz, C. S., J. Boesiger, C. H. Song, N. Mach, T. Kobayashi, R. C. Mulligan, Y. Nawa, G. Dranoff and S. J. Galli (1998). "Role for interleukin-3 in mast-cell and basophil development and in immunity to parasites." *Nature* 392(6671): 90-3.

³¹ Mach, N., C. S. Lantz, S. J. Galli, G. Reznikoff, M. Mihm, C. Small, R. Granstein, S. Beissert, M. Sadelain, R. C. Mulligan and G. Dranoff (1998). "Involvement of interleukin 3 in delayed-type hypersensitivity." *Blood* 91(3): 778-83.

³² Dirksen, U., R. Nishinakamura, P. Groneck, U. Hattenhorst, L. Noe, R. Murray and S. Burdach (1997). "Human pulmonary alveolar proteinosis associated with a defect in GM-CSF/IL-3/IL-5 receptor common beta chain expression." *J Clin Invest* 100(9): 2211-7.

³³ Liber, L. (1997). "The teratogenic effect of interleukin-3 recombinant." *Academic Journal of Guangzhou Medical University* 4: 6-10.

³⁴ Robin, C. and C. Durand (2010). "The roles of BMP and IL-3 signaling pathways in the control of hematopoietic stem cells in the mouse embryo." *Int J Dev Biol* 54(6-7): 1189-200.

highest homology and identity to humans of the species evaluated (>80%). It is known that subcutaneous injection of recombinant human IL-3 induces proliferation of immature hematopoietic cells from the myeloid, megakaryocytic, and erythroid series at the injection site in cynomolgus monkeys presumed to be related to exaggerated pharmacology. Thus, evidence supports the cynomolgus monkey as a pharmacologically relevant species for toxicology studies. (b) (4)

(b) (4) it is likely that tagraxofusp can bind to any tissues with cells that express CD123 including hematopoietic progenitors, normal myeloid derived cell types, some endothelial cells, and osteoblasts. Given the high expression in malignant cells, tumor cells may function as a target sink to reduce undesirable activity of tagraxofusp in normal tissues.

SL-401, following binding to CD123 and internalization, increases ADP-ribosylation of EF-2 in THP-1 cells (AML cell line), but not in SL-401-resistant cell lines (generated by treating cells with SL-401 at a 95% lethal concentration). (b) (4)

(b) (4) Following 18 hours of treatment with SL-401, the IC₅₀ values for expression of apoptotic markers were in the femtomolar range in peripheral blood or bone marrow aspirates with from 12 BPDCN patients and BPDCN cells were shown to be more sensitive to SL-401-mediated cytotoxicity than AML or ALL cells. The correlation between SL-401 sensitivity and CD123 receptor density was mixed (in one study there was a positive correlation, in another study there was no correlation). In BPDCN mouse xenograft models, SL-401 administered IP for 5 consecutive days at 100 µg/kg/day increased median overall survival compared to controls. In an AML mouse xenograft model, SL-401 significantly increased overall survival of mice compared with those treated with the vehicle control. Overall, the nonclinical studies support the anti-tumor mechanism of action of tagraxofusp for the proposed indication.

Pharmacokinetics/ADME/Toxicokinetics

An absorption study was conducted in female cynomolgus monkeys comparing the systemic exposure after intravenous administration of 2 formulations of SL-401, frozen solution versus a (b) (4) formulation. The systemic exposures achieved in monkeys after administering either formulation were comparable, therefore the toxicology results from earlier nonclinical assessments completed using the SL-401 frozen solution formulation (e.g., used in the GLP-compliant 3-month study) can be bridged to the (b) (4) tagraxofusp formulation. In the GLP-compliant 1-month study (5-day cynomolgus monkey toxicity study with a 3-week recovery period), ADA was detected in cynomolgus monkeys as early as the first assessment day (Day 14). On Day 5, AUC exposure (445 and 1088 h.ng/mL for the 30 and 60 µg/kg/day doses, respectively) was slightly higher than on Day 1. The C_{max} (566 and 1110 ng/mL for the 30 and 60

µg/kg/day doses, respectively) on Day 5 were generally comparable to Day 1. The SL-401 fusion protein was eliminated with a half-life of approximately ½ hour. In the GLP-complaint 3-month cynomolgus monkey toxicity study (5 consecutive SL-401 dosing days separated by 3-week off dosing periods for 3 cycles), the AUC and C_{max} for the lowest dose with toxicity (35 µg/kg/day AUC = 326 h.ng/mL and C_{max} = 542.5 ng/mL) on Day 1 was lower by half by Day 27 to, on Day 54, 3.6 h.ng/mL (AUC) and 8.8 ng/mL (C_{max}). ADA was detected in cynomolgus monkeys as early as the first assessment day (Day 14) and the decrease in SL-401 plasma concentration is likely attributable to the effects of ADAs. The half-life was an hour on Day 1. The average percentages of the nominal concentration of 50 µg/mL SL-401 in the GLP-complaint 3-month study were 89%, 86%, and 79% for Days 1, 27, and 54, respectively, and is likely a result of the instability and potential precipitation of the samples after a freeze/thaw cycle. However, the lower than nominal concentrations did not appear to impact exposure in the 3-month study on Day 1 (AUC and C_{max} are comparable between the 30 µg/kg/day dose in 1-month study and 35 µg/kg/day dose in 3-month study on Day 1). It is unclear if the lower than nominal concentrations impacted exposure on Days 27 and 54 due to the development of ADA.

