

TABLE OF CONTENTS

2.3	INTRODUCTION	6
2.3.S	DRUG SUBSTANCE (SARGRAMOSTIM, PARTNER THERAPEUTICS)	7
2.3.S.1	GENERAL INFORMATION	7
2.3.S.1.1	Nomenclature	7
2.3.S.1.2	Structure	7
2.3.S.1.3	General Properties	9
2.3.S.2	MANUFACTURE	10
2.3.S.2.1	Manufacturers	10
2.3.S.2.2	Description of Manufacturing Process and Process Controls	11
2.3.S.2.2.1	Step 1 Cell Expansion	15
2.3.S.2.2.2	Step 2 Fermentation	15
2.3.S.2.2.3	Step 3 Harvest and Recovery	16
2.3.S.2.2.4	Step 4 Purification	16
2.3.S.2.2.5	Step 5 Final Buffer Exchange and Filtration	16
2.3.S.2.3	Control of Materials	17
2.3.S.2.4	Controls of Critical Steps and Intermediates	19
2.3.S.2.5	Process Validation and/or Evaluation	21
2.3.S.2.6	Manufacturing Process Development	22
2.3.S.3	CHARACTERIZATION	25
2.3.S.3.1	Elucidation of Structure and Other Characteristics	25
2.3.S.3.2	Impurities	29
2.3.S.4	CONTROL OF DRUG SUBSTANCE	30
2.3.S.4.1	Specifications	30
2.3.S.4.1.1	Drug Substance Release Specifications	30
2.3.S.4.1.2	Drug Substance Shelf-life Specifications	31
2.3.S.4.2	Analytical Procedures	32
2.3.S.4.3	Validation of Analytical Procedures	33
2.3.S.4.4	Batch Analyses	34
2.3.S.4.5	Justification of Specifications	41
2.3.S.5	REFERENCE STANDARDS OR MATERIALS	42
2.3.S.6	CONTAINER CLOSURE SYSTEM	44
2.3.S.7	STABILITY	45

2.3.S.7.1	Stability Summary and Conclusions.....	45
2.3.S.7.2	Postapproval Stability Protocol and Stability Commitments	45
2.3.S.7.3	Stability Data	45
2.3.P	DRUG PRODUCT (LEUKINE FOR INJECTION)	49
2.3.P.1	DESCRIPTION AND COMPOSITION OF THE DRUG PRODUCT	49
2.3.P.1.1	Composition (Leukine for Injection, 250 µg/vial).....	49
2.3.P.2	PHARMACEUTICAL DEVELOPMENT	50
2.3.P.2.1	Drug Substance	50
2.3.P.2.2	Excipients	50
2.3.P.2.3	Formulation Development.....	50
2.3.P.2.4	Process Development.....	51
2.3.P.2.5	Container Closure System	51
2.3.P.2.6	Compatibility	51
2.3.P.3	MANUFACTURE	52
2.3.P.3.1	Manufacturers	52
2.3.P.3.2	Batch Formula	53
2.3.P.3.3	Description of Manufacturing Process and Process Controls.....	54
2.3.P.3.3.1	Compounding	56
2.3.P.3.3.2	Bioburden Reduction Filtration	56
2.3.P.3.3.3	Sterile Filtration and Filling.....	56
2.3.P.3.3.4	Lyophilization.....	57
2.3.P.3.3.5	Visual Inspection	58
2.3.P.3.4	Controls of Critical Steps and Intermediates	58
2.3.P.3.5	Process Validation and/or Evaluation.....	59
2.3.P.4	CONTROL OF EXCIPIENTS	60
2.3.P.5	CONTROL OF DRUG PRODUCT	61
2.3.P.5.1	Specifications.....	61
2.3.P.5.1.1	Leukine for Injection Release Specifications	61
2.3.P.5.1.2	Leukine for Injection Shelf-life Specifications.....	62
2.3.P.5.2	Analytical Procedures.....	64
2.3.P.5.3	Validation of Analytical Procedures.....	64
2.3.P.5.4	Batch Analysis	65
2.3.P.5.5	Characterization of Impurities	67

2.3.P.5.6	Justification of Specifications.....	67
2.3.P.6	REFERENCE STANDARDS OR MATERIALS	68
2.3.P.7	CONTAINER CLOSURE SYSTEM	69
2.3.P.8	STABILITY.....	71
2.3.P.8.1	Stability Summary and Conclusions.....	71
2.3.P.8.2	Postapproval Stability Protocol and Stability Commitment.....	71
2.3.P.8.3	Stability Data	71
2.3.A.1	FACILITIES AND EQUIPMENT	77
2.3.A.1.1	Drug Substance Manufacturer	77
2.3.A.1.2	Drug Product Manufacturer	77
2.3.A.2	ADVENTITIOUS AGENTS SAFETY EVALUATION	80
2.3.A.3	EXCIPENTS.....	80

LIST OF TABLES

Table 1:	Summary of product information:	6
Table 2:	Sargramostim Maximum Absorption and Extinction Coefficient.....	9
Table 3:	pI-Values for Sargramostim Isoforms	9
Table 4:	Specific Activity and Receptor Affinity of Sargramostim Glycoforms	9
Table 5:	Manufacturing Sites of Sargramostim Bulk Drug Substance.....	10
Table 6:	Sargramostim Manufacturing Process, Flow Diagram.....	14
Table 7:	Raw Materials Used in Working Cell Bank Production.....	17
Table 8:	Raw Materials Used in Cell Expansion and Fermentation.....	17
Table 9:	Raw Materials Used in Harvest & Recovery, Purification, Final Buffer Exchange & Filtration	18
Table 10:	Animal-Derived Materials Used in the Manufacture of Sargramostim.....	18
Table 11:	Critical Process Parameters and In-Process Controls for the Manufacture of Sargramostim.....	19
Table 12:	Summary of Elucidation of Structure Results for Sargramostim	26
Table 13:	Process-Related Impurities in Drug Substance Process Validation Batches.....	29
Table 14:	Release Specifications for Sargramostim Drug Substance.....	30
Table 15:	Stability Specifications for Sargramostim Drug Substance.....	31
Table 16:	List of Analytical Procedures	32
Table 17:	Analytical Procedure Method Validation Reports for Drug Substance.....	33

Table 18:	Description of Drug Substance Batches Manufactured at Partner Therapeutics Northpointe, Lynnwood WA	34
Table 19:	Reference Standard RS-121-4 Qualification Results	42
Table 20:	Stability Testing Schedule for the Drug Substance	45
Table 21:	Summary of Sargramostim Drug Substance Batches on Stability	45
Table 22:	Sargramostim Drug Substance, Batch 12840/B23076, Storage Condition -70 °C, Upright.....	46
Table 23:	Sargramostim Drug Substance, Batch 12840/B24483, Storage Condition -70 °C, Upright.....	47
Table 24:	Sargramostim Drug Substance, Batch 12840/B25981, Storage Condition -70 °C, Upright.....	48
Table 25:	Unit Formula of Leukine for Injection, 250 µg/vial	49
Table 26:	Testing Facilities and Tests Performed.....	52
Table 27:	Batch Formula of the Drug Product Leukine for Injection, 250 µg/vial	53
Table 28:	Leukine Lyophilizer Cycle	57
Table 29:	Critical Process Parameters	58
Table 30:	In-Process Specifications for Manufacture of Leukine for Injection	59
Table 31:	Endotoxin Specifications of Excipients in Leukine for Injection.....	60
Table 32:	Release Specifications, Leukine for Injection, 250 µg/vial	61
Table 33:	Shelf-life Specifications, Leukine for Injection, 250 µg/vial	62
Table 34:	Analytical Procedures for the Control of the Drug Product	64
Table 35:	Analytical Procedure Method Validation Reports for the Drug Product	64
Table 36:	Pharmacopoeia Method Suitability Reports for the Drug Product.....	65
Table 37:	Batch Analysis Results for Leukine for Injection, 250 µg/vial, Batches AR3496 and B26910.....	65
Table 38:	Description of Primary Packaging Components.....	69
Table 39:	Glass Vials (Dimensions: 22 mm x 40 mm) Specifications	69
Table 40:	Chlorobutyl Stoppers (Formulation: 4432/50) Specifications.....	70
Table 41:	Aluminum Seals (Size: 20 mm) Specifications	70
Table 42:	Stability Protocol (2 – 8 °C)	71
Table 43:	Summary of Stability Data for Leukine for Injection, 250 µg/Vial	72
Table 44:	Long-Term (2 – 8 °C) Stability Data for Batch AR3496	73
Table 45:	Accelerated (25 °C) Stability Data for Batch AR3496.....	74
Table 46:	Long-Term (2 – 8 °C) Stability Data for Batch B26910	75

Table 47: Accelerated (25 °C) Stability Data for Batch B26910.....	76
Table 48: Manufacturing Area 3 and Filling Line 3 Major Equipment.....	78
Table 49: Equipment Utilized in Leukine for Injection Drug Product Manufacturing	79

LIST OF FIGURES

Figure 1: Amino acid sequence.....	7
Figure 2: Secondary Structure.....	8
Figure 3: Tertiary Structure.....	8
Figure 4: Fermentation Batching Schematic.....	12
Figure 5: Purification Batching Schematic	13
Figure 6: Certificate of Analysis – Drug Substance Batch B25981.....	35
Figure 7: Certificate of Analysis – Drug Substance Batch B26063.....	38
Figure 8: Schematic of the Drug Product Manufacturing Process.....	55

2.3 INTRODUCTION

A summary of the drug product information is provided in [Table 1](#). Leukine for Injection is an FDA approved (1991) drug product in the United States.

Table 1: Summary of product information:

Proprietary (Brand) Name of Drug Product	Leukine (sargramostim) for injection
Non-proprietary or Common Name of Drug Product	Leukine
Non-proprietary or Common Name of Drug Substance (Medicinal Ingredient)	Sargramostim
Company (Manufacturer/Sponsor) Name	Partner Therapeutics, Inc.
Dosage Form(s)	Lyophilized powder for reconstitution for administration
Strength(s)	250 µg/ml
Route of Administration	Subcutaneous injection or intravenous infusion

2.3.S DRUG SUBSTANCE (SARGRAMOSTIM, PARTNER THERAPEUTICS)

2.3.S.1 GENERAL INFORMATION

2.3.S.1.1 Nomenclature

International Non-Proprietary Name (INN): Sargramostim

Chemical Abstracts Service (CAS) Number: 123774-72-1

Sargramostim (recombinant human Granulocyte Macrophage-Colony Stimulating Factor; rhu GM-CSF) primary structure (amino acid sequence) of is provided in Figure 1. rhu GM-CSF is a 127 amino acid glycoprotein that differs from native human GM-CSF by substitution of leucine (Leu) for arginine (Arg) at position 23. Two underlined consensus sequences represent possible N-glycosylation sites; the asparagine (Asn) at position 27 is N-glycosylated, whereas serine (Ser) at position 9 is O-glycosylated. Disulfide bridges form between Cys(54) – Cys(96) and Cys(88) – Cys(121).

2.3.S.1.2 Structure

The primary amino acid structure of sargramostim is provided in [Figure 1](#).

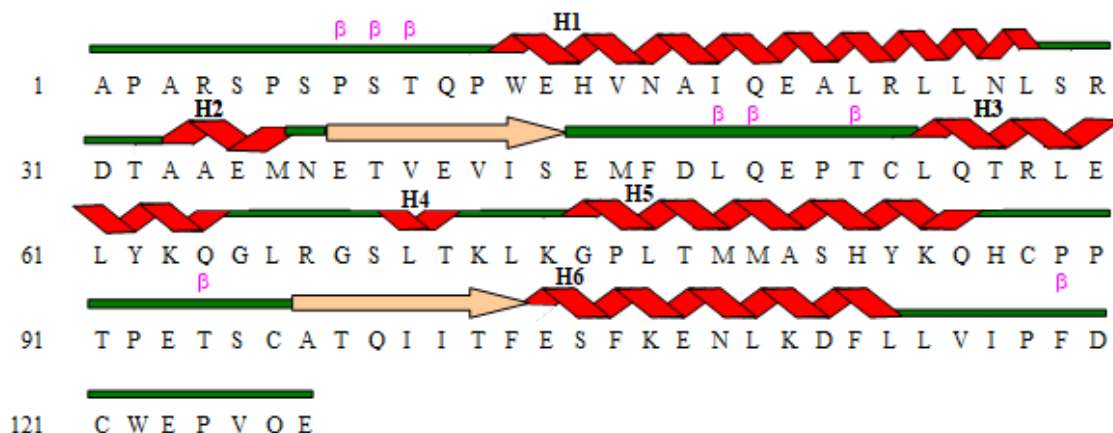
Figure 1: Amino acid sequence

1 Ala-Pro-Ala-Arg-Ser-Pro-Ser-Pro-Ser-Thr-Gln-Pro-Trp-Glu-His-Val-Asn-Ala-Ile-Gln-
21 Glu-Ala-Leu-Arg-Leu-Leu-Asn-Leu-Ser-Arg-Asp-Thr-Ala-Ala-Glu-Met-Asn-Glu-Thr-Val-
41 Glu-Val-Ile-Ser-Glu-Met-Phe-Asp-Leu-Gln-Glu-Pro-Thr-Cys-Leu-Gln-Thr-Arg-Leu-Glu-
61 Leu-Tyr-Lys-Gln-Gly-Leu-Arg-Gly-Ser-Leu-Thr-Lys-Leu-Lys-Gly-Pro-Leu-Thr-Met-Met-
81 Ala-Ser-His-Tyr-Lys-Gln-His-Cys-Pro-Pro-Thr-Pro-Glu-Thr-Ser-Cys-Ala-Thr-Gln-Ile-
101 Ile-Thr-Phe-Glu-Ser-Phe-Lys-Glu-Asn-Leu-Lys-Asp-Phe-Leu-Leu-Val-Ile-Pro-Phe-Asp-
121 Cys-Trp-Glu-Pro-Val-Gln-Glu

Secondary structure

In this model of sargramostim secondary structure (Figure 2), β -sheets are represented by orange arrows, α -helices (H1 through H6) by red spirals, and random coils by green strands.

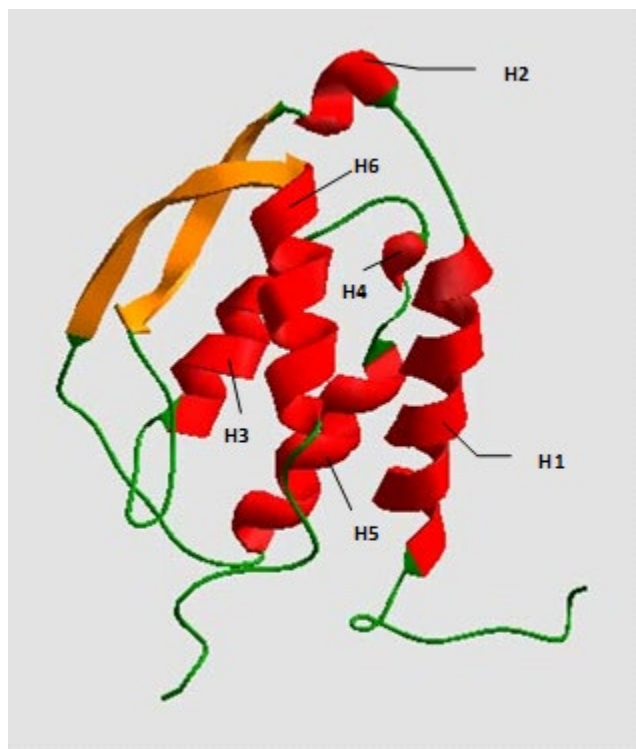
Figure 2: Secondary Structure



Tertiary structure:

In this model of non-glycosylated sargramostim tertiary structure (Figure 3), β -sheets are represented by orange arrows, α -helices (H1 through H6) by red spirals, and random coils by green strands. Two α -helices (labeled H1 and H5) form the receptor binding pocket.

Figure 3: Tertiary Structure



2.3.S.1.3 General Properties

The physicochemical properties of the drug substance sargramostim are:

Appearance: Clear, colorless to pale straw liquid.

pH: Sargramostim in 0.1 M tromethamine (tris(hydroxymethyl)aminomethane) buffer has a pH of 7.2 – 7.6.

Molecular Forms: The 4 molecular forms of sargramostim are non-glycosylated, O-glycosylated, N-glycosylated, and N- plus O-glycosylated.

UV-Spectrum and Specific Absorption: Theoretical extinction coefficient of sargramostim is $\epsilon = 14180$, $E_{0.1\% \text{ } 280 \text{ nm}} = 0.983 \text{ cm}^2/\text{mg}$; the actual extinction coefficient experimentally determined of sargramostim is provided in [Table 2](#).

Table 2: Sargramostim Maximum Absorption and Extinction Coefficient

Parameter	Result
Absorption maximum	280 nm
Specific absorption, $E_{0.1\% \text{ } 280 \text{ nm}}$	$1.10 \text{ L cm}^{-1} \text{ g}^{-1}$ to $1.16 \text{ L cm}^{-1} \text{ g}^{-1}$
Molar absorption coefficient, ϵ	15853 to $16758 \text{ L cm}^{-1} \text{ mol}^{-1}$

pI: The theoretical pI of sargramostim is 4.85. Sargramostim contains at least 5 post-translational isoforms including N- and O-linked glycoforms with pI values ranging from 4.5 – 5.3. pI-values for the major isoforms are provided in [Table 3](#).

Table 3: pI-Values for Sargramostim Isoforms

Protein Isoform	pI Value
Non-glycosylated sargramostim	5.24 ± 0.03
O-glycosylated sargramostim	5.27 ± 0.04
N-glycosylated sargramostim	5.18 ± 0.03
Mono-phosphorylated N-glycosylated sargramostim	4.97 ± 0.05
Di-phosphorylated N-glycosylated sargramostim	4.74 ± 0.05
Tri-phosphorylated N-glycosylated sargramostim	4.60 ± 0.03

Specific Activity: Specific activity of sargramostim is $5.86 \times 10^6 \pm 0.021 \text{ IU/mL}$. Estimated specific activity and relative percent of major drug substance glycoforms are listed in [Table 4](#). Estimated sargramostim activity is calculated from relative glycoform composition, resulting in drug substance biological activity of $5.7 \times 10^6 \text{ IU/mg}$.

Table 4: Specific Activity and Receptor Affinity of Sargramostim Glycoforms

Glycoform	Relative Percent	Specific Activity (IU/mg)
N- and N- plus O-glycosylated	29 %	$6.10 \pm 0.32 \times 10^6$
O-glycosylated	22 %	$6.34 \pm 0.55 \times 10^6$
Non-glycosylated	49 %	$5.17 \pm 0.41 \times 10^6$

2.3.S.2 MANUFACTURE

2.3.S.2.1 Manufacturers

The sites involved in the manufacture of sargramostim bulk drug substance (BDS) and their respective responsibilities are provided in [Table 5](#).

Table 5: Manufacturing Sites of Sargramostim Bulk Drug Substance

Site	Steps
Partner Therapeutics (Northpointe Site) 2625 162nd Street SW Lynnwood, Washington USA 98087-3263 FEI: 3007934434 DUNS: 08-105-9614	Manufacture and primary packaging of BDS Generation of working cell bank (WCB) Storage of master cell bank (MCB), WCB, and BDS In-process, release and stability testing of BDS Testing of raw materials Release and stability testing of cell banks Stability storage
Integrated Commercialization Solutions, Inc. (ICS) 420 International Blvd. Suite 500 Brooks, KY 40109 FEI: 3002478693 DUNS: 832820588	Storage of BDS
Charles River, Biopharmaceutical Services, Inc. 358 Technology Drive Malvern, PA USA 19355-1315 FEI: 1000121235 DUNS: 07-849-5006	Secondary storage of cell banks (MCB and WCB)
Nelson Laboratories 6280 South Redwood Road Salt Lake City, UT USA 84123-6600 FEI: 3000233845 DUNS: 15-166-3234	Testing of raw materials
Eurofins Lancaster Laboratories 2425 New Holland Pike Lancaster, PA USA 17605-5994 FEI: 2513291 DUNS: 06-977-7290	Testing of raw materials Gene sequence testing of cell banks
SGS Life Science Services 616 Heathrow Drive Lincolnshire, IL USA 60069-4205 FEI: 1418028 DUNS: 06-249-1980	Testing of raw materials
Pace Analytical Life Sciences, LLC 1311 Helmo Ave. North Oakdale, MN USA 55128-6023	Testing of raw materials

Site	Steps
FEI: 3001452367 DUNS: 79-790-3197	
Nitto Avecia 10 Vanderbilt Irvine, CA USA 92618-2010 FEI: 3012971227 DUNS: 11-697-5565	Testing of raw materials

FEI = FDA Establishment Identifier; DUNS = Data Universal Numbering System

2.3.S.2.2 Description of Manufacturing Process and Process Controls

The process used to manufacture the drug substance is comprised of ten-unit operations. Working cell bank (WCB) starting material is cultured through two expansion steps before transfer to a 100-L production fermentor, followed by harvest and recovery unit operations. Downstream processing consists of three reverse phase high pressure liquid chromatography columns (RP-HPLC), a low-pressure cation exchange chromatography column (LP-CEX), and filtration to yield sargramostim bulk drug substance.

Excluding WCB starting material, a total of 44 individual unit operations are required to manufacture a single BDS batch. [Figure 4](#) and [Figure 5](#) illustrate fermentation and purification batching schemes, respectively, that are required to manufacture a single batch of sargramostim BDS.

Partner Therapeutics does not engage in reprocessing of sargramostim.

Figure 4: Fermentation Batching Schematic

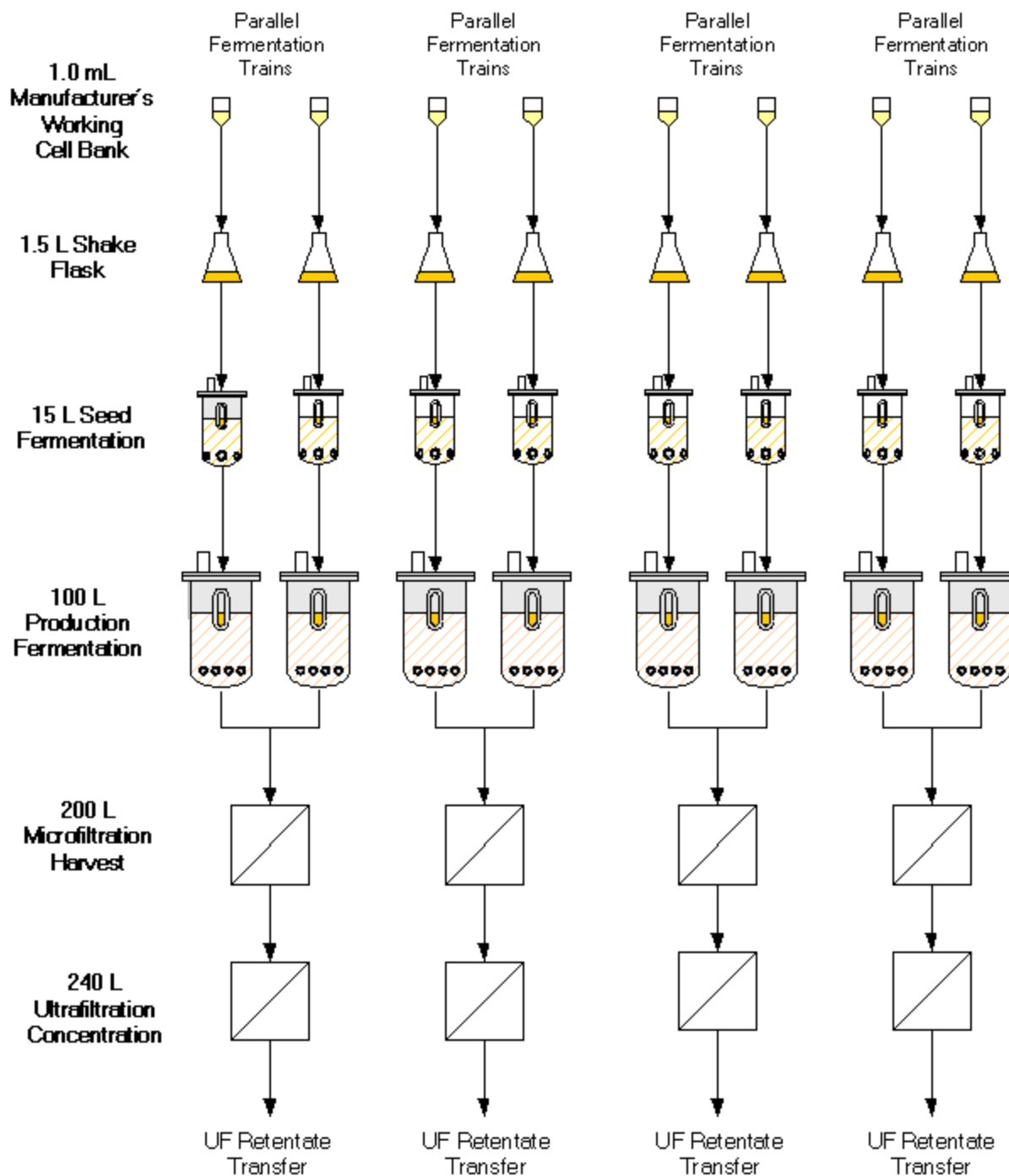
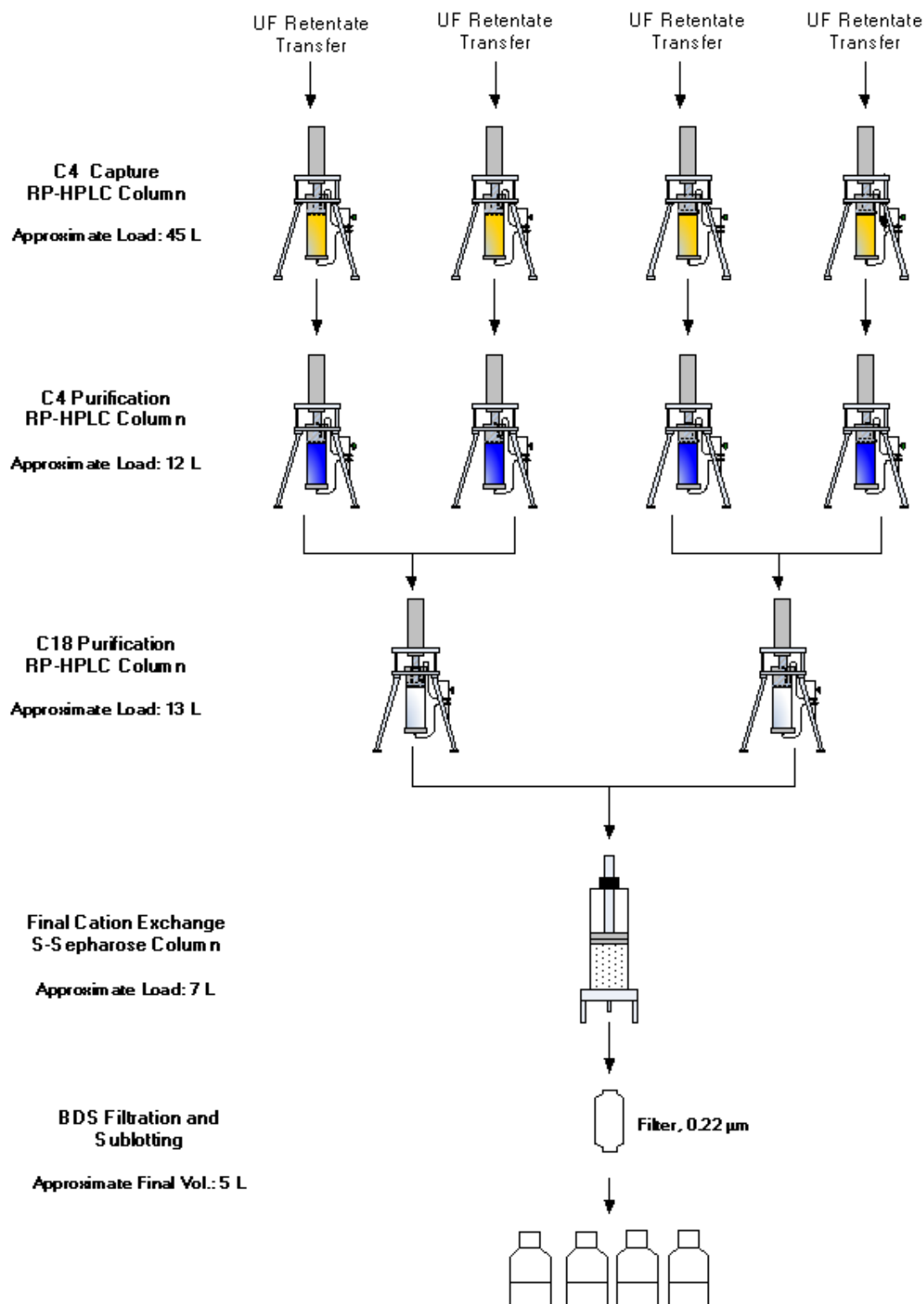


Figure 5: Purification Batching Schematic



A summary of the sargramostim manufacturing process, including critical process parameters (CPPs), process controls (PCs), and in-process controls (IPCs) is provided in [Table 6](#).

Table 6: Sargramostim Manufacturing Process, Flow Diagram

Process Step	CPP	PC	IPC
Step 1: Cell expansion			
Shake flask	None	Agitation Temperature Duration	Optical Density
Seed Fermentation	None	Agitation Aeration Backpressure pH Temperature Duration	Optical Density
Step 2: Fermentation	Temperature pH	Agitation Aeration Backpressure Duration Glucose Feed Rate Ethanol Feed Rate	Optical Density Wet Cell Weight Non-Host Contamination
Step 3: Harvest and Recovery			
Microfiltration	None	Temperature Feed Flow Rate Retentate Pressure Filtrate Pressure Final Concentration/ Diafiltration Volume Final Filtrate Volume	None
Ultrafiltration	None	Temperature Retentate Flow Rate Transmembrane Pressure Concentration Volume Final Retentate Volume	Microbial Content Calculated yield
Step 4: Purification			
C4 Capture Chromatography	Collection Start Time, UV Absorbance, UV Slope Collection End Time, UV Absorbance, UV Slope	Wash Buffer Composition Elution Gradient Buffer Composition Elution Gradient Flow Rate	None

Process Step	CPP	PC	IPC
C4 Purification Chromatography	Collection Start Time, UV Absorbance, UV Slope Collection End Time, UV Absorbance, UV Slope	Wash Buffer Composition Elution Gradient Buffer Composition Elution Gradient Flow Rate	Endotoxin Glycoform Ratio Calculated Step Yield
C18 Purification Chromatography	None	Wash Buffer Composition Elution Gradient Buffer Composition Elution Gradient Flow Rate, Collection Start, Collection End	Glycoform Ratio Calculated Step Yield
Step 5: Final Buffer Exchange and Filtration			
Final Cation Exchange	None	Load and initial wash flow rate or Maximum process flow rate, Collection Start, Collection End	None
Bulk Drug Substance Filtration	None	None	Calculated Step Yield

2.3.S.2.2.1 Step 1 Cell Expansion

Shake Flask

The Shake Flask operation expands 1.0 mL of culture from a WCB vial to volume and density sufficient to inoculate the 15-liter seed fermentor. Under laminar flow conditions, a shake flask containing media is inoculated with 1.0 mL of thawed WCB inoculum and placed in an incubator/shaker for 24 hours. After the incubation period, in-process samples are analyzed for optical density.

Seed Fermentation

The Seed Fermentation operation expands the culture to a density that is sufficient to inoculate the production fermentation process. A 15-liter fermentor is inoculated with shake flask contents and cultivated in culture medium at controlled conditions. After 14 hours post-inoculation, seed fermentor contents are transferred to the production fermentor.

2.3.S.2.2.2 Step 2 Fermentation

The fermentation process increases biomass and volume of cells, followed by promotion of expression and secretion of sargramostim into the medium for harvest and purification. A 100-liter production fermentor is inoculated from the seed fermentor and cultivated in culture medium at controlled conditions in fed-batch mode. Beginning at 1.5 hours post-inoculation, 50 % glucose is aseptically added at a continuous rate of 15.0 g/min. At 12.5 hours post-inoculation, the glucose feed is stopped, and 50 % ethanol is aseptically added at a continuous feed rate of 5.6 g/min. The ethanol feed is stopped 24 hours post inoculation, and fermentor contents are cooled and transferred to the harvest vessel. The production fermentation yields approximately 100 L of harvest material per fermentor.

2.3.S.2.2.3 Step 3 Harvest and Recovery

Microfiltration

The microfiltration harvest process is the initial product recovery stage and separates yeast cells from secreted sargramostim in culture medium. The microfiltration harvest process is performed using a tangential flow filtration (TFF) system. Once target volume is reached, a constant volume diafiltration is initiated by addition of purified water to the concentrated biomass.

Ultrafiltration

The Ultrafiltration (UF) unit operation concentrates MF filtrate for the first chromatographic operation. The ultrafiltration process is performed using a TFF system; UF retentate is concentrated to approximately one sixth of the starting volume.

2.3.S.2.2.4 Step 4 Purification

C4 Capture Chromatography

The C4 capture process collects and concentrates sargramostim and reduces process related impurities from UF retentate. A column containing C4 resin is equilibrated with Trifluoroacetic acid in Water for Injection (TFA/WFI) and Trifluoroacetic acid in Acetonitrile (TFA/ACN); UF retentate is loaded onto the column; the column is washed and product eluted with a linear gradient of TFA/WFI and TFA/ACN that ascends from 35 – 60 % TFA/ACN and collected. Automated collection starts and ends at set points for collection window time, UV absorption, and slope (UV %/min).

C4 Purification Chromatography

The C4 purification process separates 3 sargramostim glycoforms from the hyperglycosylated form that directly impacts drug substance glycoform composition. A column containing C4 resin is equilibrated with Pyridine/ Acetic Acid/ WFI (PAP A) and Pyridine/ Acetic Acid/ n-Propanol/ WFI (PAP B) buffers; C4C fluid is loaded onto the column; the column is washed and product eluted with PAP A and PAP B that ascends from 35 – 70 % PAP B and collected. Automated collection starts and ends at set points for collection window time, UV absorption, and slope (UV %/min).

C18 Purification Chromatography

The C18 purification step further reduces process related impurities. A column containing C18 resin is equilibrated with TFA/WFI and TFA/ACN; C4P fluid is loaded onto the column; the column is washed and product eluted with a linear gradient of TFA/WFI and TFA/ACN that ascends from 25 – 70 % TFA/ACN and collected. Automated collection starts and ends at set points for collection window time, UV absorption, and slope (UV %/min).

2.3.S.2.2.5 Step 5 Final Buffer Exchange and Filtration

Final Cation Exchange

The final chromatography step is a cation exchange operation that combines two C18 purification product eluates and exchanges buffer into 0.1 M TRIS. A column containing S-sepharose resin is equilibrated with 0.5 M β-Alanine followed with 0.05 M β-Alanine; C18 fluid is loaded onto the column; the column is washed and product eluted with 0.1 M TRIS and collected. Manual collection of the sargramostim peak is performed by monitoring UV absorption during the process at a target product elution volume calculated according to the batch record.

Bulk Drug Substance Filtration

The Bulk Drug Filtration unit operation is the last process step in fermentation, isolation, and purification of sargramostim that yields bulk drug substance. Sargramostim eluate from the cation exchange chromatography process is 0.22 µm filtered into fluorinated ethylene propylene (FEP) containers for storage.

2.3.S.2.3 Control of Materials

There are no plans to generate another Master Cell Bank (MCB); current stock MCB inventory used for working cell bank generation is sufficient to provide ongoing manufacturing operations at least 100 years.

Raw materials used in the manufacture of sargramostim are tested by suppliers and accepted on a COA or tested in-house and listed in [Table 7](#), [Table 8](#), and [Table 9](#).

Table 7: Raw Materials Used in Working Cell Bank Production

Product Name	Grade
Master Cell Bank	Non-compendial
Flask Media	Non-compendial
Glycerol	Non-compendial

Table 8: Raw Materials Used in Cell Expansion and Fermentation

Product Name	Grade
Adenine	Non-compendial
Ammonium Hydroxide, 30 %	ACS
Ammonium Sulfate	ACS
Bacto-Peptone	Non-compendial
Bacto-Yeast Extract	Non-compendial
Biotin	USP
Boric Acid	NF
Calcium Chloride, 2H	USP
Calcium Pantothenate	USP
Cupric Sulfate, 5H	USP
Dextrose, AH	USP
Ethyl Alcohol, 190 Proof	USP
Ferric Chloride, 6H	ACS
Glycerin	USP
Hy-Case SF	Non-compendial
Hydrochloric Acid	NF
Manganese Sulfate, 1H	ACS
Magnesium Sulfate, 7H	USP
Meso-inositol	FCC
Niacin	USP
Nitrogen	NF
Phosphoric Acid	NF
Potassium Phosphate, IB	NF
Pyridoxine Hydrochloride	USP
Sodium Chloride	USP
Sodium Molybdate, 2H	ACS
Surfactant, Pluronic L-61	Non-compendial
Thiamine Hydrochloride	USP

Product Name	Grade
L-Tyrosine	USP
Uracil	Non-compendial
Zinc Sulfate, 7H	USP

Table 9: Raw Materials Used in Harvest & Recovery, Purification, Final Buffer Exchange & Filtration

Product Name	Grade
Acetonitrile	ACS
Acetic Acid, Glacial	USP
β-Alanine	Non-compendial
Bakerbond: Silica, C4, 15 µm	Non-compendial
Bakerbond: Silica, C18, 15 µm	Non-compendial
Hydrochloric Acid	NF
Methanol Cycle-tainer	ACS
Methylparaben	NF
N-Propanol	Non-compendial
Nitrogen	NF
Pyridine	ACS
S-Sepharose, Fast Flow	Non-compendial
Sodium Hydroxide, 50 %	Non-compendial
Trifluoroacetic Acid	Non-compendial
TRIS-Hydrochloride	Non-compendial
Tromethamine	USP

The sargramostim fermentation process utilizes three animal derived raw materials: BactoPeptone, Hy-Case SF, and L-Tyrosine. There are no animal derived raw materials in the downstream process or the drug product fill-finish process. [Table 10](#) summarizes these materials:

Table 10: Animal-Derived Materials Used in the Manufacture of Sargramostim

Biological Raw Material	Biological Source	Country of Origin	Manufacturer	Step	Suitability for Use
Bacto-Peptide	bovine tissue	USA	Becton, Dickenson and Company	Production Fermentation Media	Certified by the European Directorate for the Quality of Medicines (EDQM) to comply with the criteria set forth in Ph.Eur. general monograph 1483: Products with risk of transmitting agency of animal spongiform encephalopathies.
Hy-Case SF	bovine milk	Australia, New Zealand	Kerry Bioscience	Production Shake Flask Media and Seed Fermentation Media	As stated in the Ph.Eur. general monograph 1483: In the light of the current scientific knowledge and irrespective of the geographical origin, milk is unlikely to present any risk of TSE contamination.

Biological Raw Material	Biological Source	Country of Origin	Manufacturer	Step	Suitability for Use
L-Tyrosine	poultry feathers	USA	JT Baker, Millipore Sigma	Production Shake Flask Media	As provided in the 2007 European Food Safety Journal (596, 1-45), scientific studies have shown there is no epidemiological proof that poultry are susceptible to contracting TSE or have been affected by TSE. This is confirmed in the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3).

2.3.S.2.4 Controls of Critical Steps and Intermediates

In-process controls are designed to measure process outputs and are used to monitor the manufacturing process performance and ensure specifications are met. In-process controls are summarized in [Table 11](#).