General Toxicology

In the GLP-compliant 1-month, 5-day toxicity study with a 3-week recovery period, cynomolgus monkeys were administered SL-401 intravenously at doses of 0 (5% dextrose), 30, and 60 µg/kg/day for 5 consecutive days (n=3/sex/group, with 2/sex/group for recovery in control and high dose monkeys). On Day 6, one female at 60 µg/kg/day was euthanized in extremis, due to severe necrosis of renal cortical tubules (kidneys) with clinical pathology correlations indicative of decreased glomerular filtration rate. Effects on body weight (irreversible), suppression of adaptive response to decreased red cell mass, prolonged coagulation times, and toxicity to the kidney (some findings irreversible) and thymus (irreversible) were noted at doses ≥ 30 µg/kg/day. Minimal to moderate degeneration/necrosis of the choroid plexus of the brain was observed at doses ≥ 30 µg/kg/day that increased to severe during the recovery period at 60 µg/kg/day. The progressive nature of the choroid plexus degeneration/necrosis is unknown at 30 µg/kg/day since recovery was not assessed at this dose level. At 60 mg/kg/day, liver toxicity and mild lung (irreversible) microscopic findings were observed as well as findings consistent with inflammatory response (and further supported by the presence of Döhle bodies noted microscopically on blood smears among individual animals) (see Table 5 for more details). Perivascular hemorrhage of the injection site and erosion/ulcers were noted at higher incidence and severity in treated monkeys compared to controls.

In a GLP-compliant 3-month toxicity study, cynomolgus monkeys were administered SL-401 intravenously at doses of 0 (0.9% NaCl), 35, and 45 µg/kg/day for 5 consecutive days (n=3/sex/group) separated by 3-week periods without dosing. On Day 9, one female at 45 µg/kg/day was euthanized in extremis, due to clinical signs of moderate decreased activity, thin, moderate dehydration, increased body temperature, bruising of the abdomen and hind limbs, and swelling of the right inguinal area hind limbs and dosing site. The overall cause for morbidity for this animal could not be determined. At

doses ≥ 35 $\mu\text{g/kg/day}$, clinical signs were observed (more so than in the 1-month study). However, organ toxicities were overall consistent (see Table 5 for more details). As noted in the toxicokinetics summary above, neutralizing ADA was observed in the 3-month study that impacted exposure, with low exposure to SL-401 detected at the end of the study. While SL-401 is pharmacologically active at low serum levels, the impact this low exposure would have on the 3-month study results would, in theory, be less toxicity.

See Executive Summary for the summary of genetic toxicology, carcinogenicity, and reproductive and developmental toxicology studies.

12 Appendix/Attachments

Table 5 Summary of Significant Findings in Repeated Dose Studies with Tagraxofusp (SL-401)

Species (Study No.)	Route & Duration	Dose $\mu\text{g/kg}$ (N/dose)	Critical Doses	$\mu\text{g/kg}$	Significant findings
Cynomolgus Monkey (2231-001) Non-GLP	IV; 5 days (PBS, pH 7.4)	40, 60, 80 (N=2/sex/group)	LD MTD	80 60	On Day 5, a male at 80 $\mu\text{g/kg/day}$ was euthanized in extremis, due to test article-related clinical signs (hunched posture, severely decreased activity, trouble gripping, decreased motor skills, limbs cold to touch, and no pain response in feet). All doses, both sexes: decreased activity, hunched posture, and hair sparse. BW $\downarrow$ (all doses, both sexes; MD/HD females); inappetence (HD both sexes) All doses: GLOB $\uparrow$, ALB $\downarrow$
Cynomolgus Monkey (2231-004) Non-GLP	IV; 5 days (5% dextrose vehicle)	30, 60 (N=1/sex/group)			Dose-dependent BW $\downarrow$; inflammatory response; RETIC $\downarrow$; creatinine $\uparrow$; and hepatic effects. The actual dose received by the animals could not be confirmed using the qualified low-concentration HPLC analytical method because of a previously unknown stability issue with the test article after freezing it in vehicle as diluted material.
Cynomolgus Monkey (2231-002) GLP (1-month)	IV; 5 days and 3-week recovery (5% dextrose vehicle)	0, 30, 60 (N=3/sex/group, terminal; N=2/sex/group, recovery for controls and HD)	LD	60	On Day 6, one female at 60 $\mu\text{g/kg/day}$ was euthanized in extremis, due to severe necrosis of renal cortical tubules (kidneys), with clinical signs of blood collection site redness, inappetence, and cold to touch and clinical pathology findings of $\uparrow$ BUN and creatinine. <u>Both doses (≥ 30 $\mu\text{g/kg/day}$):</u> BW $\downarrow$; inappetence (irreversible at HD); RETIC $\downarrow$ (lack of adaptive response to decreased red cell mass); APTT $\uparrow$, PT $\uparrow$, and FIB $\uparrow$; and AST(>100% $\uparrow$); creatinine $\uparrow$; GLOB $\uparrow$ (irreversible at HD), ALB $\downarrow$, A:G $\downarrow$, Cl $\downarrow$, Ca $\downarrow$, PHOS $\downarrow$; thymus wt $\downarrow$ (irreversible at HD) with correlated microscopic findings of lymphoid depletion; adrenal wt $\uparrow$, kidney, liver, spleen wt $\uparrow$ (males only); microscopic findings of renal tubular degeneration and necrosis, and increased hyaline droplets with proteinuria