Table 11: Critical Process Parameters and In-Process Controls for the Manufacture of Sargramostim

Step	Description	Control	Operating Range/Limit
Step 1: Cell Expansion			
Shake Flask	Agitation Temperature Duration Optical Density	PC PC PC IPC	250 rpm 30 °C 24 hrs 6.53 – 8.611
Seed Fermentation	Agitation Aeration Backpressure pH Temperature Duration Optical Density	PC PC PC PC PC PC IPC	375 rpm 10.0 slpm 0.10 bar 5.5 29.0 °C 14 hrs 9.10 – 11.76
Step 2: Fermentation			
	Temperature pH Agitation Aeration Backpressure Duration Glucose Feed Rate Ethanol Feed Rate Optical Density	CPP CPP PC PC PC PC PC PC IPC	27.6 ± 0.5 °C 5.5 ± 0.2 325 rpm 70.0 slpm 0.15 bar 24 hours 15.0 g/min 5.6 g/min 50.32 – 77.75

Step	Description	Control	Operating Range/Limit
	Wet Cell Weight Product Concentration Calculated Yield Non-Host Contamination	IPC IPC IPC	68.14 – 88.61 g/L 5.70 – 9.82 g/fermentation No non-host organisms detected
Step 3: Harvest and Recovery			
Microfiltration	Temperature Feed Flow Rate Retentate Pressure Filtrate Pressure Final Concentration/ Diafiltration Volume Final Filtrate Volume	PC PC PC PC PC PC	15 °C 60 lpm 5.0 psi 5.5 psi 70 L 240 L
Ultrafiltration	Temperature Retentate Flow Rate Transmembrane Pressure Concentration Volume Final Retentate Volume Microbial Content Protein Concentration	PC PC PC PC PC IPC IPC	15 °C 25 lpm 20 psi 40 L 45 L < 10 CFU/mL Report results for C4 Capture step yield calculation
Step 4: Purification			
C4 Capture Chromatography	Collection Start Time UV Absorbance UV Slope Collection End Time UV Absorbance UV Slope Wash Buffer Composition Elution Gradient Buffer Composition Elution Gradient Flow Rate Calculated Step Yield	CPP CPP CPP CPP CPP CPP PC PC PC PC	206 – 251 min ≥ 2 % ≥ 3 %/min 236 – 281 min ≤ 2.50 % ≤ -1.0 %/min 0 %, 0 – 35 %, 35 % organic buffer 35 – 60 % organic buffer 0.230 L/min 76.39 – 103.70 %
C4 Purification Chromatography	Collection Start Time UV Absorbance UV Slope Collection End Time UV Absorbance UV Slope Wash Buffer Composition Elution Gradient Buffer Composition Elution Gradient Flow Rate Endotoxin Glycoform Ratio	CPP CPP CPP CPP CPP CPP PC PC PC IPC IPC	22.1 – 32.1 min ≥ 2 % ≥ 7 %/min 30.5 – 40.5 min ≤ 2.70 % ≤ -0.9 %/min 35 % organic buffer 35 – 70 % organic buffer 0.285 L/min < 25 EU/mL Peak 1: ≤ 5.6 % Peak 2: 24 – 41 %

Step	Description	Control	Operating Range/Limit
	Calculated Step Yield	IPC	Peak 3: 13 – 31 % Peak 4: 34 – 52 % 84.70 – 105.32 %
C18 Purification Chromatography	Wash Buffer Composition Elution Gradient Buffer Composition Elution Gradient Flow Rate Collection Start UV Absorbance UV Slope Collection End UV Absorbance UV Slope Glycoform Ratio Calculated Step Yield	PC PC PC PC PC PC PC PC PC IPC IPC	25 % organic buffer 25 – 70 % organic buffer 0.250 L/min Time: 43 – 51 min ≤ 1 % ≤ 1 %/min Time: 54 – 62 min ≤ 1.50 % ≤ -0.1 %/min Peak 1: ≤ 5.6 % Peak 2: 24 – 41 % Peak 3: 13 – 31 % Peak 4: 34 – 52 % 92.45 – 103.85 %
Step 5: Final Buffer Exchange and Filtration			
Final Cation Exchange	Load and initial wash flow rate or Maximum process flow rate Collection Start Collection End	PC PC PC	Load and initial wash flow rate based on a 1.4 min residence time for a given packed bed height or ≤ 392.5 mL/min First sign of UV upward deflection Target eluate volume is reached and the UV absorbance is at or below ~0.40 CU
Bulk Drug Substance Filtration	Calculated Step Yield	IPC	77.98 – 98.50 %

2.3.S.2.5 Process Validation and/or Evaluation

The sargramostim manufacturing process was initially validated at the Seattle WA manufacturing facility (51U) at the time of product approval (1991). The Seattle facility was decommissioned when sargramostim manufacturing moved to the new Northpointe (NP) facility in Lynnwood WA in 2010, and the process was re-validated pursuant to facility licensure. A total of 5 consecutive BDS conformance runs were performed in support of process validation; the decision to execute more than 3 BDS batches was based on transfer of the manufacturing process to the new and automated facility. This transfer justified collection of additional data points to demonstrate a consistent manufacturing process.

Process validation acceptance criteria were calculated from 2-sided 99 % prediction intervals based on data available from NP Engineering runs and the number of data points for each unit operation. Fermentation, harvest and recovery and purification processes were evaluated per the following conditions:

1. All process validation lots were produced according to the approved batch record.

2. All protocol process parameters were maintained within their operating ranges during production.
3. All Quality and Performance Attributes met their respective acceptance criteria.

Shake Flask, Seed Fermentation and Production Fermentation unit operations were performed according to approved protocol and batch records. Each conformance BDS included 8 Shake Flasks, 8 Seed Fermentations, and 8 Production Fermentations, for a total of 40 individual unit operations. Review and evaluation of Shake Flask, Seed Fermentation, and Production Fermentation unit operations verified that all process parameters were maintained within their operating ranges. All product quality and process performance attributes met their respective acceptance criteria. Fermentation unit operations are considered under effective and consistent control.

Harvest and Recovery unit operations microfiltration/diafiltration (MF/DF) and ultrafiltration (UF) were performed according to approved protocols and batch records. Each conformance BDS run included 4 MF/DF and 4 UF, for a total of 20 sets each of MF/DF and UF material delivered to the first of 3 Purification unit operations, C4 Capture. Membrane lifetime studies were also performed for microfiltration and ultrafiltration membranes. MF/DF and UF unit operations verified that all process parameters were maintained within their operating ranges. All product quality and process performance attributes met their respective acceptance criteria. MF/DF and UF unit operations are considered under effective and consistent control.

Purification unit operations were performed according to approved protocols and batch records. Each conformance BDS run included 4 C4 captures, 4 C4 purifications, and 2 C18 purifications. Combined C18 purifications were further processed in the BDS Cation Exchange/Final Filtration unit operation. Column Lifetime qualification studies verified maximum column lifetimes for C4 Capture, C4 Purification and C18 Purification. Purification process unit operations verified that all process parameters were maintained within their operating ranges. All product quality and process performance attributes met their respective acceptance criteria. Purification unit operations met are considered under effective and consistent control.

Partner Therapeutics maintains a lifecycle approach to manufacture of sargramostim through continuous process verification. The validated state is maintained through process monitoring, investigations as needed, updates based on regulatory expectations, and continuous process improvements. Drug substance process validation and verification demonstrate that the manufacturing process successfully produces sargramostim in a controlled and consistent manner.

2.3.S.2.6 Manufacturing Process Development

Leukine®(sargramostim) was initially approved in 1991; manufacturing process development at the Northpointe (NP) facility was based on the knowledge and experience of the commercial process previously performed at the Seattle facility (51U).

Throughout process transfer from 51U to NP, efforts were made at the NP site to minimize process differences by using comparable equipment, maintaining similar processing procedures, and employing a similar scale and batching scheme. Some changes were required, however, to accommodate the automated NP facility, and to improve process robustness and control.

A major improvement at the NP facility is increased use of equipment automation throughout the upstream process. Equipment utilizing significant levels of automation include: Media Compounding system, Seed Fermentors, Production Fermentors, MF system, UF system, and Clean-in-Place system. The level of automation encompasses local controllers with connection to a supervisory control and data acquisition system comprised of multiple servers. This allows for both data over time and discrete time data to be

gathered and stored for control, monitoring, and evaluation. Additionally, automation enables ability to use pre-programmed “recipes” resulting in consistent and robust process operation.

New NP seed fermentors were designed to match dimensions of the seed fermentor at 51U. One element of fermentor design that could not be incorporated into NP fermentors was a similar air sparger, due to complexity of the 51U sparger (four-armed, rotating sparger integral to agitation assembly) and sub-optimal design. For these reasons, an alternate sparger (single, non-rotating perforated tube) was implemented during NP fermentor design.

NP production fermentors were designed to match dimensions of production fermentors at 51U. For the same reasons described above for seed fermentors, a similar sparger design (single, non-rotating perforated tube) was used for NP production fermenters. Agitation, aeration, and vessel back pressure were increased to achieve similar oxygen transfer characteristics. Initial engineering runs showed glycoform results for BDS that did not meet acceptance criteria. Adjustments were made to sparger and agitator designs, and agitation, aeration and vessel back pressure were returned to levels equivalent to 51U. Subsequent engineering runs continued to show glycoform results for BDS that did not meet acceptance criteria. Investigation concluded that BDS glycoform failures were most appropriately addressed by adjustment of fermentation temperature.

Multiple fermentation media were autoclaved at 51U, however all media at NP (except for autoclaved glycerol solution and steam-in-place fermentation media) are filtered through a 0.2-µm filter. The 0.2-µm filters maintain compatibility to process stream and are suited for filtration of increased volumes with potentially higher throughput.

The NP MF operation uses permeate pressure control, as opposed to permeate flow rate control used in the 51U process. It was observed that MF membranes were less prone to fouling when process control was based on permeate pressure rather than permeate flow rate; permeate pressure allowed consistent pressure across the MF membranes throughout the cell separation process. Another modification to MF unit operation was removal of permeate hold time prior to subsequent unit operation (Ultrafiltration process). MF permeate was held for either 1 or 3 days at the 51U facility for process scheduling. To provide better operational efficiency, the MF permeate hold was removed and the MF and subsequent UF process are performed sequentially on the same day.

The NP UF skid was designed with greater control capabilities (automated valves, pumps, and control loops) than equipment used at 51U. To take advantage of these enhancements, the UF process was programmed to automatically control Trans-Membrane Pressure (TMP) as opposed to manual retentate pressure control that was used for the 51U process. Automated TMP control provides more consistent process performance. Changes were also made to upgrade to a newer filter technology. The NP process uses a cassette type filter (Pellicon 2) with a 10-kD nominal molecular weight cut-off. The Pellicon 2 filter uses composite regenerated cellulose that is free of voids and defects that may have existed in previously used, regenerated cellulose spiral filters. Additionally, higher throughput capacity and shorter path length associated with Pellicon 2 filter modules result in less required membrane area than 51U and lower feed flow rates. Finally, the concentration factor during the UF process was increased to approximately 6x compared to 4x concentration performed in the 51U process. The change provides a smaller and more concentrated load for the subsequent C4 Capture step. This provides operational benefits, such as shorter C4 Capture processing time, while meeting comparability acceptance criteria for post UF protein concentration and normalized yield results.

Several changes to downstream buffer compounding operations improved operational efficiency: implementation of single-use, gamma-irradiated storage bags with attached filters for aqueous buffers used

in the cation exchange unit operation; implementation of different filters for all solvent buffers, including the introduction of 0.1% TFA/ACN (trifluoroacetic acid/ acetonitrile) filtration, which maintain process compatibility and bioburden reduction, and is better suited for increased volumes; and increased compounded buffer volumes and expiry for aqueous and solvent buffers to allow for use in multiple purification processes.

There were 3 changes associated with transfer of UF product from upstream to downstream: different configuration in-line filter, different collection container, and smaller transfer volume. The first 2 changes are incorporated in a disposable, pre-packaged, gamma-irradiated product bag with attached filter. Due to the increased concentration factor during UF processing in the NP process, product transfer volume from upstream to downstream operations is less than the 51U process (approximately 45 L versus approximately 60 L, respectively). The smaller transfer volume leads to a smaller load volume which provides an operational benefit of shorter C4 Capture processing time. The implementation of these changes, including the smaller transfer volume, improves operational efficiency.

The NP HPLC system is used to perform 3 purification processes: C4 Capture, C4 Purification, and C18 Purification; the skid has 3 distinct product flow paths, 1 for each unit operation, an improvement over the 51U skid in which all 3 HPLC processes used the same piping. An additional equipment improvement for the NP HPLC system was implementation of dynamic axial compression columns (DAC) columns with qualified in-house packing procedures, rather than contractor supplied fixed bed columns used in the 51U process. DAC columns apply constant pressure to the packed resin bed, resulting in greater stability by eliminating channeling or voids that may form within the column. The more stable resin bed improves process-to-process chromatography consistency, leading to more reproducible process performance.

In addition to the use of the DAC column, the following process improvements were made to the C4 Capture process. A guard column used in the 51U process to eliminate excessive column back pressure and extend column lifetime was eliminated as the DAC technology produces a more stable resin bed and increased column lifetime. A post-column conditioning “sawtooth” gradient was added to reduce column carryover. Modification of the post-load wash sequence improved the separation of process related impurities; the resulting improvement in a clean, consistent, and reproducible elution profile resulted in the implementation of automated collection of a single product eluate peak instead of a collection of fractions. These cumulative changes resulted in a more consistent unit operation.

The following process improvements were made to the C4 Purification process. Changes to the organic buffer concentration of column equilibration step, load material and post load wash step resulted in improved, consistent chromatography and increased column longevity. The UV monitoring wavelength was increased from 280 nm to 300 nm to minimize background absorbance associated with buffers. As with C4C, these changes resulted in automated collection of a single eluate peak, resulting in a more consistent unit operation. Automated collection of eluate was also implemented in the C18 operation.

2.3.S.3 CHARACTERIZATION

2.3.S.3.1 Elucidation of Structure and Other Characteristics

Characterization of sargramostim was performed using reference standards 0088-006, 0088-001, and 0088-006 and sargramostim batches B18168, B18242, and B18313 produced at the NP facility. A summary of the tests and results are listed in [Table 12](#).

Table 12: Summary of Elucidation of Structure Results for Sargramostim

Parameter	Methods	Ref. Std. 6268-044 & 008-001	Ref. Std. 0088-006	NP B18168	NP B18242	NP B18313
Confirmation of Primary Sequence	Neutral pH Glu-C Map/MS/MS	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed
	Reduced Tryptic Map/MS/MS	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed
	Acid pH Glu-C Map/MS/MS	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed
Amino Acid Composition	Amino Acid Analysis	Results of a typical analysis provided				
Molecular Formula	Primary Sequence	C ₆₃₉ H ₁₀₀₂ N ₁₆₈ O ₁₉₆ S ₈				
Theoretical Molecular Weight	Primary Sequence	14430 g/mole				
Extinction Coefficient	Calculated based on Trp, Tyr, Cys.	Theoretical: $\epsilon = 14180 \text{ cm}^{-1}\text{moles}^{-1}\text{liters}$, $E^{0.1\%}_{280 \text{ nm}} = 0.983 \text{ cm}^2/\text{mg}$				
	Spectrophotometry/Protein Conc	Not required	Not required	1.12 cm ² /mg	1.10 cm ² /mg	1.16 cm ² /mg
Carboxy-terminal Analysis	Neutral pH Glu-C Map/MS/MS	C-terminus intact	C-terminus intact	C-terminus intact	C-terminus intact	C-terminus intact
	Reduced Tryptic Map/MS/MS	Not done	no result -assay problems	no result -assay problems	no result -assay problems	no result -assay problems
Assignment of Disulfide Bonds	Acidic pH HPLC Glu-C Map/MS	Cys ⁵⁴ -Cys ⁹⁶ Cys ⁸⁸ -Cys ¹²¹	Cys ⁵⁴ -Cys ⁹⁶ Cys ⁸⁸ -Cys ¹²¹	Cys ⁵⁴ -Cys ⁹⁶ Cys ⁸⁸ -Cys ¹²¹	Cys ⁵⁴ -Cys ⁹⁶ Cys ⁸⁸ -Cys ¹²¹	Cys ⁵⁴ -Cys ⁹⁶ Cys ⁸⁸ -Cys ¹²¹
Methionine Oxidation	Neutral pH Glu-C Map	Met ⁷⁹ ~ 5-7%	Met ⁷⁹ 4.79%	Met ⁷⁹ 2.70%	Met ⁷⁹ 2.64%	Met ⁷⁹ 2.55%
	Acid pH Glu-C Map/MS/MS	Met ³⁶ , Met ⁴⁶ no ox	Not required	Not required	Not required	Not required
	Tryptic Map/MS/MS	Not done	Met ⁷⁹ 5%, Met ⁴⁶ ~1%	Met ⁷⁹ 3%, Met ⁴⁶ ~1%	Met ⁷⁹ 3%, Met ⁴⁶ ~1%	Met ⁷⁹ 3%, Met ⁴⁶ ~1%
Glycosylation Site Occupancy	Neutral pH Glu-C Map/MS	Consistent with Ser ⁹	Ser ⁹ 46%	Ser ⁹ 49%	Ser ⁹ 49%	Ser ⁹ 49%
	Acid pH Glu-C Map/MS/MS	with Asn ²⁷	Asn ²⁷ 26%	Asn ²⁷ 25%	Asn ²⁷ 25%	Asn ²⁷ 24%
	Reduced Tryptic Map/MS/MS	Asn ³⁷ none detected	Asn ³⁷ none detected	Not available	Not available	Not available

Parameter	Methods	Ref. Std. 6268-044 & 008-001	Ref. Std. 0088-006	NP B18168	NP B18242	NP B18313
Aspartate Isomerization (Asp120)	Neutral pH Glu-C Map	≤ 1%	0%	0%	0%	0%
Deamidation (Asn or Gln residues)	Neutral pH Glu-C Map/MS	No deamidation	N ¹⁷ , Q ²⁰ , N ²⁷ <1%	N ¹⁷ , Q ²⁰ , N ²⁷ <1%	N ¹⁷ , Q ²⁰ , N ²⁷ <1%	N ¹⁷ , Q ²⁰ , N ²⁷ <1%
Molecular Masses of Glycoforms	MALDI TOF MS	Comparable MALDI TOF MS profile and masses	Comparable MALDI TOF MS profile and masses	Comparable MALDI TOF MS profile and masses	Comparable MALDI TOF MS profile and masses	Comparable MALDI TOF MS profile and masses
Asn27 N-linked Oligosaccharide Structures	CGE N-linked Oligosaccharide Map MALDI TOF MS TOF ESI/MS & CID ESI/MS/MS	Comparable profile & quantitation Comparable profile & masses N-glycan structures described	Comparable profile & quantitation Comparable profile & masses Not done	Comparable profile & quantitation Comparable profile masses Not done	Comparable profile & quantitation Comparable profile & masses Not done	Comparable profile & quantitation Comparable profile & masses Not done
Asn37 N-linked Oligosaccharide Structures	Glu-C Maps/MS/MS Tryptic Maps/MS/MS MALDI TOF MS	No evidence of N ³⁷ -glycosylation present in 51U commercial or NP process BDS				
O-linked Oligosaccharide Structures	MALDI T OF MS (intact glycoforms) MALDI T OF MS (released) TOF ESI/MS & CID ESI/MS/MS	Comparable profile & masses Comparable intact glycoforms O-glycan structures described	Comparable profile & masses Comparable intact glycoforms Not required	Comparable profile & masses Not done Not required	Comparable profile & masses Not done Not required	Comparable profile & masses Not done Not required
pI Value Major Glycoforms BDS N- Glycoforms O-Glycoforms Non-glycosylated	Isolated Glycoforms/IEF	Not required	Not required	Five distinct bands, PI 5.3 – 4.6 4 bands, PI 5.2 - 4.6 2 bands, PI 5.3 - 5.0 3 bands, PI 5.3- 4.7	Five distinct bands, PI 5.3 – 4.6 4 bands, PI 5.2 - 4.6 2 bands, PI 5.3 - 5.1 3 bands, PI 5.3- 4.8	Five distinct bands, PI 5.3 – 4.6 4 bands, PI 5.2 - 4.6 2 bands, PI 5.3 - 5.0 3 bands, PI 5.3- 4.7
Specific Activity Major Glycoforms	Isolated Glycoforms/bioassay	Not required	Not required	All glycoforms comparable to parent BDS	All glycoforms comparable to parent BDS	N- and O-glycoforms comparable to parent BDS; non-glycosylated inconclusive

Parameter	Methods	Ref. Std. 6268-044 & 008-001	Ref. Std. 0088-006	NP B18168	NP B18242	NP B18313
Phosphorylation (qualitative)	ICPMS and MAS ¹ MALDI TOF MS	Phosphorous confirmation -PO ₃ glycoforms, similar masses	Not required -PO ₃ glycoforms, similar masses	Not required -PO ₃ glycoforms, similar masses	Not required -PO ₃ glycoforms, similar masses	Not required -PO ₃ glycoforms, similar masses
Secondary Structure and Thermodynamic Stability	Circular Dichroism CD Spectra 222nm Ellipticity	Not done Not done	Similar Secondary Structure Comparable CD Temperature Prof	Similar Secondary Structure Comparable CD Temperature Prof	Similar Secondary Structure Comparable CD Temperature Prof	Similar Secondary Structure Comparable CD Temperature Prof
Tertiary Structure and Thermodynamic Stability	Intrinsic Fluorescence Profile Inflection in Fluorescence Intensity	Not done Not done	Similar Tertiary Structure Comparable Temperature Profile	Similar Tertiary Structure Comparable Temperature Profile	Similar Tertiary Structure Comparable Temperature Profile	Similar Tertiary Structure Comparable Temperature Profile
RPC ELISA ²	ELISA (ng/mg sargramostim)	Not required	Not required	10.56 ng/mg (ppm)	13.68 ng/mg (ppm)	6.44 ng/mg (ppm)

1) Analysis of small scale BDS produced by the currently licensed process confirmed the presence of phosphorous (not sulfur) in sargramostim.

2) RPC – Residual Process Components ELISA. Non-product related impurities including Host Cell Protein and fermentation media components.

2.3.S.3.2 Impurities

Raw materials used in fermentation, fermentation by-products, and residual solvents from the purification process were identified as process related impurities in the manufacture of drug substance.

Process-related substances, host cell components, host cell DNA, and residual solvents were analyzed during process validation. Amounts of process-related impurities that were present in validation batches of Sargramostim bulk drug substance are provided in [Table 13](#). Data demonstrated no need to control the impurities in the final drug substance.

Table 13: Process-Related Impurities in Drug Substance Process Validation Batches

Batch Number	Host Cell Components (ng/mg) ¹	Host DNA (pg/mg) ²	Acetic Acid (µg/mL)	Ethanol (µg/mL) ³	Trifluoroacetic Acid (µg/mL) ⁴
B18168	11	< 1.7	< 50	87	< 50
B18242	14	< 2.5	< 50	78	< 50
B18313	6	< 2.5	< 50	87	< 50
B18378	17	< 2.5	< 50	78	< 50
B18486	17	< 2.3	< 50	72	< 50

¹ ICH Q3A reporting limit is 125 pg/mg.

² Limit is ≤ 10 pg/mg (based on 1985 FDA Guidance for Industry, FDA Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology).

³ Limit for a Class 3 solvent (i.e., includes ethanol) is ≤ 5000 ppm (0.05 %).

⁴ No adequate toxicological data available to determine Class [ICH Q3C(R6)].

2.3.S.4 CONTROL OF DRUG SUBSTANCE

2.3.S.4.1 Specifications

2.3.S.4.1.1 Drug Substance Release Specifications

The drug substance will comply with the release specifications provided in [Table 14](#).

Table 14: Release Specifications for Sargramostim Drug Substance

Test	Analytical Procedure	Acceptance Limit
Physical Appearance		
Appearance/Color/Clarity	T-0023	Clear, colorless to pale straw liquid
Identification		
Isoelectric Focusing	T-0114	Major species migrates at pI 5.2 ± 0.2 with no more than 3 minor species evident in the pI range 4.5 to 5.2.
Glu-C Peptide Mapping	T-0323	<p>N-Terminal Species Ala1: 65 – 76 % Ala3: 24 – 35 % Arg4: ≤ 1 % Ser5: ≤ 5 %</p> <p>No new peaks detected in the 280 nm chromatogram. Report Peak Area Ratios for peaks: G2 + G7p, Ala3, Ala1, G7p + G8, G10, G9, and G12p + G13. All Peak Area Ratios must lie within the range of 0.7 – 1.3. Elution profile at 214 nm comparable to Ref. Std.</p>
Quantity		
Protein Concentration	T-0315	5.00 – 8.30 mg/mL
Biological Activity		
Potency	T-0091	$4.0 - 6.9 \times 10^6$ IU/mg
Purity		
SDS-PAGE, Silver Stain (reduced, non-reduced)	T-0002	The mobility of the 3 bands of the test sample must correspond to the molecular weights based on comparison to MW markers and a rhu GM-CSF Ref. Std. run on the same gel. Test sample displays no extra bands that are not present in the Ref. Std.
Glycosylated Variants	T-0075	<p>Peak 1 (related protein impurity): ≤ 4.0 %.</p> <p>Peak area percentages of the 3 glycosylated components must be within the following ranges:</p> <p>Peak 2 (N-linked glycoform): 24 – 37 % Peak 3 (O-linked glycoform): 15 – 27 % Peak 4 (non-glycosylated glycoform): 38 – 52 %</p>

Test	Analytical Procedure	Acceptance Limit
		Retention times for glycosylated component peaks 2, 3, and 4 of the test sample are within ± 0.5 minutes of those for the Ref. Std. analyzed in the same run.
High Molecular Weight Component	T-0154	≤ 1.0 %; Retention time for monomer peak is within ± 0.5 minutes of Ref. Std and elution profile comparable to Ref. Std.
Protein Purity	T-0013	≥ 99 % by area
General		
Monosaccharide Composition	T-0108	3.63 – 5.22 moles of mannose/mole of sargramostim 0.326 – 0.433 moles of N-acetylglucosamine/mole of sargramostim
pH	T-0019	7.2 – 7.6
Microbial		
Endotoxin	T-3007	≤ 1.25 EU/mg
Microbial Content TAMC TYMC	T-3011	< 1 cfu/mL < 1 cfu/mL

IU = International units; MW = Molecular weight; EU = Endotoxin units; TAMC = Total aerobic microbial count; TYMC = Total yeast and mold count; cfu= Colony forming units

2.3.S.4.1.2 Drug Substance Shelf-life Specifications

The drug substance on stability will comply with the shelf-life specifications provided in [Table 15](#).

Table 15: Stability Specifications for Sargramostim Drug Substance

Test	Analytical Procedure	Acceptance Limit
Physical Appearance		
Appearance/Color/Clarity	T-0023	Clear, colorless to pale straw liquid
Identification		
Glu-C Peptide Mapping	T-0323	N-Terminal Species Ala1: 65 – 76 % Ala3: 24 – 35 % Arg4: ≤ 1 % Ser5: ≤ 5 % No new peaks detected in the 280 nm chromatogram. Report Peak Area Ratios for peaks: G2 + G7p, Ala3, Ala1, G7p + G8, G10, G9, and G12p + G13. All Peak Area Ratios must lie within the range of 0.7 – 1.3. Elution profile at 214 nm comparable to Ref. Std.

Test	Analytical Procedure	Acceptance Limit
Biological Activity		
Potency	T-0091	4.0 – 6.9 x 10 ⁶ IU/mg
Purity		
SDS-PAGE, Silver Stain (reduced, non-reduced)	T-0002	The mobility of the 3 bands of the test sample must correspond to the molecular weights based on comparison to MW markers and a rhu GM-CSF Ref. Std. run on the same gel. Test sample displays no extra bands that are not present in the Ref. Std.
Glycosylated Variants	T-0075	Peak 1 (related protein impurity): ≤ 4.0 %. Peak area percentages of the 3 glycosylated components must be within the following ranges: Peak 2 (N-linked glycoform): 24 – 37 % Peak 3 (O-linked glycoform): 15 – 27 % Peak 4 (non-glycosylated glycoform): 38 – 52 % Retention times for glycosylated component peaks 2, 3, and 4 of the test sample are within ± 0.5 minutes of those for the Ref. Std. analyzed in the same run.
High Molecular Weight Component	T-0154	≤ 1.0 % for HMWC; Retention time for monomer peak is within ± 0.5 minutes of Ref. Std and elution profile comparable to Ref. Std.
Other		
pH	T-0019	7.2 – 7.6

IU = International units; MW = Molecular weight

2.3.S.4.2 Analytical Procedures

The analytical procedures for sargramostim drug substance are provided in [Table 16](#).

Table 16: List of Analytical Procedures

Test	Method	Methodology
SDS-PAGE, Silver Stain (reduced, non-reduced)	T-0002	SDS-PAGE Silver Stain Procedure
Protein Purity	T-0013	Scanning Densitometry for rhu- GM-CSF
pH	T-0019	pH Determinations
Appearance/ Color/ Clarity	T-0023	Appearance/ Color/ Clarity Test Procedure
Glycosylated Variants	T-0075	Quantitative Analysis of rhu GM-CSF Glycosylated Variants by High Performance Liquid Chromatography (HPLC)
Potency	T-0091	TF-1 Bioassay for rhu GM-CSF

Monosaccharide Composition	T-0108	Monosaccharide Compositional Analysis for rhu GM-CSF
Isoelectric Focusing	T-0114	Isoelectric Focusing (IEF) Testing Procedure for rhu GM-CSF
High Molecular Weight Component	T-0154	Size Exclusion Chromatography of GM-CSF
Protein Concentration	T-0315	Leukine (sargramostim) UV Spectrophotometer Scan
Glu-C Peptide Mapping	T-0323	Glu-C Peptide Mapping Procedure for rhu GM-CSF
Endotoxin	T-3007	Endotoxin Assay – KQCL Method
Microbial Content	T-3011	Microbial Content Assay

2.3.S.4.3 Validation of Analytical Procedures

Analytical method validations for sargramostim, as appropriate, have included accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range. Compendial methods have been subject to method verification.

A list of method validation or qualification documents for performing drug substance testing using analytical procedures described is provided in [Table 17](#).

Table 17: Analytical Procedure Method Validation Reports for Drug Substance

Analytical Procedure	Validation Report Number	Validation Report Title
T-0002	QCMV-T0002-041718R	Method Validation Report: Validation of Test Method T-0002, SDS-PAGE Silver Stain Procedure using WedgeWell™ gels
T-0013	QCMV-0185	Test Method Validation Report - Scanning Densitometry for GM-CSF
T-0019	QCMV-T0019-102913	Compendial verification of pH Determination, SOP T-0019, for Qualification of Genzyme Northpointe as an Alternative Testing Site
T-0023	QCMT-052813R	Leukine Bulk Drug Substance Alternative Testing Site Qualification Report, Version 2 and Leukine Drug Product Alternative Testing Site Qualification Report, Version 2
T-0075	MVR-0004	Validation of Test Method T-0075, Quantitative Analysis of rhu GM-CSF Glycosylated Variants by High Performance Liquid Chromatography (HPLC)
T-0091	QCMV-0132.01	Immunex Quality Control Test Method Validation Report, TF-1 Bioassay for GM-CSF
T-0108	QCMV-T0108-061715	Method Validation Report: Validation of Test Method T-0108, Monosaccharide Composition Analysis of rhu GM-CSF
T-0114	QCMV-0013.01	Validation of IEF for rhu GM-CSF
T-0154	QCMV-T0154-101812	Test Method SOP T-0154 “Size Exclusion Chromatography of GM-CSF” Using the Shodex PROTEIN KW 802.5 analytical column

Analytical Procedure	Validation Report Number	Validation Report Title
T-0315	QCMV-T0315-051414R	Compendial Verification of Leukine (sargramostim) UV Spectrophotometer Scan, SOP T-0315 for Qualification of Northpointe as an Alternative Testing Site
T-0323	QCMV-0174	Validation of the Glu-C Peptide Map Method for Analysis of GM-CSF
T-3007	QCMV-T3007-102913R	Northpointe In-Process and Sargramostim BDS Materials Confirmation Study
T-3011	QCMV-T3011-090208R	Final Report: Sargramostim Product Testing at the Northpointe Facility


2.3.S.4.4 Batch Analyses

Certificates of Analysis of the batches of sargramostim bulk drug substance used in the manufacture of the Investigational Medicinal Product (IMP) are described in [Table 18](#). The batches met the sargramostim bulk drug substance release specifications.

Table 18: Description of Drug Substance Batches Manufactured at Partner Therapeutics Northpointe, Lynnwood WA

Drug Substance Batch No.	Date of Manufacture	Expiry Date	Reference
B25981	28 October 2020	October 2025	Figure 6
B26063	19 November 2020	November 2025	Figure 7

Figure 6: Certificate of Analysis – Drug Substance Batch B25981



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CERTIFICATE OF ANALYSIS

<div> <div>Leukine® (sargramostim)</div> <div>Recombinant Human Granulocyte Macrophage Colony Stimulating Factor Bulk Drug Substance</div> </div>			
Material Number: 12840		Batch Number: B25981	
Date of Manufacture: 28Oct2020		Expiration Date: October, 2025	
Specification: SPEC-0012-10		Effective Date: 10JUN2020	
Partner Therapeutics Northpointe • 2625 162 nd St SW, Lynnwood WA 98087 • 425-245-1245			

ASSAY	METHOD	SPECIFICATION	RESULTS																
Appearance/Color/Clarity	T-0023	Clear, colorless liquid to pale straw liquid	Clear, colorless liquid.																
pH	T-0019	7.2-7.6	7.4																
Protein Concentration: UV Spectrophotometer Scan	T-0315	5.00-8.30 mg/mL	6.40 mg/mL																
Potency TF-1 Bioassay	T-0091	4.0 – 6.9 x 10 ⁶ IU/mg	6.0 x 10 ⁶ IU/mg																
Glu-C Peptide Mapping	T-0323	N-Terminal Species Ala1: 65 – 76% Ala3: 24 – 35% Arg4: ≤1% Ser5: ≤5% No new peaks detected in the 280 nm chromatogram. Report Peak Area Ratios for peaks: G2 +G7p, Ala3, Ala1, G7p+G8, G10, G9, and G12p+G13. All Peak Area Ratios must lie within the range of 0.7 - 1.3. Elution profile at 214 nm comparable to Reference Standard.	N-Terminal Species Ala1: 71 % Ala3: 29 % Arg4: <QL % Ser5: <QL % Pass <table> <tr> <th>Peak</th> <th>Ratio</th> </tr> <tr> <td>G2+G7p</td> <td>1.0</td> </tr> <tr> <td>Ala3</td> <td>0.9</td> </tr> <tr> <td>Ala1</td> <td>1.0</td> </tr> <tr> <td>G7p+G8</td> <td>1.0</td> </tr> <tr> <td>G10</td> <td>1.0</td> </tr> <tr> <td>G9</td> <td>1.1</td> </tr> <tr> <td>G12p+G13</td> <td>1.0</td> </tr> </table> Pass	Peak	Ratio	G2+G7p	1.0	Ala3	0.9	Ala1	1.0	G7p+G8	1.0	G10	1.0	G9	1.1	G12p+G13	1.0
Peak	Ratio																		
G2+G7p	1.0																		
Ala3	0.9																		
Ala1	1.0																		
G7p+G8	1.0																		
G10	1.0																		
G9	1.1																		
G12p+G13	1.0																		
Monosaccharide Compositional Analysis	T-0108	3.63 – 5.22 moles of mannose/mole of Sargramostim 0.326 – 0.433 moles of N-acetylglucosamine/mole of Sargramostim	4.65 moles of mannose/mole of Sargramostim 0.386 moles of N-acetylglucosamine/mole of Sargramostim																

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Page 1 of 3



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Leukine® (sargramostim) Recombinant Human Granulocyte Macrophage Colony Stimulating Factor Bulk Drug Substance	
Material Number: 12840	Batch Number: B25981
Date of Manufacture: 28Oct2020	Expiration Date: October, 2025
Specification: SPEC-0012-10	Effective Date: 10JUN2020
Partner Therapeutics Northpointe • 2625 162 nd St SW, Lynnwood WA 98087 • 425-245-1245	

ASSAY	METHOD	SPECIFICATION	RESULTS								
SDS-PAGE, Silver Stain (reduced, non-reduced)	T-0002	The mobility of the 3 bands of the test sample must correspond to the molecular weights based on comparison to MW markers and a rhu GM-CSF Reference Standard run on the same gel. Test sample displays no extra bands that are not present in the Ref Std.	Pass								
Isoelectric Focusing	T-0114	Major species migrates at pI 5.2 ± 0.2 with no more than 3 minor species evident in the pI range 4.5 to 5.2.	Pass								
Quantitative Analysis of Glycosylated Variants by RP-HPLC	T-0075	Peak 1 (related protein impurity): ≤ 4.0%. Peak area percentages of the three glycosylated components must be within the following ranges: Peak 2 (N-linked glycoform): 24-37% Peak 3 (O-linked glycoform): 15-27% Peak 4 (non-glycosylated glycoform): 38-52% The retention times for glycosylated component peaks 2, 3, and 4 of the test sample are within ± 0.5 minutes of those for the Reference Standard analyzed in the same run.	Peak 1: 2.4% Peak 2: 28% Peak 3: 23% Peak 4: 46% Comparison between test sample and Reference Standard <table><tr><th>Peak</th><th>Retention Time Difference (min)</th></tr><tr><td>2</td><td>0.0</td></tr><tr><td>3</td><td>0.0</td></tr><tr><td>4</td><td>0.0</td></tr></table>	Peak	Retention Time Difference (min)	2	0.0	3	0.0	4	0.0
Peak	Retention Time Difference (min)										
2	0.0										
3	0.0										
4	0.0										
Size Exclusion HPLC	T-0154	≤1.0% higher molecular weight component. Retention time for monomer peak is within ±0.5 minutes of Reference Standard. Elution profile is comparable to Reference Standard.	< QL % higher molecular weight component. 0.0 minutes retention time difference. Pass								
Scanning Densitometry	T-0013	Protein purity is ≥99% by area	Pass								

07/01/21 2:10 PM

CONFIDENTIAL – PROPRIETARY INFORMATION OF PARTNER THERAPEUTICS

Page 2 of 3

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Leukine® (sargramostim) Recombinant Human Granulocyte Macrophage Colony Stimulating Factor Bulk Drug Substance	
Material Number: 12840	Batch Number: B25981
Date of Manufacture: 28Oct2020	Expiration Date: October, 2025
Specification: SPEC-0012-10	Effective Date: 10JUN2020
Partner Therapeutics Northpointe • 2625 162 nd St SW, Lynnwood WA 98087 • 425-245-1245	

ASSAY	METHOD	SPECIFICATION	RESULTS
Endotoxin (LAL) Assay	T-3007	≤ 1.25 EU/mg	<0.05 EU/mg
Microbial Content	T-3011	<1 CFU/mL	0 CFU/mL on TSA media 0 CFU/mL on SDA media

The signatures below indicate that the results meet specifications

Compiled by:		Date:	01Jul2021
Approved by:		Date:	06Jul2021

07/01/21 2:10 PM

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Page 3 of 3

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Figure 7: Certificate of Analysis – Drug Substance Batch B26063



CERTIFICATE OF ANALYSIS

Leukine® (sargramostim) Recombinant Human Granulocyte Macrophage Colony Stimulating Factor Bulk Drug Substance	
Material Number: 12840	Batch Number: B26063
Date of Manufacture: 19Nov2020	Expiration Date: November, 2025
Specification: SPEC-0012-10	Effective Date: 10JUN2020
Partner Therapeutics Northpointe • 2625 162 nd St SW, Lynnwood WA 98087 • 425-245-1245	

ASSAY	METHOD	SPECIFICATION	RESULTS																
Appearance/Color/Clarity	T-0023	Clear, colorless liquid to pale straw liquid	Clear, colorless liquid.																
pH	T-0019	7.2-7.6	7.4																
Protein Concentration: UV Spectrophotometer Scan	T-0315	5.00-8.30 mg/mL	6.63 mg/mL																
Potency TF-1 Bioassay	T-0091	4.0 – 6.9 x 10 ⁶ IU/mg	5.8 x 10 ⁶ IU/mg																
Glu-C Peptide Mapping	T-0323	<p>N-Terminal Species Ala1: 65 – 76% Ala3: 24 – 35% Arg4: ≤1% Ser5: ≤5%</p> <p>No new peaks detected in the 280 nm chromatogram.</p> <p>Report Peak Area Ratios for peaks: G2 +G7p, Ala3, Ala1, G7p+G8, G10, G9, and G12p+G13. All Peak Area Ratios must lie within the range of 0.7 - 1.3.</p> <p>Elution profile at 214 nm comparable to Reference Standard.</p>	<p>N-Terminal Species Ala1: 71 % Ala3: 29 % Arg4: <QL % Ser5: <QL %</p> <p>Pass</p> <table><thead><tr><th>Peak</th><th>Ratio</th></tr></thead><tbody><tr><td>G2+G7p</td><td>1.0</td></tr><tr><td>Ala3</td><td>0.9</td></tr><tr><td>Ala1</td><td>1.0</td></tr><tr><td>G7p+G8</td><td>1.0</td></tr><tr><td>G10</td><td>1.0</td></tr><tr><td>G9</td><td>1.0</td></tr><tr><td>G12p+G13</td><td>1.0</td></tr></tbody></table> <p>Pass</p>	Peak	Ratio	G2+G7p	1.0	Ala3	0.9	Ala1	1.0	G7p+G8	1.0	G10	1.0	G9	1.0	G12p+G13	1.0
Peak	Ratio																		
G2+G7p	1.0																		
Ala3	0.9																		
Ala1	1.0																		
G7p+G8	1.0																		
G10	1.0																		
G9	1.0																		
G12p+G13	1.0																		
Monosaccharide Compositional Analysis	T-0108	3.63 – 5.22 moles of mannose/mole of Sargramostim 0.326 – 0.433 moles of N-acetylglucosamine/mole of Sargramostim	4.92 moles of mannose/mole of Sargramostim 0.404 moles of N-acetylglucosamine/mole of Sargramostim																