Species (Study No.)	Route & Duration	Dose µg/kg (N/dose)	Critical Doses	µg /kg	Significant findings
					and decreased urine volume; degeneration/ necrosis of choroid plexus (progressive at HD); reversible degeneration/ necrosis of the rectus femoris muscle <u>High dose (≥ 60 µg/kg/day):</u> NEU↑ (inflammatory response), followed by ~80% decrease that persisted through recovery; BASO↑; ALT (>100%↑); BUN↑ (irreversible in HD females); TP↓; TRIG↑; lung wt↑ (recovery period only, no microscopic correlates but mild lung edema was observed microscopically in the female with early mortality); hepatocellular vacuolation and necrosis correlated with ALT and AST↑; spleen vacuolation; mineralization of the ovaries in 4/5 females.
Cynomolgus Monkey (2231-007) GLP (3-month)	IV; 3 cycles of 5 consecutive daily doses, separated by 3-week periods without dosing (0.9% NaCl vehicle)	0, 35, 45 (N=3/sex/group)	LD	45	On Day 9, one female at 45 µg/kg/day was euthanized in extremis, due to clinical signs of moderate decreased activity, thin, moderate dehydration, increased body temperature, bruising of the abdomen and hind limbs, and swelling of the right inguinal area hind limbs and dosing site. The overall cause for morbidity for this animal could not be determined. Clinical signs including hunched posture, tremors, inappetence, eyelids closed, decreased activity, dry/scabbed skin, and discoloration at the injection site were seen at both doses with hypersensitivity with suppressed erythropoiesis at the HD. <u>Toxicities at both doses:</u> BW↓; inflammatory response (FIB↑; GLOB↑; ALB↓); prolonged coagulation; decreased platelets (HD only); red cell mass↓ and suppressed adaptive response (RETIC↓); hepatic effects (ALT and AST (>100%↑)); renal effects (proteinuria), Ca↓ (HD only); PHOS↓; microscopic changes primarily in the brain choroid plexus, thymus, kidney, injection site, and liver as observed in the 1-month study.

The same test article lot 184-TT1002-P001-13 was used for studies 2231-002 and -004; test article lot 184-TT1002-P002-13 was used for studies 2231-001 and -007.

LD=lethal dose; MTD = maximum tolerated dose; MD = mid dose; HD = high dose; IV = intravenous; GLOB = globulin; ALB = albumin; BW = body weight; RETIC = reticulocytes ; BUN = urea nitrogen; APTT = Activated Partial Thromboplastin Time; PT = prothrombin time; FIB = fibrinogen; AST = aspartate aminotransferase; ALT = alanine aminotransferase; A:G = albumin to globulin ratio; Cl = chloride; Ca = calcium; PHOS = phosphorus; wt = weight; NEU = neutrophils; BASO = basophils; TP = total protein; TRIG = triglycerides

List of Studies Not Fully Reviewed (from Section 3.2):**Pharmacokinetics**

Study #	Title	Module
Analytical Methods and Validation Reports		
11-144	An Immunoassay (ECL-IA) for the Quantitative Determination of SL-401 in Cynomolgus Monkey K2EDTA Plasma (plus Validation Report Addendum 1)	4.2.2.1
12-129	Validation of Bridging Immunoassay (ECL-IA) for the Detection of Anti-SL-401 Antibodies in Cynomolgus Monkey Serum	4.2.2.1
GCL-279	ECL Method for the Quantitative Determination of SL-401 in Cynomolgus Monkey EDTA Plasma	4.2.2.1.
GCL-320	Bridging ECL for the Detection of Anti-SL-401 Antibodies in Cynomolgus Monkey Serum	4.2.2.1.

Repeat Dose Toxicology

Study#	Title	Module
2231-001	SL-401: An Intravenous Dose Range Finding Study in Cynomolgus Monkeys	4.2.3.2
2231-004	SL-401: An Intravenous Pilot Dose Confirmation Study in Cynomolgus Monkeys	4.2.3.2

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/s/

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I concur