05/07/21 12:48 PM

CONFIDENTIAL – PROPRIETARY INFORMATION OF PARTNER THERAPEUTICS

Page 1 of 3



Leukine® (sargramostim) Recombinant Human Granulocyte Macrophage Colony Stimulating Factor Bulk Drug Substance	
Material Number: 12840	Batch Number: B26063
Date of Manufacture: 19Nov2020	Expiration Date: November, 2025
Specification: SPEC-0012-10	Effective Date: 10JUN2020
Partner Therapeutics Northpointe • 2625 162 nd St SW, Lynnwood WA 98087 • 425-245-1245	

ASSAY	METHOD	SPECIFICATION	RESULTS								
SDS-PAGE, Silver Stain (reduced, non-reduced)	T-0002	The mobility of the 3 bands of the test sample must correspond to the molecular weights based on comparison to MW markers and a rhu GM-CSF Reference Standard run on the same gel. Test sample displays no extra bands that are not present in the Ref Std.	Pass								
Isoelectric Focusing	T-0114	Major species migrates at pI 5.2 ± 0.2 with no more than 3 minor species evident in the pI range 4.5 to 5.2.	Pass								
Quantitative Analysis of Glycosylated Variants by RP-HPLC	T-0075	Peak 1 (related protein impurity): ≤ 4.0%. Peak area percentages of the three glycosylated components must be within the following ranges: Peak 2 (N-linked glycoform): 24-37% Peak 3 (O-linked glycoform): 15-27% Peak 4 (non-glycosylated glycoform): 38-52% The retention times for glycosylated component peaks 2, 3, and 4 of the test sample are within ± 0.5 minutes of those for the Reference Standard analyzed in the same run.	Peak 1: 2.3% Peak 2: 28% Peak 3: 24% Peak 4: 46% Comparison between test sample and Reference Standard <table><tr><th>Peak</th><th>Retention Time Difference (min)</th></tr><tr><td>2</td><td>0.0</td></tr><tr><td>3</td><td>0.0</td></tr><tr><td>4</td><td>0.0</td></tr></table>	Peak	Retention Time Difference (min)	2	0.0	3	0.0	4	0.0
Peak	Retention Time Difference (min)										
2	0.0										
3	0.0										
4	0.0										
Size Exclusion HPLC	T-0154	≤1.0% higher molecular weight component. Retention time for monomer peak is within ±0.5 minutes of Reference Standard. Elution profile is comparable to Reference Standard.	< QL % higher molecular weight component. 0.0 minutes retention time difference. Pass								
Scanning Densitometry	T-0013	Protein purity is ≥99% by area	Pass								

05/07/21 12:48 PM

CONFIDENTIAL – PROPRIETARY INFORMATION OF PARTNER THERAPEUTICS

Page 2 of 3



Leukine® (sargramostim) Recombinant Human Granulocyte Macrophage Colony Stimulating Factor Bulk Drug Substance	
Material Number: 12840	Batch Number: B26063
Date of Manufacture: 19Nov2020	Expiration Date: November, 2025
Specification: SPEC-0012-10	Effective Date: 10JUN2020
Partner Therapeutics Northpointe • 2625 162 nd St SW, Lynnwood WA 98087 • 425-245-1245	

ASSAY	METHOD	SPECIFICATION	RESULTS
Endotoxin (LAL) Assay	T-3007	≤ 1.25 EU/mg	<0.05 EU/mg
Microbial Content	T-3011	<1 CFU/mL	0 CFU/mL on TSA media 0 CFU/mL on SDA media

The signatures below indicate that the results meet specifications

Compiled by:	<i>Manda Clark</i>	Date:	<i>19 May 2021</i>
Approved by:	<i>REC</i>	Date:	<i>19 May 2021</i>

05/07/21 12:48 PM

CONFIDENTIAL – PROPRIETARY INFORMATION OF PARTNER THERAPEUTICS

Page 3 of 3

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2.3.S.4.5 Justification of Specifications

The specifications for sargramostim drug substance are based on manufacturing and development experience, ICH Guidance, and the capabilities of the analytical methods. Manufacturing and stability data are monitored and specifications are evaluated and revised, as appropriate.

2.3.S.5 REFERENCE STANDARDS OR MATERIALS

The sargramostim Reference Standard is selected from a released batch of drug substance; it is used to generate standard curves in quantitative assays, as a qualitative comparability standard, and as a control in assays required for release, as well as stability testing of drug substance and drug product batches.

Table 19 summarizes the results of testing and qualification of the current reference standard RS-121-4.

Table 19: Reference Standard RS-121-4 Qualification Results

Test Parameter	Acceptance Limit	Assay 1	Assay 2	Assay 3
Protein Concentration (UV Spectrophotometer Scan)	5.00 to 8.30 mg/mL	6.670 mg/mL 6.696 mg/mL 6.646 mg/mL	6.734 mg/mL 6.736 mg/mL 6.717 mg/mL	6.716 mg/mL 6.720 mg/mL 6.712 mg/mL
1Neutral Sugar Content (Acid H ydrolysis/A-415 nm)	40 to 159 µg hexose/mg rhu GM-CSF Ref. Std.	74 µg	86 µg	91 µg
Monosaccharide Compositional Analysis (Acid Hydrolysis/IEX)	Ratio of moles mannose to moles N- Acetylglucosamine is ≥ 5:1 and ≤ 15:1	8:1	12:1	12:1
Identity (SDS-PAGE; Silver Stain: Reduced and Non- Reduced)	Three bands compare to MW markers and rhu GM- CSF Ref. Std.; no extra bands not present in the Ref. Std.	Pass	Pass	Pass
Quantitative Analysis of rhu GM- CSF Glycosylated Variants (RP- HPLC)	Peak 1: ≤ 4.0%. Peak 2: 24 % to 37 % Peak 3: 15 % to 27 % Peak 4: 38 % to 52 % The retention times for Peaks 2, 3, and 4 are ± 0.5 minutes of Ref. Std.	Peak 1: 2.4 % Peak 2: 29 % Peak 3: 21 % Peak 4: 48 % Pass	Peak 1: 2.6 % Peak 2: 28 % Peak 3: 21 % Peak 4: 49 % Pass	Peak 1: 2.4 % Peak 2: 28 % Peak 3: 21 % Peak 4: 48 % Pass
High Molecular Weight Compon ent (SE-HPLC)	≤ 1.0 % HMWC. Elution profile and RT of monomer peak is ± 0.5 minutes of Ref. Std.	0.0 %	0.0 %	0.0 %
Relative Protein Composition and Purity (Scanning Densitometry)	Protein purity is ≥ 99 % by area	> 99 %	99 %	99 %
Isoelectric Focusing (Gel Electrophoresis)	Major species migrates at pI 5.2 ± 0.2 with no more than 3 minor species evident in the range pI 4.5 to 5.2	Pass	Pass	Pass
N-terminal glycosylate variants (Glu-C Peptide Mapping)	Ala1: 65 % to 76 % Ala3: 24 % to 35 % Arg4: ≤ 1% Ser5: ≤ 5% No new peaks detected at 280 nm in profile. Peak area ratios must be 0.7 to 1.3 for peaks: [G2+G7P] Ala3 Ala1 [G7P+G8] G10 G9 [G12+G13]	Ala1: 71 % Ala3: 29 % Arg4: < QL Ser5: < QL Pass 1.0 1.0 1.0 1.0 1.1 1.0 1.0 Pass	Ala1: 70 % Ala3: 30 % Arg4: < QL Ser5: < QL Pass 1.0 1.1 1.0 1.0 1.0 0.9 1.0 Pass	Ala1: 70 % Ala3: 30 % Arg4: < QL Ser5: < QL Pass 1.0 1.1 1.0 1.0 1.0 1.0 1.0 Pass

Test Parameter	Acceptance Limit	Assay 1	Assay 2	Assay 3
	214 nm elution profile compares to Ref. Std.			
Content Uniformity (UV Spectrophotometer Scan)	Aliquots selected at beginning, middle and end of aliquoting process. Mean protein concentration, SD and RSD are calculated. RSD must be \leq 6.0 %	Mean Protein Concentration = 6.68 mg/mL SD = 0.03 mg/mL RSD = 0.4 %		
TF-1 Bioassay	4.0 to 6.9 x 10 ⁶ IU/mg rhu GM- CSF	Log10 IU/mg Assay 1: 6.770 Assay 2: 6.931 Assay 3: 6.714	Log10 IU/mg Assay 1: 6.735 Assay 2: 6.725 Assay 3: 6.716	Log10 IU/mg Assay 1: 6.747 Assay 2: 6.679 Assay 3: 6.920
		Mean Log10 IU/mg = 6.81 Reported Specific Activity = 6.4 x 10 ⁶ IU/mg	Mean Log10 IU/mg = 6.73 Reported Specific Activity = 5.3 x 10 ⁶ IU/mg	Mean Log10 IU/mg = 6.78 Reported Specific Activity = 6.1 x 10 ⁶ IU/mg
Protein concentration	Calculated from 19 individual test values (3 x 3 values for UV Spec Scan assays and 10 x 1 values for the Content Uniformity assay).	Mean Protein Concentration = 6.69 mg/mL		

2.3.S.6 CONTAINER CLOSURE SYSTEM

The BDS container closure system is a narrow mouth, 2000-mL Nalgene® Teflon® bottle with a linerless screw cap closure. The container is molded from DuPont Teflon FEP (fluorinated ethylene propylene) 100J resin, and the closure is molded from Dupont Tefzel® ETFE (ethylenetetrafluoroethylene) HT2181 resin. Qualification and safety testing have been conducted on the container closure system to support storage of sargramostim bulk drug substance. Container closure materials have low extraction characteristics and meet requirements for use as articles or components of articles intended to contact food (21CFR§177.1550). In addition, the container closure meets USP <88> Class VI and USP <87> requirements for materials acceptable for use as articles or components of articles intended to contact intravenous solutions.

2.3.S.7 STABILITY

2.3.S.7.1 Stability Summary and Conclusions

Stability data of sargramostim drug substance through 96 months support a 60-month expiry when stored at long-term $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$ storage condition. Accelerated stability data at 5, 15, 25 and 40°C storage conditions were collected to understand susceptibility to degradation, to demonstrate the capability of analytical methods to detect degradation, and to support excursions during handling, shipping, and storage.

2.3.S.7.2 Postapproval Stability Protocol and Stability Commitments

The long-term storage condition for drug substance is $-70^{\circ}\text{C} \pm 10^{\circ}\text{C}$. At least one batch of sargramostim drug substance, manufactured annually, is placed on stability studies each calendar year (unless a batch is not manufactured in that year). The stability tests and schedule are presented in [Table 20](#). The stability specifications are presented in [Table 15](#).

Table 20: Stability Testing Schedule for the Drug Substance

Test	Analytical Procedure	Storage Time (months)				
		0	12	36	48	60
Appearance (Visual)	T-0023	I	x	x	x	x
Potency (Bioassay)	T-0091	I	x	x	x	x
Purity (Glu-C Peptide Mapping)	T-0323	I	x	x	x	x
Purity (SDS-PAGE)	T-0002	I	x	x	x	x
Purity (Glycosylated Variants)	T-0075	I	x	x	x	x
Purity (High Molecular Weight Components)	T-0154	I	x	x	x	x
pH	T-0019	I	x	x	x	x

I = Initial release data

2.3.S.7.3 Stability Data

[Table 21](#) summarizes representative batches of sargramostim drug substance on stability, storage conditions, available stability data, and purpose of the study. All stability data meet applicable specifications within the expiry period under long-term storage conditions (-70°C). There is no evidence of any significant physical or chemical changes in sargramostim drug substance at the long-term storage condition.

Table 21: Summary of Sargramostim Drug Substance Batches on Stability

Batch Number	Storage Condition	Stability Data	Purpose	Reference
B23076	Long-term (-70°C)	60 months	2016 Annual stability	Table 22
B24483	Long-term (-70°C)	36 months	2019 Annual stability	Table 23
B25981	Long-term (-70°C)	12 months	2020 Annual Stability	Table 24

Table 22: Sargramostim Drug Substance, Batch 12840/B23076, Storage Condition -70 °C, Upright

Date Manufactured: 11/17/2016

Date Study Started: 12/15/2016

Date of Expiry: 11/17/2021

Purpose of Study: Annual stability

Attribute/Test	Specification	Time (Months)					
		0	12	24	36	48	
Appearance/Color/ Clarity	Clear, colorless to pale straw liquid (CCL-PSL)	CCL	CCL	CCL	CCL	CCL	CCL
TF-1Bioassay	4.0-6.9 E+06 IU/mg	6.4	5.3	6.3	5.9	6.4	6.6
SDS-Page Silver Stain (Reduced, Non- Reduced)	No new bands/ equivalent to reference standard (NB/Equiv)	NB/ Equiv	NB/ Equiv	NB/ Equiv	NB/Equiv	NB/ Equiv	NB/Equiv
RP-HPLC	[SC:4/11/16]						
Peak 1	≤ 4.0% ≤ 4.0%	2.4	2.4	2.4	2.4	2.5	2.5
Peak 2	25-38% 24-37%	29	28	28	28.0	27.5	28.5
Peak 3	15-27% 15-27%	21	21	21	21.6	21.8	20.7
Peak 4	39-53% 38-52%	48	48	49	48.1	48.2	48.3
SE-HPLC	≤ 1.0%	0.0	0.0	< 0.1	< 0.1	<0.1	<0.1
pH	7.2 -7.6	7.4	7.4	7.4	7.4	7.4	7.5
Glu-C Peptide Mapping							
% Ala1	65-76%	70.8	70.6	70.3	70.2	69.9	70.0
% Ala3	24-35%	29.2	29.4	29.7	29.8	30.1	30.0
% Arg4	≤ 1%	<QL	<QL	<QL	<QL	<QL	<QL
% Ser5	≤ 5%	<QL	<QL	<QL	<QL	<QL	<QL
Scheduled Test Date		Dec-16	Dec-17	Dec-18	Dec-19	Dec-20	Dec-21

QL=Quantitation Limit

Table 23: Sargramostim Drug Substance, Batch 12840/B24483, Storage Condition -70 °C, Upright

STABILITY SUMMARY DATA TABLE					
MATERIAL NUMBER	12840	BATCH NUMBER	B24483	STORAGE CONDITIONS	-70 C
DATE MANUFACTURED	07AUG2019	DATE STUDY STARTED	07AUG2019	DATE OF EXPIRATION	07AUG2024
PURPOSE OF STUDY	ANNUAL STABILITY				

TEST	SPECIFICATION	TIMEPOINT (MONTHS)					
		0	12	24	36	48	60
Appearance/Color/Clarity	CCL	CCL	CCL	CCL	CCL		
TF-1Bioassay	4.0-6.9 E+06 IU/mg	6.4	6.4	6.2	6.3		
SDS-Page Silver Stain (Reduced, Non-Reduced)	No new bands/ equivalent to reference standard (NB/Equiv)	NB/Equiv	NB/Equiv	NB/Equiv	NB/Equiv		
RP-HPLC							
Peak 1	≤ 4.0%	2.5	2.5	2.5	2.8		
Peak 2	24-37%	28.8	27.9	29.4	28.7		
Peak 3	15-27%	20.9	22.4	20.6	21.2		
Peak 4	38-52%	47.9	47.2	47.4	47.3		
SE-HPLC	≤ 1.0%	<0.1	<0.1	<0.1	<0.1		
Glu-C Peptide Mapping							
% Ala1	65-76%	70.4	70.3	70.1	70.7		
% Ala3	24-35%	29.6	29.7	29.9	29.3		
% Arg4	≤ 1%	<QL	<QL	<QL	<QL		
% Ser5	≤ 5%	<QL	<QL	<QL	<QL		
pH	7.2 – 7.6	7.42	7.43	7.38	7.37		
SCHEDULED TEST DATE		AUG19	AUG20	AUG21	AUG22	AUG23	AUG24

Table 24: Sargramostim Drug Substance, Batch 12840/B25981, Storage Condition -70 °C, Upright

Date Manufactured: 10/28/2020

Date Study Started: 10/30/2020

Date of Expiry: 10/28/2025

Purpose of Study: 2020 Annual stability

Attribute/Test	Specification	Time (Months)					
		0	12	24	36	48	
Appearance/Color/ Clarity	Clear, colorless to pale straw liquid (CCL-PSL)	CCL	CCL				
TF-1Bioassay	4.0-6.9 E+06 IU/mg	6.0	6.9				
SDS-Page Silver Stain (Reduced, Non- Reduced)	No new bands/ equivalent to reference standard (NB/Equiv)	NB/ Equiv	NB/ Equiv				
RP-HPLC Peak 1	≤ 4.0%	2.4	2.2				
Peak 2	24-37%	28.1	28.3				
Peak 3	15-27%	23.1	23.1				
Peak 4	38-52%	46.4	46.3				
SE-HPLC	≤ 1.0%	<0.1	<0.1				
pH	7.20 -7.60	7.44	7.43				
Glu-C Peptide Mapping % Ala1	65-76%	71.0	72.1				
% Ala3	24-35%	29.0	27.9				
% Arg4	≤ 1%	<QL	<QL				
% Ser5	≤ 5%	<QL	<QL				
Scheduled Test Date		Oct-20	Oct-21	Oct-22	Oct-23	Oct-24	Oct-25

2.3.P DRUG PRODUCT (LEUKINE FOR INJECTION)

2.3.P.1 DESCRIPTION AND COMPOSITION OF THE DRUG PRODUCT

Leukine for Injection, 250 µg/vial, drug product is provided as a sterile, preservative-free, lyophilized powder in an 8 mL, Type I glass vial. A vial of drug product includes the active ingredient sargramostim (recombinant DNA human granulocyte macrophage colony-stimulating factor) and the excipients Mannitol USP, Sucrose NF, and Tromethamine USP. During compounding 1 N Hydrochloric Acid is added to adjust the pH of the formulation buffer. The vial contains an overfill of 14 µg. The lyophilized drug product is reconstituted with either 1 mL Sterile Water for Injection USP (SWFI) or 1 mL Bacteriostatic Water for Injection USP (BWFI) for a 250 µg sargramostim/mL solution for administration. After reconstitution the volume of the drug product is approximately 1.05 mL.

2.3.P.1.1 Composition (Leukine for Injection, 250 µg/vial)

The quantitative formulation of Leukine for Injection, 250 µg/vial, is presented in [Table 25](#).

Table 25: Unit Formula of Leukine for Injection, 250 µg/vial

Ingredients	Quantity/vial ¹	Quantity/mL ²	Pharmaceutical Function	Quality Standards
Active Ingredient				
Sargramostim	264 µg	250 µg	Active ingredient	In-house ³
Inactive Ingredients				
Mannitol	42.0 mg	40.0 mg	Bulking agent	USP
Sucrose	10.5 mg	10.0 mg	Stabilizer	NF
Tromethamine ⁴	1.27 mg	1.21 mg	Buffer component	USP
1 N Hydrochloric Acid	q.s. ⁵	NA	pH adjustment	Footnote 6
Water for Injection	NA ⁷	1 mL	Solvent	USP
Nitrogen	q.s. ⁸	NA	Vacuum neutralization	NF

q.s. = Quantity sufficient; NA = Not applicable

¹ The vial includes an overfill of 14 µg sargramostim.

² The active ingredient and excipients in 1 mL after reconstitution with 1 mL Sterile Water for Injection USP (SWFI) or 1 mL Bacteriostatic Water for Injection USP (BWFI).

³ Refer to Section 3.2.S.5, Reference Standards or Materials.

⁴ The quantity includes Tromethamine from the drug substance sargramostim, which is in 0.1 M Tromethamine buffer, pH 7.4 (7.2 – 7.6).

⁵ Quantity sufficient to adjust the pH to 7.2 – 7.6.

⁶ For adjusting the pH, a 1 N Hydrochloric Acid solution is prepared with Hydrochloric Acid NF and Water for Injection USP.

⁷ The water is essentially removed during lyophilization.

⁸ The vials are backfilled with nitrogen prior to complete stopper insertion.

2.3.P.2 PHARMACEUTICAL DEVELOPMENT

2.3.P.2.1 Drug Substance

The active ingredient in the drug product Leukine for Injection is sargramostim, a recombinant human granulocyte macrophage colony-stimulating factor (rhu GM-CSF) that functions as an immunostimulator. Sargramostim is used for myeloid reconstitution after autologous or allogeneic bone marrow transplantation. It is also used to treat neutropenia induced by chemotherapy during treatment of acute myeloid leukemia and as a medical countermeasure for treating people who have been exposed to sufficient radiation to suppress bone marrow myelogenesis. All indications approved for Leukine for Injection are provided in the United States Prescribing Information (USPI).

2.3.P.2.2 Excipients

Excipients present in Leukine for Injection are pharmacopoeia grade. These excipients were selected based on compatibility with sargramostim drug substance, dosage form, manufacturing process, and administration method. There has been no evidence of incompatibility between sargramostim glycosylated drug substance isoforms and drug product excipients tromethamine, sucrose, and mannitol based on review of drug product release data of glycosylated variants.

2.3.P.2.3 Formulation Development

Development of drug product Leukine for Injection, a legacy product, started after identification of the human granulocyte-macrophage colony-stimulating factor (hu GM-CSF) sequence in 1985. A recombinant form of human GM-CSF (rhu GM-CSF) was produced in yeast with the resulting drug substance sargramostim formulated into the drug product Leukine for Injection. The Leukine for Injection marketing authorization application (MAA) was submitted by Immunex Corp., Seattle WA, USA, as a Process License Application (PLA) and Establishment License Application (ELA), which were documents required for a biologic drug product registration in the United States prior to 2000. The initial MAA was approved by the U.S. FDA Center for Biologics Evaluation and Research (CBER) in 1991. Formulation development of Leukine for Injection was not included in the initial MAA since it was not required in an MAA at that time. In addition, the Leukine for Injection MAA has passed through multiple owners with minimal development work performed on drug product formulation since initial approval, except for introduction of a liquid formulation that was subsequently withdrawn from commercialization. The PLA and ELA were converted and submitted as a BLA (103362) in CTD format after the U.S. Federal Register regulation effective date of 30 December 1999.

The formulation of Leukine for Injection, as originally developed, consists of excipients tromethamine, sucrose, and mannitol in water for injection (WFI). Drug product manufacture begins with addition of excipients to WFI followed by adjusting pH to approximately 7.4 (range 7.2 – 7.6) with 1 N hydrochloric acid (prepared using concentrated hydrochloric acid and water for injection). Tromethamine provides buffering while sucrose and mannitol act as stabilizers and bulking agents. This formulation is considered robust after three decades of commercialization.

Leukine for Injection drug product includes an overfill of 14 µg sargramostim per vial. During manufacturing, the batch is formulated as 264 µg sargramostim per mL of formulation solution with each vial filled with 1 mL of solution and lyophilized. Drug product is reconstituted with either 1 mL sterile

water for injection or 1 mL bacteriostatic water for injection with a resulting volume of approximately 1.05 mL, which comprises the diluent volume plus the lyophilized cake mass).

2.3.P.2.4 Process Development

The Leukine for Injection manufacturing process comprises formulation (compounding) of excipients, pH adjustment of the compounded solution, addition of bulk drug substance sargramostim, sterile filtration, filling of vials, lyophilization, capping, and 100 % visual inspection. This drug product manufacturing process has remained as originally submitted with only minor process revisions (i.e., increased process time, elimination of bulk density testing, and bulk sterility testing). The manufacturing process is appropriate for a sterile, lyophilized drug product.

2.3.P.2.5 Container Closure System

The tubing glass vials were selected due to the superior dimensional tolerances of tubing glass over molded glass vials, making them more suitable for a lyophilization process. Tubing vials have a low coefficient of expansion with uniform thickness on the surface allowing less resistance to heat transfer during lyophilization, which results in a more effective freeze dry cycle. Because the drug product formulation is relatively neutral pH (7.2 – 7.6) and the storage temperature is refrigerated (2 – 8 °C), there is low risk of glass delamination with tubing vials.

The stoppers are coated on the product contact side with FluroTec (Daikyo-Fluro Resin D) and on the bottom side of the stopper flange (land seal) with a B2 coating. The FluroTec barrier film minimizes interaction between the drug product and the stopper reducing the absorption and adsorption of the drug product while maintaining container closure integrity. The B2 coating is a cross-linked mixture of high- and low-molecular weight silicone oils that have lower levels of particles compared to conventional silicone oil. The stoppers were selected to eliminate natural latex rubber that has the potential to result in allergic reactions and to reduce potential particulates in the drug product. The seals used for Leukine for Injection are 20 mm West Flip-Off seals that have a dark blue matte 6 bridge plastic button.

2.3.P.2.6 Compatibility

Leukine for Injection drug product is formulated in 0.1 M tromethamine buffer, pH 7.4 (7.2 – 7.6). The active ingredient sargramostim, with a theoretical pI of 4.85, is completely soluble in the formulation buffer. Upon reconstitution with either Sterile Water for Injection USP (SWFI) or Bacteriostatic Water for Injection USP (BWFI), with both diluents in the pH range of 5.0 – 7.0, the amount of buffer capacity of the 0.1 M tromethamine is sufficient to maintain the reconstituted drug product at a pH range of 7.1 – 7.7. The pH of reconstituted Leukine for Injection is optimal for a drug product administered by either SC injection or IV infusion, which is within the physiological pH range of 7 – 8.

Results of chemical and microbial in-use stability studies when Leukine is reconstituted with Sterile Water for Injection USP support an in-use hold time of not more than 24 hours when held at 2 – 8 °C. Results of chemical and microbial in-use stability studies when Leukine is reconstituted with Bacteriostatic Water for Injection USP support an in-use hold time of not more than 20 days when held at 2 – 8 °C.

2.3.P.3 MANUFACTURE

2.3.P.3.1 Manufacturers

Leukine for Injection drug product manufacturing, primary and secondary packaging (with labeling to identify as active) are performed in accordance with current Good Manufacturing Practices at the following facility:

Patheon Manufacturing Services LLC (Patheon)
5900 Martin Luther King Jr. Highway
Greenville, NC 27834
United States

FDA Facility Establishment Identifier (FEI): 1018495
Data Universal Numbering System (DUNS): 079415560

Leukine for Injection drug product storage, primary and secondary labeling, and assembly, QP release and distribution are performed in accordance with current Good Manufacturing Practices at the following facility:

Victoria Pharmaceuticals
The Plenum Building
Royal Group of Hospitals Site
Grosvenor Road
Belfast BT12 6BA
United Kingdom

MHRA Site Number: 1683129
MIA (IMP) Number: 32485

Leukine for Injection drug product is imported by:

Tanner Pharma UK Limited
The Tithe Barn
Harpendenbury Farm, Harpendenbury
Redbourn, St. Albans AL13 7QA
United Kingdom

In-process testing including identity of the bulk drug substance, endotoxin, and sterility are performed at Patheon. The release and stability testing of Leukine for Injection are performed at the following facilities:

Table 26: Testing Facilities and Tests Performed

Testing Facility	Release Testing	Stability Testing
Partner Therapeutics, Inc. 2625 162 nd Street SW Lynnwood, WA 98087 United States FDA FEI: 3007934434 DUNS: 081059614	All tests except for USP <790>, bacterial endotoxins, sterility, and particulate matter	All tests except for particulate matter.
Patheon Manufacturing Services LLC 5900 Martin Luther King Jr. Highway Greenville, NC 27834 United States	USP <790>, bacterial endotoxins, and sterility	Not Applicable

Testing Facility	Release Testing	Stability Testing
Nitto Avecia Pharma Services, Inc. 10 Vanderbilt Irvine, CA 92618 United States FDA FEI: 3012971227 DUNS:116975565	Particulate matter	Particulate matter

2.3.P.3.2 Batch Formula

The batch formula for the manufacture of Leukine for Injection drug product is provided in Table 27. The quantities provided in the batch formula are provided on a per liter basis of approximately 1,000 vials and nominal commercial batch size of approximately 54,000 vials. Batches may be between 48,000 and 59,000 vials (approximately $\pm 10\%$ of 54,000 vials). A batch of drug product will be compounded with an excess of approximately 4 liters, which is used to flush the bioburden reducing and sterilizing filters. After flushing the filters, the flushes are discarded.

Table 27: Batch Formula of the Drug Product Leukine for Injection, 250 µg/vial

Ingredient	Reference to Standards	Per Liter Quantity ¹	Nominal Commercial Batch Quantity per 58 L ^{1,2}
Sargramostim ³	In-house	0.264 g ⁴ (approximately 0.04 L) ⁵	15.312 g ⁴ (approximately 2.32 L) ⁵
Mannitol	USP	42 g	2.436 kg
Sucrose	NF	10.5 g	609.0 g
Tromethamine ⁶	USP	0.77 g ⁷	44.66 g ⁷
1 N Hydrochloric Acid	Footnote 8	q.s. ⁹	q.s. ⁹
Water for Injection	USP	q.s. to 1 kg (approximately 0.98 L)	q.s. to 58.93 kg (approximately 58 L)
Nitrogen ¹⁰	NF	q.s.	q.s.

q.s. = Quantity sufficient

¹ Actual quantities of excipients dispensed may vary slightly from the theoretical batch formula (i.e., cumulative variation not more than $\pm 10\%$).

² A volume of 58 liters is compounded to manufacture approximately 54,000 vials that includes approximately 4 liters for filter flushes.

³ The drug substance is provided in 0.1 M tromethamine buffer, pH 7.4 (7.2 – 7.6).

⁴ The drug product is formulated with an overfill of 14 µg/vial (compounding concentration of 264 µg/mL).

⁵ The quantity of drug substance solution used is based on the assay value of sargramostim; the drug substance target assay is 7.0 mg/mL (historical range is 6.04 – 6.98 mg/mL with an average of 6.6 mg/mL). The quantity of drug substance included in Table 1 uses the historical average of 6.6. mg/mL.

⁶ Also referred to as tris(hydroxymethyl)aminomethane (i.e., TRIS).

⁷ The quantity (grams) of Tromethamine provided is based on a drug substance concentration of 6.6 mg/mL and a volume of 2.32 liters. The actual amount of Tromethamine added is dependent upon the volume of the bulk drug substance and is calculated for each batch.

⁸ For adjusting the pH, a 1 N Hydrochloric Acid solution is prepared with Hydrochloric Acid NF and Water for Injection USP.

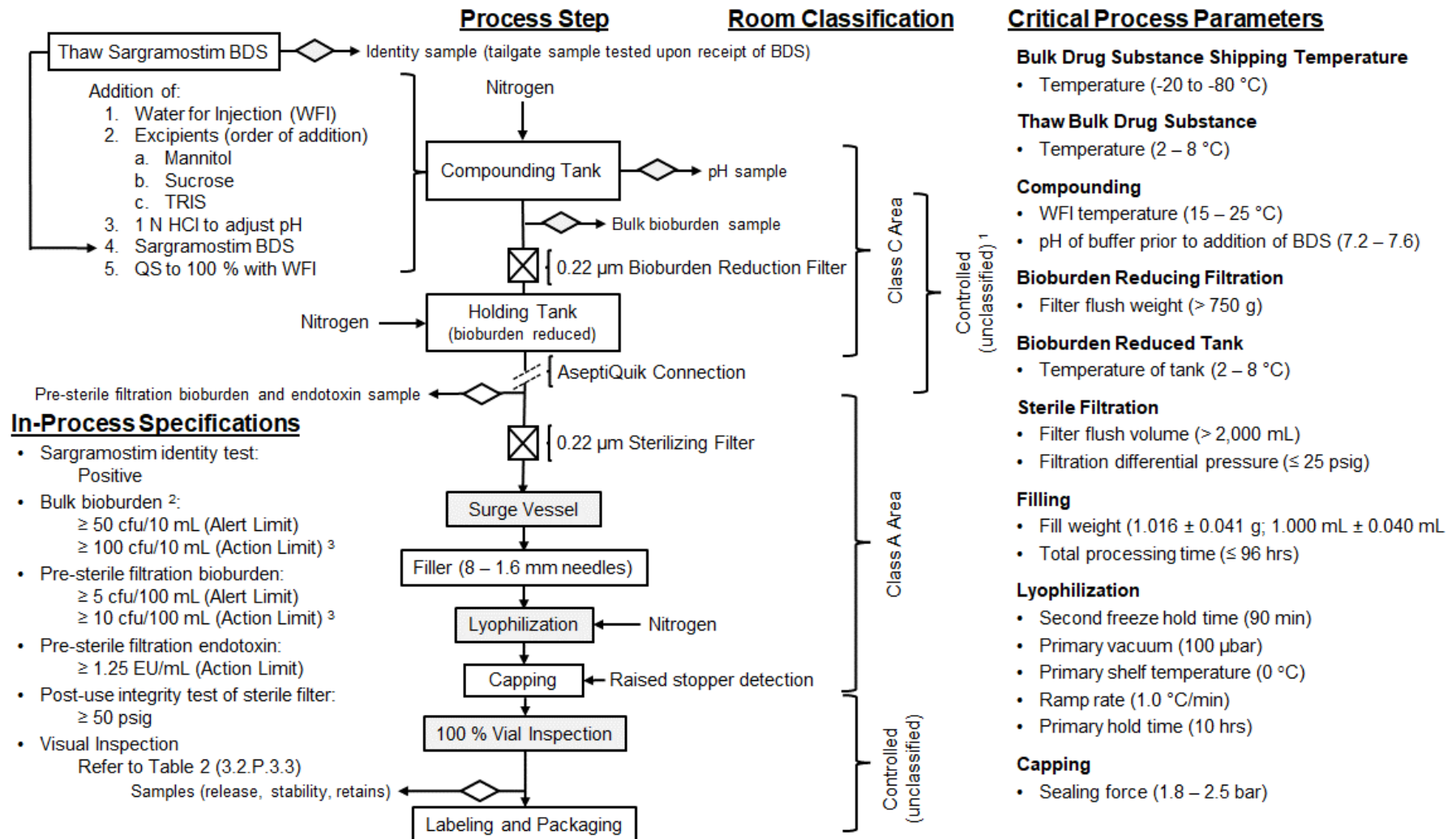
⁹ Quantity sufficient to adjust pH 7.2 – 7.6.

¹⁰ The vials are backfilled with nitrogen to neutralize the vacuum of the lyophilization step prior to stopper insertion.

2.3.P.3.3 Description of Manufacturing Process and Process Controls

A schematic of the manufacturing process, including critical process parameters (CPPs), is provided in [Figure 8](#).

Figure 8: Schematic of the Drug Product Manufacturing Process



¹ The bioburden reduced tank, after filtration, is moved from Class C area into Controlled (unclassified) area and connected to a control panel for sterile filtration in a Class A area.

² Bulk bioburden is listed as an IPS but not identified as a CPP.

³ Should the Action Limit meet or exceed the limit, the results will be investigated, and a determination made as to the disposition of the batch (i.e., ability to forward process).

2.3.P.3.3.1 Compounding

Frozen sargramostim bulk drug substance is thawed at 2 – 8 °C for ≥ 56 and ≤ 152 hr (time from thaw to addition of BDS to the compounding vessel).

In a Class C area, a dedicated stainless-steel 120-liter compounding tank is placed on a floor scale and $80\% \pm 0.2$ kg of the calculated amount of Water for Injection (WFI) at 15 – 25 °C is added to the tank. The quantity of excipients Mannitol USP, Sucrose NF, and Tromethamine USP are calculated on a per L basis for the intended batch.

Mannitol, sucrose, and TRIS (tromethamine) are added individually to the compounding tank in the order presented. After each addition the solution is mixed (200 ± 10 rpm) and dissolution is confirmed by visual observation. With mixing (200 ± 10 rpm) 1 N HCl is added to the compounding tank to adjust the pH of the bulk excipient buffer to 7.2 – 7.6 prior to addition of sargramostim BDS. The solution is mixed, the tank is sampled, and the pH measured. If the pH is not within range, incremental amounts of 1 N HCl are added to the bulk solution. After each addition of 1 N HCl the tank is mixed (200 ± 10 rpm) prior to measuring the pH. When the pH is within range (7.2 – 7.6) the mixing speed is decreased to 85 ± 10 rpm. After the pH of the buffer solution has been adjusted, the thawed BDS is added to the compounding tank with mixing (85 ± 10 rpm).

WFI (15 – 25 °C) is added to the compounded drug product (DP) solution to the batch weight (± 0.2 kg). After WFI addition is complete, the solution is mixed at 85 ± 10 rpm. The maximum allowed time from start of filling the tank with WFI to the bioburden reduction filtration process step is ≤ 24 hr.

2.3.P.3.3.2 Bioburden Reduction Filtration

The compounding tank is connected to a 0.22 µm hydrophilic cartridge filter which leads to a holding tank. Using filtered nitrogen, the compounding tank is pressurized to 27.5 psia, the bottom valve opened, and the DP solution filtered into the holding tank. A bubble point integrity test is performed on the 0.22 µm filter pre- and post-filtration. To remove any potential oxidizable substances from the bioburden reducing filter, the initial > 750 g of filtered DP solution is collected immediately after the filter and discarded. When filtration is complete the holding tank is pressurized to 16 – 20 psia with nitrogen and cooled to 2 – 8 °C. The maximum allowable hold time in the holding tank is ≤ 72 hr.

2.3.P.3.3.3 Sterile Filtration and Filling

A pre-filtration bubble point integrity test is performed on the sterilizing 0.22 µm hydrophilic cartridge filter prior to equipment set-up. The holding tank is transferred to a controlled but unclassified area outside of the Class A filling area. The holding tank outlet valve is connected to the sterilizing 0.22 µm filter in the Class A area, which flows into a surge vessel. The holding tank is pressurized with filtered nitrogen to 18 – 22 psia (3.3 – 7.3 psig; ≤ 25 psig), the bottom valve of the holding tank is opened to allow DP solution to pass through the sterilizing filter and fill the surge vessel.

To remove any potential oxidizable substances from the sterilizing filter, the initial $> 2,000$ mL of sterile filtered DP solution is purged from the surge vessel. The surge vessel is refilled, and the sterile DP solution is filled into 8 mL, Type I glass vials. The filler is equipped with an

automated in-line non-destructive weight check scale that collects vial tare and gross weight to achieve fill weights within 1.016 ± 0.041 g.

Filled vials are partially stoppered on the filling line as they exit the filling area and are transported via transfer belt to the automated lyophilizer loading table. All transfer and automated loading system components are located in a Class A area. The processing time from addition of WFI to the compounding tank to the last vial loaded into the lyophilizer is ≤ 96 hr.

2.3.P.3.3.4 Lyophilization

The lyophilizer shelves are cooled and controlled to 5 °C and loaded. Upon completion of the lyophilizer loading, the door is closed, and a fully automated cycle (Table 28) is initiated via the lyophilizer control system.

Table 28: Leukine Lyophilizer Cycle

Step	Step Description	Cycle Setting
1	Product load temp	5 °C
2	Product hold time	60 min
3	Ramp down to	-40 °C
4	Ramp rate	0.5 °C/min
5	1st freeze hold time	90 min
6	Annealing temp	-20 °C
7	Ramp rate	0.5 °C/min
8	Annealing hold time	120 min
9	Ramp down to	-40 °C
10	Ramp rate	0.5 °C/min
11	2nd freeze hold time	90 min.
12	Primary vacuum	100 µbar (75 µm)
13	Primary shelf temp	0 °C
14	Ramp rate	1.0 °C/min
15	Primary hold time	10 hr
16	Secondary dry temp	35 °C
17	Ramp rate	0.5 °C/min.
18	Secondary vacuum	100 µbar (75 µm)
19	Secondary hold time	14.8 hr
20	Vacuum break	800 mbar (11.6 psia)
21	Seat stoppers	1900 psig
22	Hold temp	5 °C

Step	Step Description	Cycle Setting
23	Hold pressure	Atmospheric

When capping starts the vials are fed to the capper that is fitted with a “no stopper” and “high stopper” detector so that triggering either of these sensors will reject the vial. Capped vials are transported to a tray loader located in a non-classified area, loaded into trays, and palletized. The pallets are stored at 2 – 8 °C until visually inspected.

2.3.P.3.3.5 Visual Inspection

All vials are manually visually inspected by qualified inspectors.

2.3.P.3.4 Controls of Critical Steps and Intermediates

Table 29 provides the critical process parameters (CPP), which are operating parameters controlled during manufacturing of Leukine for Injection drug. Also included are in-process specifications (IPS), which are tests that confirm the drug product is acceptable to forward process (Table 30).

Table 29: Critical Process Parameters

Critical Process Parameter	Limit
BDS thaw temperature	2 – 8 °C
WFI temperature filled into compounding tank	15 – 25 °C
pH of buffer after HCl addition prior to addition of BDS	7.2 – 7.6
Bioburden reduction filter purge weight	> 750 g
Temperature of bioburden reduced holding tank	2 – 8 °C
Sterile filtration pressure	≤ 25 psig
Sterilization filter purge volume	> 2,000 mL
Fill volume per vial	1.000 ± 0.040 mL
Formulated bulk hold time (i.e., total time from addition of WFI to compounding tank to last vial loaded into lyophilizer)	≤ 96 hrs
2nd freeze hold time	90 min
Primary vacuum	100 µbar (75 µ)
Primary shelf temperature	0 °C
Ramp rate	1.0 °C/min
Primary hold time	10 hrs

BDS = Bulk drug substance; WFI = Water for injection; psig = Pounds per square inch gauge

¹ The limit is a set number of the validated lyophilization computer program that runs the process

Table 30: In-Process Specifications for Manufacture of Leukine for Injection

Parameter	Analytical Method	Acceptance Criteria
Identity of sargramostim bulk drug substance	Immuno-dot blot	Identity confirmed
Pre-sterile filtration bioburden TAMC and TYMC	USP <61>	≥ 5 cfu/100 mL (Alert Limit) ≥ 10 cfu/100 mL (Action Limit)
Pre-sterile filtration endotoxin	USP <85>	≥ 1.25 EU/mL (Action Limit)
Post-use membrane integrity test of sterilization filter	Bubble point test	≥ 50 psig

TAMC = Total aerobic microbial count; TYMC = Total yeast and mold count; psig = Pounds per square inch gauge; EU = Endotoxin units

2.3.P.3.5 Process Validation and/or Evaluation

Process validation of Leukine for Injection drug product has been completed at Patheon Manufacturing Services LLC, Greenville, North Carolina, USA. All CPPs, IPCs, and analytical data have met the validation criteria approved in the Process Validation Protocol. Partner Therapeutics will maintain a lifecycle approach to manufacture of Leukine for Injection, a legacy product, through continuous process verification at Patheon. The validated state will be maintained through process monitoring, investigations as needed, updates based on regulatory expectations, and continuous process improvements. The current drug product process validation results demonstrate that the manufacturing process successfully produces Leukine for Injection in a controlled and consistent manner.

2.3.P.4 CONTROL OF EXCIPIENTS

The excipients in Leukine for Injection drug product comply with the current requirements of the United States Pharmacopoeia (USP) or National Formulary (NF) monographs. However, the acceptance limits for bacterial endotoxins of Mannitol USP and Sucrose NF are tighter than their respective monograph limits. In addition, a bacterial endotoxin limit for Tromethamine USP has been added. The acceptance criteria for bacterial endotoxins (USP <85>) for mannitol, sucrose, and tromethamine are included in [Table 31](#).

In addition, Hydrochloric Acid NF, used to manufacture a 1 N solution for pH adjustment, Water for Injection USP, which is the drug product compounding solvent, and Nitrogen NF, which is for vacuum neutralization, are used in the manufacture of Leukine for Injection.

Table 31: Endotoxin Specifications of Excipients in Leukine for Injection

Excipient	Reference to Standards	Bacterial Endotoxin Limits
Mannitol	USP	< 2.5 IU/g (< 0.0025 IU/mg)
Sucrose	NF	< 2 IU/g (< 0.002 IU/mg)
Tromethamine ¹	USP	≤ 2.5 IU/g (≤ 0.0025 IU/mg)

¹ Also referred to as tris(hydroxymethyl)aminomethane (i.e., TRIS).

2.3.P.5 CONTROL OF DRUG PRODUCT

2.3.P.5.1 Specifications

2.3.P.5.1.1 Leukine for Injection Release Specifications

Drug product batches of Leukine for Injection are tested and must conform to the specifications provided in [Table 32](#).

Table 32: Release Specifications, Leukine for Injection, 250 µg/vial

Test	Analytical Procedure	Acceptance Criteria
Appearance and Description		
Lyophilized Product	T-0023	White cake
Reconstituted Solution	T-0023 USP <790>	Clear, colorless liquid Essentially free of visible particulates
Identity and Purity		
SDS-PAGE	T-0002	The mobility of 3 bands must correspond to molecular weight as compared to MW markers and sargramostim Ref. Std. run on the same gel; sample displays no extra bands not present in Ref. Std.
Peptide Mapping	T-0323	N-terminal species: Ala1: 60 – 85 % Ala3: 15 – 40 % Arg4: ≤ 2 % Ser5: ≤ 5 % Elution profile comparable to Ref. Std.
Quantity		
Protein Concentration	T-0397	225 – 275 µg/mL (± 10 % label claim)
Potency		
Bioassay	T-0091	4.0 – 6.9 x 10 ⁶ IU/mg
Purity and Related Substances		
Glycosylated Variants	T-0075	Peak 1 (related protein substance): ≤ 5.6 % Peak area percentages of 3 glycosylated variants: Peak 2 (N-linked glycoform): 24 – 41 % Peak 3 (O-linked glycoform): 13 – 31 % Peak 4 (non-glycosylated glycoform): 34 – 52 % The retention times of peaks 2, 3, and 4 ± 0.5 min. of Ref. Std.
HMW Components	T-0154	≤ 4.0 %

Test	Analytical Procedure	Acceptance Criteria
		The retention time of monomer peak is ± 0.5 min. of Ref. Std. and elution profile comparable to Ref. Std.
General		
Reconstitution Time	T-0057	≤ 120 seconds
pH of Reconstituted Solution	USP <791> (T-0019)	7.1 – 7.7
Water ¹	USP <921> ² (T-0022)	Mean of 20 vials ≤ 2.0 %; no individual vial > 3.0 %
Particulate Matter Particles ≥ 10 µm Particles ≥ 25 µm	USP <788> ³ (T-0033)	$\leq 6,000$ particles/vial ≤ 600 particles/vial
Uniformity of Dosage Units	USP <905> ⁴ (T-0010)	Pass
Safety		
Bacterial Endotoxins	USP <85> ⁵	≤ 1.25 EU/mL
Sterility	USP <71> ⁶	No Growth

Ref. Std. = Reference standard; IU = International unit; HMW = High molecular weight; EU = Endotoxin unit

¹ Reported as % moisture

² Karl Fischer method

³ Light obscuration method

⁴ Weight variation method

⁵ Kinetic chromogenic method

⁶ Membrane filtration method

2.3.P.5.1.2 Leukine for Injection Shelf-life Specifications

Drug product batches of Leukine for Injection are tested throughout shelf-life and must conform to the specifications provided in [Table 33](#).

Table 33: Shelf-life Specifications, Leukine for Injection, 250 µg/vial

Test	Analytical Procedure	Acceptance Criteria
Appearance and Description		
Lyophilized Product	T-0023	White cake
Reconstituted Solution	T-0023	Clear, colorless liquid
Quantity		
Protein Concentration	T-0397	225 – 275 µg/mL (± 10.0 % label claim)
Potency		
Bioassay	T-0091	4.0 – 6.9 x 10 ⁶ IU/mg

Test	Analytical Procedure	Acceptance Criteria
Purity and Related Substances		
SDS-PAGE	T-0002	The mobility of 3 bands must correspond to molecular weight as compared to MW markers and sargramostim Ref. Std. run on the same gel; sample displays no extra bands not present in Ref. Std.
Peptide Mapping	T-0323	N-terminal species: Ala1: 60 – 85 % Ala3: 15 – 40 % Arg4: ≤ 2 % Ser5: ≤ 5 % Elution profile comparable to Ref. Std.
Glycosylated Variants	T-0075	Peak 1 (related protein substance): ≤ 6.8 % Peak area percentages of 3 glycosylated variants: Peak 2 (N-linked glycoform): 24 – 41 % Peak 3 (O-linked glycoform): 13 – 31 % Peak 4 (non-glycosylated glycoform): 34 – 52 % The retention times of peaks 2, 3, and 4 ± 0.5 min. of Ref. Std.
HMW Components	T-0154	≤ 4.0 % The retention time of monomer peak is ± 0.5 min. of Ref. Std. and elution profile comparable to Ref. Std.
General		
pH of Reconstituted Solution	USP <791> (T-0019)	7.1 – 7.7
Water ¹	USP <921> ² (T-0022)	Mean of 5 vials ≤ 2.5 %; no individual vial > 3.5 %
Particulate Matter Particles ≥ 10 µm Particles ≥ 25 µm	USP <788> ³ (T-0033)	≤ 6,000 particles/vial ≤ 600 particles/vial
Safety		
Container Closure Integrity ⁴	T-0402	Pass

Ref. Std. = Reference standard; IU = International unit; HMW = High molecular weight

¹ Reported as % moisture

² Karl Fischer method

³ Light obscuration method

⁴ Performed in lieu of sterility

2.3.P.5.2 Analytical Procedures

A list of analytical procedure numbers and titles used for release and stability testing of Leukine for Injection drug product, 250 µg/vial, are provided in [Table 34](#). Analytical procedures for pharmacopeia methods which have been harmonized between Ph.Eur., USP, and JP reference the USP General Chapters and not the sections of the other pharmacopeia.

Table 34: Analytical Procedures for the Control of the Drug Product

Analytical Procedure	Title of Analytical Procedure
T-0023	Appearance/Color/Clarity Test Procedure
T-0002	SDS-PAGE Silver Stain Procedure
T-0323	Glu-C Peptide Mapping Procedure for rhu GM-CSF
T-0397	GM-CSF Quantitation by Size Exclusion High Performance Liquid Chromatography
T-0091	TF-1 Bioassay for rhu GM-CSF
T-0075	Quantitative Analysis of rhu GM-CSF Glycosylated Variants by High Performance Liquid Chromatography (HPLC)
T-0154	Size Exclusion Chromatography of GM-CSF
T-0057	Reconstitution Time
T-0402	Container Closure Integrity Test ²

² Container Closure Integrity testing is used throughout stability in lieu of sterility testing.

2.3.P.5.3 Validation of Analytical Procedures

Method validations demonstrate suitability of analytical procedures used for release and stability testing of Leukine® for Injection drug product. Analytical method validations, as appropriate, have included accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range. A list of qualification documents for performing drug product testing is provided in [Table 35](#).

Suitability reports of pharmacopoeia methods for testing drug product for pH (<791>), Water Determination (<921>), and Uniformity of Dosage Units (<905>), Subvisible Particulate Matter in Therapeutic Protein Injections (<787>), Bacterial Endotoxins Test (<85>), and Sterility Tests (<71>) are listed in [Table 36](#).

Table 35: Analytical Procedure Method Validation Reports for the Drug Product

Analytical Procedure	Validation Report Number	Validation Report Title
T-0023	QCMT-052813P ¹	Qualification of Northpointe as an Alternative Testing Site for T-0023 "Appearance/Color/Clarity Test Procedure"
T-0397	QCMV-T0397-040114R	QC Method Validation Report: SOP T-0397 "GM-CSF Quantitation by Size Exclusion High Performance Liquid Chromatography"

T-0057	QCMT-052813P ¹	Qualification of Northpointe as an Alternative Testing Site for Test Method T-0057 "Reconstitution Time"
T-0402	QCMV-2010-3871	Container Closure Integrity Test (Validation)
	QCMT-052813P ¹	Qualification of Northpointe as an Alternative Testing Site for Test Method T-0402 "Container Closure Integrity"

¹ Validation reports were included in the Leukine Drug Product Alternative Testing Site Qualification Report QCMT-052813R Version 2.

Table 36: Pharmacopoeia Method Suitability Reports for the Drug Product

Analytical Procedure	Suitability Report Number	Suitability Report Title
USP <905>	QCMV-T0010-070819R	Uniformity of Dosage Units Testing by Weight Variation Compendial Verification
USP <791>	QCMV-T0019-102913R	Compendial Verification of pH Determinations, SOP T-0019, for Qualification of Genzyme Northpointe as an Alternative Testing Site
USP <921>	QCMV-T0022-051314R	Compendial Verification of Determination of Moisture Content for Lyophilized Products Using Karl Fischer Coulometry, SOP T-0022, for Qualification of Northpointe as an Alternative Testing Site
USP <788>	G9SBF69.725	Validation of Test Method T 033 Particulate Matter In Small Volume Parenterals
USP <85>	2020/00923/00	Validation Report of the Kinetic-Chromogenic Bacterial Endotoxins Test for Leukine (sargramostim) for Injection, 250 mcg.
USP <71>	2021/01341/01	Validation Report of the Sterility Test Method for Leukine (Sargramostim) for Injection, 250 mcg

2.3.P.5.4 Batch Analysis

The batch analysis data for development batch AR3496 and clinical batch B26910 are provided in Table 37. These drug product batches were manufactured as described in 3.2.P.3.3, Description of Manufacturing Process and Process Controls, and packaged in a container closure system as described in 3.2.P.7, Container Closure System.

Table 37: Batch Analysis Results for Leukine for Injection, 250 µg/vial, Batches AR3496 and B26910

Drug Product Batch			AR3496	B26910
Test	Analytical Procedure	Acceptance Criteria	Results	
Lyophilized Product	T-0023	White cake	Pass	Pass
Reconstituted Solution	T-0023 USP <790>	Clear, colorless liquid	Pass Pass	Pass Pass

Drug Product Batch			AR3496	B26910
Test	Analytical Procedure	Acceptance Criteria	Results	
		Solution is essentially free of visible particulates		
SDS-PAGE	T-0002	The mobility of 3 bands must correspond to molecular weight as compared to MW markers and sargramostim Ref. Std. run on the same gel; sample displays no extra bands not present in Ref. Std.	Pass	Pass
Peptide Mapping	T-0323	N-terminal species: Ala1: 60 – 85 % Ala3: 15 – 40 % Arg4: ≤ 2 % Ser5: ≤ 5 % Elution profile comparable to Ref. Std.	71 29 < DL ¹ < DL ² Compares	71 27 < DL ¹ < QL ² Compares
Protein Content	T-0397	225 – 275 µg/mL (90.0 – 110.0 % label claim)	252 (100.8)	254 (101.6)
Biological Activity	T-0091	4.0 – 6.9 x 10 ⁶ IU/mg	6.3	6.4
Glycosylated Variants	T-0075	Peak 1: ≤ 5.6 % Peak area percentages of 3 glycosylated variants: Peak 2: 24 – 41 % Peak 3: 13 – 31 % Peak 4: 34 – 52 % The retention times of peaks 2, 3, and 4 ± 0.5 min. of Ref. Std.	2.8 29 22 46 Pass	2.8 30 23 45 Pass
HMW Components	T-0154	≤ 4.0 % The retention time of monomer peak is ± 0.5 min. of Ref. Std. and elution profile comparable to Ref. Std.	0.4 Pass	< 0.3 Pass
Reconstitution Time	T-0057	≤ 120 seconds	33	27
pH of Reconstituted Solution	USP <791> (T-0019)	7.1 – 7.7	7.3	7.4
Water	USP <921> (T-0022)	Mean of 20 vials ≤ 2.0 %; no individual vial > 3.0 %	1.1 Footnote 3	0.9 Footnote 4
Particulate Matter Particles ≥ 10 µm Particles ≥ 25 µm	USP <788> (T-0033)	≤ 6,000 particles/vial ≤ 600 particles/vial	18 1	152 6
Uniformity of Dosage Units	USP <905> (T-0010)	Pass	Pass	Pass

Drug Product Batch			AR3496	B26910
Test	Analytical Procedure	Acceptance Criteria	Results	
Bacterial Endotoxins	USP <85>	≤ 1.25 EU/mL	0.30	< 0.30
Sterility	USP <71>	Pass	Pass	Pass

Ref. Std. = Reference standard; DL = Detection limit; QL = Quantitation limit; IU = International unit; HMW = High molecular weight; EU = Endotoxin unit

¹ The limit of detection for Arg4 (T-0323) is < 0.85 %.

² The limit of quantitation for Ser5 (T-0323) is < 2.41 %.

³ Individual water results: 1.13, 0.81, 0.87, 0.91, 0.93, 0.97, 0.84, 0.96, 0.91, 1.06, 0.90, 0.94, 1.16, 1.08, 0.82, 0.99, 1.00, 0.93, 0.92, 1.10

⁴ Individual water results: 0.83, 0.86, 0.86, 0.78, 0.81, 0.83, 0.97, 0.93, 1.00, 0.86, 0.95, 0.83, 0.80, 0.82, 0.89, 0.84, 0.79, 0.85, 0.81, 0.84

2.3.P.5.5 Characterization of Impurities

There are no new impurities present in the drug product, Leukine for Injection, that are not present in the drug substance, sargramostim.

2.3.P.5.6 Justification of Specifications

The specifications for drug product are based on manufacturing and development experience, ICH Guidance, and the capabilities of the analytical methods. Manufacturing and stability data are monitored and specifications are evaluated and revised, as appropriate.

2.3.P.6 REFERENCE STANDARDS OR MATERIALS

The reference standard for testing drug product Leukine for Injection is the same reference standard used for testing drug substance sargramostim.

2.3.P.7 CONTAINER CLOSURE SYSTEM

The primary container closure system for Leukine for Injection drug product consists of a clear, colorless, Type I borosilicate glass vial closed with a chlorobutyl stopper fastened by an aluminum crimp seal with a dark blue plastic flip-off cap. The description of the components of the primary packaging system (i.e., glass vials, stoppers, and aluminum seals), materials of construction, and manufacturer are listed in [Table 38](#).

Table 38: Description of Primary Packaging Components

Description	Materials of Construction	Manufacturer
Clear, colorless, 20 mm glass vial	Tubing, Type I borosilicate glass	Ompi North America ¹ Canadá 130, Parque Nacional Industrial 65550 Ciénega de Flores, Nuevo Leon Mexico
Gray, 20 mm lyophilization stopper	4432/50 chlorobutyl formulation, FluroTec® and B2 coating	West Pharmaceutical Services, Inc. Jersey Shore, PA 17740 United States
Aluminum seal with plastic dark blue flip-off cap	Aluminum and plastic	West Pharmaceutical Services of Florida, Inc. Clearwater, FL 33760 United States

¹ Depending upon supply and product demand the vials may be manufactured by Nuovo Ompi S.r.l., Piombino Dese, Padova, 35017, Italy or Nuovo Ompi S.r.l., Borgo Tor Tre Ponti, Latina, 04013, Italy.

The glass vials (6R) are manufactured in compliance with ISO 9001:2008, Quality Management Systems, and meet the requirements of the USP General Chapter <660>, Containers-Glass. The specifications for the clear, colorless Type I glass vials manufactured by Ompi North America are provided in [Table 39](#).

Table 39: Glass Vials (Dimensions: 22 mm x 40 mm) Specifications

Test	Test Method	Acceptance Criteria
Appearance	Visual examination	Clear, colorless
Volume	USP <660>	≥ 5.0 and ≤ 10.0 mL
Alkaline Release	USP <660>	≤ 1.0 mL 0.01 M HCl
Dimensions Body Diameter Collar Diameter Neck Diameter Collar Height Neck Height	COC	21.80 – 22.20 mm 19.70 – 20.20 mm 0.00 – 16.50 mm 3.40 – 3.80 mm 8.00 – 9.00 mm
Glass type	Glass Supplier COA	Borosilicate type I

COC = Certificate of Conformance; COA = Certificate of Analysis

The chlorobutyl stoppers meet the requirements of the USP General Chapter <381>, Elastomeric Closures for Injections. The specifications for the gray, chlorobutyl stoppers manufactured by West Pharmaceutical Services are provided in [Table 40](#).

Table 40: Chlorobutyl Stoppers (Formulation: 4432/50) Specifications

Test	Test Method	Acceptance Criteria
Appearance	Visual examination	Gray
Total Bioburden	USP <61>	≤ 12.09 cfu/20 stoppers ≤ 5 cfu/100 m ²
Bacterial Endotoxin	USP <85>	≤ 0.10 EU/mL/10 stoppers ≤ 1.0 EU/stopper
Particulates > 25 – 50 µ > 50 – 100 µ > 100 µ	QC	≤ 13.0 particles/10 cm ² ≤ 3.5 particles/10 cm ² ≤ 0.9 particles/10 cm ²
Elastomer Type	Supplier COA	Chlorobutyl 4432/50

cfu = Colony forming units; EU = Endotoxin units; QC = Quality Certificate

The specifications for the aluminum seals manufactured by West Pharmaceutical Services are provided in [Table 41](#).

Table 41: Aluminum Seals (Size: 20 mm) Specifications

Test	Test Method	Acceptance Criteria
Appearance	Visual examination	Silver with dark blue cap
Flip-off Force	QC	3.6 – 5.4 lbs.
Overall Height	QC	0.370 – 0.394 in.
Skirt Length	QC	0.293 – 0.291 in.

QC = Quality Certificate

2.3.P.8 STABILITY

2.3.P.8.1 Stability Summary and Conclusions

Leukine for Injection proposed shelf-life of 48-months at long-term storage (5 °C) is based on the approved commercial drug product shelf-life of 48 months (U.S. BLA 103362) and the results of the 12-month long-term stability data of development batch AR3496 and clinical batch B26910. The development (AR3496) and clinical (B26910) batches were manufactured according to the process provided in 2.3.P.3.3 and meet the specifications provided in 2.3.P.5.1. The stability data of AR3496 and B26910 are provided in 2.3.P.8.3.

2.3.P.8.2 Postapproval Stability Protocol and Stability Commitment

Drug product batches will be tested according to the protocol provided in Table 41. The stability specifications are presented in [Table 42](#).

Table 42: Stability Protocol (2 – 8 °C)

Test	Timepoint (months)								
	0 ¹	6	9	12	18	24	36	48	60
Appearance – Lyophilized (T-0023)	x	x	x	x	x	x	x	x	x
Appearance – Reconstituted (T-0023)	x	x	x	x	x	x	x	x	x
Protein Content (T-0397)	x	x	x	x	x	x	x	x	x
Biological Activity (T-0091)	x	x	x	x	x	x	x	x	x
SDS-PAGE (T-0002)	x	x	x	x	x	x	x	x	x
Peptide Mapping (T-0323)	x	x	x	x	x	x	x	x	x
Glycosylated Variants (T-0075)	x	x	x	x	x	x	x	x	x
HMW Components (T-0154)	x	x	x	x	x	x	x	x	x
pH of Reconstituted Solution (T-0019)	x	x	x	x	x	x	x	x	x
Water (T-0022)	x	x	x	x	x	x	x	x	x
Particulate Matter (T-0033)	x	--	--	x	--	x	x	x	x
Container Closure Integrity (T-0402) ²	x	--	--	x	--	x	x	x	x

¹ Zero (0) timepoint is the release result

² Performed in lieu of sterility

2.3.P.8.3 Stability Data

Table 43 provides a summary of the lyophilized drug product Leukine for Injection, 250 µg/mL, stability data available for development batch AR3496 and clinical batch B26910 manufactured at Patheon, Greenville, North Carolina, USA. All stability data meet shelf-life specifications under long-term and accelerated storage conditions. There is no evidence of any significant physical or chemical changes in Leukine for Injection drug product under the long-term and accelerated storage conditions.

Table 43: Summary of Stability Data for Leukine for Injection, 250 µg/Vial

Lot Number	Storage Condition	Stability Data	Reference
AR3496 (Devo 8)	2 – 8 °C	12 months	Table 44
	25 °C/60 % RH	12 months	Table 45
B26910 (clinical)	2 – 8 °C	12 months	Table 46
	25 °C/60 % RH	12 months	Table 47

Table 44: Long-Term (2 – 8 °C) Stability Data for Batch AR3496

STABILITY SUMMARY DATA TABLE						
MATERIAL NUMBER	022310	BATCH NUMBER	AR3496	STORAGE CONDITIONS	2-8 C	
DATE MANUFACTURED	16DEC2021	DATE STUDY STARTED	12JAN2022	DATE OF EXPIRATION	16DEC2025	
DRUG SUBSTANCE LOT	B24954/B25085	PURPOSE OF STUDY	QCSR-2135, SUPPORT PROCESS VALIDATION AT PATHEON			

TEST	SPECIFICATION	TIMEPOINT (MONTHS)				
		0	3	6	9	12
Appearance/Color/Clarity	White Cake; Clear, colorless liquid (WC/CCL)	WC/CCL	WC/CCL	WC/CCL	WC/CCL	WC/CCL
TF-1 Bioassay	4.0 – 6.9 E+06 IU/mg	6.3	6.9	6.2	6.3	6.3
SDS-PAGE Silver Stain (Reduced/Non-Reduced)	No new bands/equivalent to reference standard (NB/Equiv)	NB/Equiv	NB/Equiv	NB/Equiv	NB/Equiv	NB/Equiv
RP-HPLC						
Peak 1	≤ 6.8 %	2.8	2.8	2.6	2.6	2.8
Peak 2	24-41 %	29.3	29.4	29.2	29.5	29.9
Peak 3	13-31 %	21.9	21.8	21.6	21.6	21.5
Peak 4	34-52%	46.0	46.0	46.7	46.3	45.9
Glu-C Peptide Mapping						
% Ala1	60-85 %	71.2	69.8	71.6	71.7	71.9
% Ala3	15-40 %	28.8	28.0	28.4	28.4	28.1
% Arg4	≤ 2 %	<QL	<QL	<QL	<QL	<QL
% Ser5	≤ 5 %	<QL	<QL	<QL	<QL	<QL
SE-HPLC	≤ 4.0 %	0.4	<0.3	<0.3	<0.3	0.3
pH	7.1 – 7.7	7.3	7.4	7.4	7.4	7.4
Conc. SE HPLC	225 - 275 µg/mL	252	253	249	250	258
Particulate Matter						
≥ 10 µm	≤ 6000 particles	18	NRQ	NRQ	NRQ	417
≥ 25 µm	≤ 600 particles	1				9
Container/Closure Integrity	No compromise to integrity	NRQ	NRQ	NRQ	NRQ	NCI
% Moisture	Release Specification: Mean of 20 vials ≤ 2.0 % with no individual vial > 3.0 %. Stability Specification: Mean of 5 vials ≤ 2.5 % with no individual vial > 3.5 %	1.0	1.2	1.3	1.5	1.4
SCHEDULED TEST DATE		JAN22	APR22	JUL22	OCT22	JAN23

Table 45: Accelerated (25 °C) Stability Data for Batch AR3496

STABILITY SUMMARY DATA TABLE						
MATERIAL NUMBER	022310	BATCH NUMBER	AR3496	STORAGE CONDITIONS	25 C	
DATE MANUFACTURED	16DEC2021	DATE STUDY STARTED	12JAN2022	DATE OF EXPIRATION	16DEC2025	
DRUG SUBSTANCE LOT	B24954/B25085	PURPOSE OF STUDY	QCSR-2135, SUPPORT PROCESS VALIDATION AT PATHEON			

TEST	SPECIFICATION	TIMEPOINT (MONTHS)				
		0	3	6	9	12
Appearance/Color/Clarity	White Cake; Clear, colorless liquid (WC/CCL)	WC/CCL	WC/CCL	WC/CCL	WC/CCL	WC/CCL
TF-1 Bioassay	4.0 – 6.9 E+06 IU/mg	6.3	6.5	6.1	6.3	6.2
SDS-PAGE Silver Stain (Reduced/Non-Reduced)	No new bands/equivalent to reference standard (NB/Equiv)	NB/Equiv	NB/Equiv	NB/Equiv	NB/Equiv	NB/Equiv
RP-HPLC						
Peak 1	≤ 6.8 %	2.8	3.2	3.7	3.8	3.6
Peak 2	24-41 %	29.3	29.2	29.0	28.6	29.7
Peak 3	13-31 %	21.9	21.3	21.2	21.6	21.2
Peak 4	34-52%	46.0	46.3	46.1	46.0	45.5
Glu-C Peptide Mapping						
% Ala1	60-85 %	71.2	69.9	70.8	70.9	71.2
% Ala3	15-40 %	28.8	28.0	29.2	29.1	28.8
% Arg4	≤ 2 %	<QL	<QL	<QL	<QL	<QL
% Ser5	≤ 5 %	<QL	<QL	<QL	<QL	<QL
SE-HPLC	≤ 4.0 %	0.4	0.3	0.5	1.0	1.2
pH	7.1 – 7.7	7.3	7.4	7.4	7.4	7.4
Conc. SE HPLC	225 - 275 µg/mL	252	253	246	250	254
Particulate Matter						
≥ 10 µm	≤ 6000 particles	18	NRQ	NRQ	NRQ	79
≥ 25 µm	≤ 600 particles	1				2
Container/Closure Integrity	No compromise to integrity	NRQ	NRQ	NRQ	NRQ	NCI
% Moisture	Release Specification: Mean of 20 vials ≤ 2.0 % with no individual vial > 3.0 %. Stability Specification: Mean of 5 vials ≤ 2.5 % with no individual vial > 3.5 %	1.0	1.5	1.4	1.0	1.4
SCHEDULED TEST DATE		JAN22	APR22	JUL22	OCT22	JAN23

Table 46: Long-Term (2 – 8 °C) Stability Data for Batch B26910

STABILITY SUMMARY DATA TABLE											
MATERIAL NUMBER	13085	BATCH NUMBER	B26910/AR8010 (PPQ #2)		STORAGE CONDITIONS		2-8 C				
DATE MANUFACTURED	12APR2022	DATE STUDY STARTED	19MAY2022		DATE OF EXPIRATION		12APR2026				
DRUG SUBSTANCE LOT	B25345, B25452, B25533	PURPOSE OF STUDY	QCSR-2137, LYO LEUKINE DP PATHEON PROCESS VALIDATION								
TEST	SPECIFICATION	TIMEPOINT (MONTHS)									
		0	3	6	9	12	18	24	36	48	60
Appearance/Color/Clarity	White Cake. Clear, Colorless Liquid (WC/CCL)	WC/CCL	WC/CCL	WC/CCL	WC/CCL	WC/CCL					
TF-1 Bioassay	4.0 – 6.9 E+06 IU/mg	6.4	6.5	6.5	6.8	6.8					
SDS-Page Silver Stain (Reduced, Non-Reduced)	No new bands/ equivalent to reference standard (NB/Equiv)	NB/Equiv	NB/Equiv	NB/Equiv	NB/Equiv	NB/Equiv					
RP-HPLC											
Peak 1	≤ 6.8%	2.8	2.6	2.5	2.3	2.8					
Peak 2	24-41%	29.6	29.2	29.7	29.4	29.2					
Peak 3	13-31%	22.5	22.8	22.4	22.5	22.2					
Peak 4	34-52%	45.1	45.5	45.3	45.8	45.8					
Peptide Mapping GM-CSF											
Ala1	60-85%	70.5	72.5	72.3	71.4	71.4					
Ala3	15-40%	27.4	27.6	27.7	28.6	28.6					
Arg4	≤ 2%	<QL	<QL	<QL	<QL	<QL					
Ser5	≤ 5%	<QL	<QL	<QL	<QL	<QL					
Conc. SE HPLC	225 - 275µg/mL	254	256	253	252	251					
SE-HPLC	≤ 4.0%	< 0.3	< 0.3	0.7	0.3	0.4					
pH	7.1 - 7.7	7.38	7.37	7.37	7.39	7.39					
% Moisture	Release specification: Mean of 20 vials ≤ 2.0%, with no individual vial > 3.0% Stability specification: Mean of 5 vials ≤ 2.5% with no individual vial >3.5%	0.86	1.20	1.13	1.32	1.48					
Container Closure Integrity	No Compromise of Integrity	NRQ	NRQ	NRQ	NRQ	Pass					
Particulate Matter											
Particles ≥ 10µm	≤ 6000 particles/vial	152	NRQ	NRQ	NRQ	360					
Particles ≥ 25µm	≤ 600 particles/vial	6				4					
SCHEDULED TEST DATE		MAY22	AUG22	NOV22	FEB23	MAY23	NOV23	MAY24	MAY25	MAY26	MAY27

Table 47: Accelerated (25 °C) Stability Data for Batch B26910

STABILITY SUMMARY DATA TABLE						
MATERIAL NUMBER	13085	BATCH NUMBER	B26910/AR8010 (PPQ #2)	STORAGE CONDITIONS	25 C	
DATE MANUFACTURED	12APR2022	DATE STUDY STARTED	19MAY2022	DATE OF EXPIRATION	12APR2026	
DRUG SUBSTANCE LOT	B25345, B25452, B25533	PURPOSE OF STUDY	QCSR-2137, LYO LEUKINE DP PATHEON PROCESS VALIDATION			

TEST	SPECIFICATION	TIMEPOINT (MONTHS)				
		0	3	6	9	12
Appearance/Color/Clarity	White Cake. Clear, Colorless Liquid (WC/CCL)	WC/CCL	WC/CCL	WC/CCL	WC/CCL	WC/CCL
TF-1 Bioassay	4.0 – 6.9 E+06 IU/mg	6.4	6.1	6.1	6.6	6.4
SDS-Page Silver Stain (Reduced, Non-Reduced)	No new bands/ equivalent to reference standard (NB/Equiv)	NB/Equiv	NB/Equiv*	NB/Equiv	NB/Equiv	NB/Equiv
RP-HPLC						
Peak 1	≤ 6.8%	2.8	2.7	2.8	4.0	4.1
Peak 2	24-41%	29.6	29.3	29.7	28.5	29.1
Peak 3	13-31%	22.5	22.3	21.9	22.4	21.9
Peak 4	34-52%	45.1	45.7	45.6	45.1	44.9
Peptide Mapping GM-CSF						
Ala1	60-85%	70.5	72.0	71.6	71.2	71.0
Ala3	15-40%	27.4	28.0	28.4	28.8	29.0
Arg4	≤ 2%	<QL	<QL	<QL	<QL	<QL
Ser5	≤ 5%	<QL	<QL	<QL	<QL	<QL
Conc. SE HPLC	225 - 275µg/mL	254	255	250	253	247
SE-HPLC	≤ 4.0%	< 0.3	0.7	1.2	1.1	2.7
pH	7.1 - 7.7	7.38	7.43	7.45	7.47	7.47
% Moisture	Release specification: Mean of 20 vials ≤ 2.0%, with no individual vial > 3.0% Stability specification: Mean of 5 vials ≤ 2.5% with no individual vial >3.5%	0.86	1.5	1.42	1.51	1.60
Container Closure Integrity	No Compromise of Integrity	NRQ	NRQ	NRQ	NRQ	Pass
Particulate Matter						
Particles ≥ 10µm	≤ 6000 particles/vial	152	NRQ	NRQ	NRQ	436
Particles ≥ 25µm	≤ 600 particles/vial	6				9
SCHEDULED TEST DATE		MAY22	AUG22	NOV22	FEB23	AUG23

*Silver Stain tested at 4MO due to an invalid assay at 3MO.

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2.3.A.1 FACILITIES AND EQUIPMENT

2.3.A.1.1 Drug Substance Manufacturer

The Partner Therapeutics Northpointe facility is located at 2625 162nd Street SW, Lynnwood WA, USA. The facility is dedicated solely to sargramostim manufacturing.

The facility is a 4-level building (3 floors plus a mechanical mezzanine) of approximately 100,000 ft². The first floor includes plant and process utility areas, process development laboratories, and support areas for engineering and administrative use. The second floor includes production and warehouse areas and quality control laboratories. The third floor includes a quality control laboratory, administrative support areas and an interstitial space that allows access to heating, ventilation, and air conditioning (HVAC) ducting and utilities. The mezzanine includes HVAC and engineering support areas.

Segregation and containment to mitigate potential for cross-contamination are achieved by: dedicated air handling units; personnel training and gowning; restricted personnel flow; procedural and physical control of materials, product, clean and dirty equipment and waste flow; dedicated equipment; and cleaning, monitoring and preventative maintenance. Specific cleaning measures are based upon operational, process, and product risks. PTx utilizes a risk-based program of air and surface monitoring in the clean rooms to ensure the facility cleaning program is effective.

The HVAC systems are under the dynamic management of the Building Management System (BMS). This control system manages the HVAC systems by continuously scanning information supplied by sensors for temperature, humidity, and differential pressure.

Equipment in the Northpointe facility is designed to meet or exceed minimum regulatory requirements or expectations. Equipment surfaces that contact the product or process stream are fabricated to be non-reactive. Surfaces are smooth and seamless for easy cleaning, sanitization, and to reduce the possibility of microbial contamination.

2.3.A.1.2 Drug Product Manufacturer

Patheon Manufacturing Services LLC, contract manufacturing facility of Leukine for Injection drug product, is located at 5900 Martin Luther King Jr Hwy, Greenville, NC 27834, USA. The Patheon Steriles – North manufacturing facility includes compounding, sterile filtration, filling, lyophilization, capping, inspection, and packaging of the drug product. The Steriles – North areas and rooms used for the manufacture of Leukine for Injection including the equipment description, room number, and room environmental controls are provided in [Table 48](#).

Specific equipment/parts that are used in the manufacture of Leukine for Injection drug product and information on product contact and sterilization of direct product-contact parts are provided in [Table 49](#).

Table 48: Manufacturing Area 3 and Filling Line 3 Major Equipment

Equipment Description	Equipment No.	Room No.	Room Classification
120 L Stainless Steel Compounding Tank (Portable)	800885	4F014 or 4F011	Grade C
120 L Stainless Steel Holding Tank (Portable)	800886	4F014 or 4F011/2F118	Grade C/ Unclassified
Smeja 280 Stopper Processor ¹ & C/D Containers	97279	2F324	Grade D
Steris Sterilizer	127883	2F104/ 2F105 ²	Unclassified/ Grade B/ Grade A ³
Pharmetics Sterilizer	97258	2F121/ 2F147 ²	Grade D/ Grade A ⁴
Bosch Rotary Vial Washer	128856	2F144	Grade D
Bosch Depyrogenation Tunnel	128852	2F144	Grade D
Smeja Isolator	128208	2F118	Unclassified
Portable Tank Docking Station/Spool Piece	PTDSL3B16	2F118	NA
Bosch Line 3 Filler	128853	2F109	Grade A
Loading Accumulation Table (LAT)	127708	2F109	Grade A
Loading Transfer Cart (LTCAR)	127701	2F109	Grade A
Unloading Transfer Cart (UTCAR)	127704	2F113	Grade B
Unloading Accumulation Buffer (UAB)	127703	2F111	Grade B
BOC Edwards 300 Sq. Ft. Freeze Dryer (VL7)	127705	2F113/ 2F124C ²	Grade B/ Grade A
Bosch Line 2/3 Lyo Capper	128022	2F111	Grade A Air Supply
Bosch Line 2/3 Lyo Trayloader	128021	2F112	Unclassified

NA = Not applicable

¹ The Smeja 280 Stopper Processor serves Lines 1, 2, 3, and 4.

² Location of Equipment/Sterile Access.

³ Sterile unloading area is a Portable Laminar Flow Module Unit located within Room 2F105 to provide a Grade A environment

⁴ Sterile unloading area is a Portable Laminar Flow Module Unit located within Room 2F147 to provide a Grade A environment.

Table 49: Equipment Utilized in Leukine for Injection Drug Product Manufacturing

Manufacturing Step	Major Equipment and Parts	Product-dedicated, Shared, or Disposable Equipment	Sterilization Method (for Product Contact)
Equipment and Component Preparation	Autoclave Vial washer Vial depyrogenation tunnel Stopper processor	Shared – not product contact Shared – not product contact Shared – not product contact Shared – not product contact	NA
Thawing BDS	NA	NA	NA
Compounding (excipient and BDS addition, dilution, mixing)	120 L Compounding tank	Dedicated – product contact	CIP
Bioburden Reduction Filtration and Holding	120 L Holding tank 0.22 µm bioburden reduction (filter housing, solution filter) Flexible tubing	Dedicated – product contact Filter housing - dedicated product contact part Filter membrane – disposable Disposable – product contact	CIP and SIP Autoclave and SIP SIP Gamma irradiated
Sterile filtration	AseptiQuik G connector 0.22 µm redundant sterile filtration (filter housing, solution filters)	Disposable – product contact Filter housing - dedicated product contact part Filter membrane - disposable	Gamma irradiated and SIP Autoclave and SIP Autoclave (final filter only) and SIP
Filling and Partial Stoppering	Filling equipment and tubing: Filler supply piping AseptiQuik G connector Reservoir (surge vessel) Tubing filling needles Stopper transfer chute and bowl	Dedicated – product contact Dedicated – product contact Dedicated – product contact Dedicated – product contact Dedicated – not product contact	SIP Gamma irradiated and SIP SIP Autoclave and SIP Autoclave and SIP
Lyophilization and Stoppering	Loading accumulation table Loading transfer cart Freeze dryer (VL7)	Shared – not product contact Shared – not product contact Shared – not product contact	NA
Capping	Capper	Shared – not product contact	NA

NA = Not applicable; BDS = Bulk drug substance; CIP = Clean-in-place; SIP = Steam-in-place

2.3.A.2 ADVENTITIOUS AGENTS SAFETY EVALUATION

Sargramostim is expressed in yeast fermentations (*Saccharomyces cerevisiae*). Therefore, risk from non-viral adventitious agents and mammalian viruses is very low. PTx ensures that TSE causative agents are not introduced in manufacturing by reducing the number of animal-derived raw materials in the process. Any animal-derived raw materials are evaluated for origin, tissue type, and processing of the material to reduce risk from adventitious agents. Bacterial and fungal contamination is controlled by bioburden control (autoclaving and filtration) and testing throughout the process. Due to selection of yeast as an expression system (non-animal origin), viral validation studies were not performed.

The sargramostim fermentation process utilizes three animal derived raw materials: BactoPeptone, Hy-Case SF, and L-Tyrosine. There are no animal derived raw materials in the downstream process or the drug product fill-finish process.

2.3.A.3 EXCIPENTS

As noted previously in this document, all excipients for Leukine for Injection drug product are inactive ingredients contained in the Ph. Eur., USP/NF, and JP pharmacopeia.