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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](#F1).

Figure : Amino acid sequence

Text, letter

Description automatically generated

Secondary structure

In this model of sargramostim secondary structure ([Figure 2](#F2)), β-sheets are represented by orange arrows, α-helices (H1 through H6) by red spirals, and random coils by green strands.

Figure : Secondary Structure

A picture containing diagram

Description automatically generated

**Tertiary structure:**

In this model of non-glycosylated sargramostim tertiary structure ([Figure 3](#F3)), β-sheets are represented by orange arrows, α-helices (H1 through H6) by red spirals, and random coils by green strands. Two alpha-helices (labeled H1 and H5) form the receptor binding pocket.

Figure : Tertiary Structure

Diagram

Description automatically generated

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 ε = 14180, E 0.1 % 280 nm = 0.983 cm2/mg; the actual extinction coefficient experimentally determined of sargramostim is provided in [Table 2](#T2).

Table : Sargramostim Maximum Absorption and Extinction Coefficient

|  |  |
| --- | --- |
| Parameter | Result |
| Absorption maximum | 280 nm |
| Specific absorption, E 0.1 %280 nm | 1.10 L cm-1 g-1 to 1.16 L cm-1 g-1 |
| Molar absorption coefficient, ε | 15853 to 16758 L/ cm-1 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](#T3).

Table : 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 x 106 ± 0.021 IU/mL. Estimated specific activity and relative percent of major drug substance glycoforms are listed in [Table 4](#T4). Estimated sargramostim activity is calculated from relative glycoform composition, resulting in drug substance biological activity of 5.7 x 106 IU/mg.

Table : Specific Activity and Receptor Affinity of Sargramostim Glycoforms

| Glycoform | Relative Percent | Specific Activity (IU/mg) |
| --- | --- | --- |
| N- and N- plus O-glycosylated | 29 % | 6.10 ± 0.32 x 106 |
| O-glycosylated | 22 % | 6.34 ± 0.55 x 106 |
| Non-glycosylated | 49 % | 5.17 ± 0.41 x 106 |

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](#T5).

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  FEI: 3001452367  DUNS: 79-790-3197 | Testing of raw materials |
| 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.

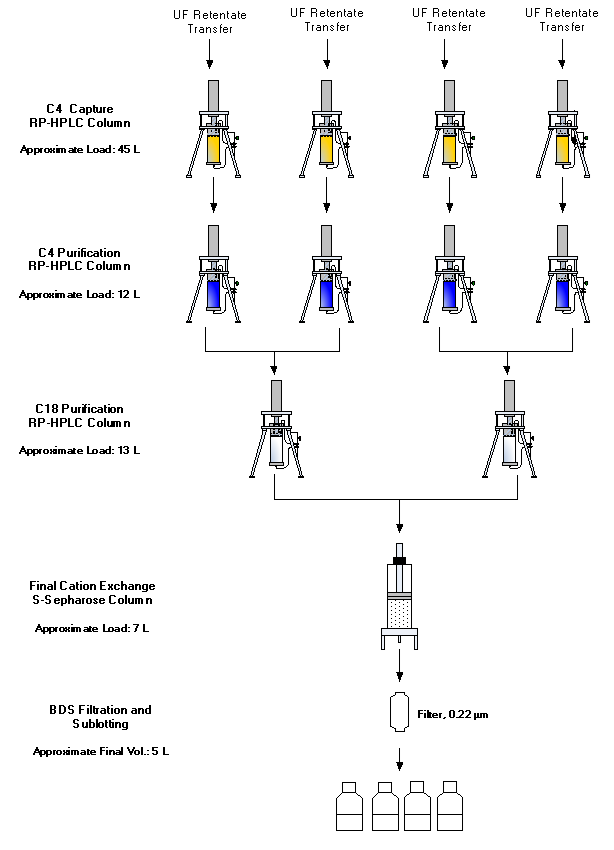
Excluding WCB starting material, a total of 44 individual unit operations are required to manufacture a single BDS batch. [Figure 4](#F4) and [Figure 5](#F5) illustrate fermentation and purification batching schemes, respectively, that are required to manufacture a single batch of sargramostim BDS.

Partner Therapeutics does not engage in reprocessing of sargramostim.

Figure : Fermentation Batching Schematic

**A picture containing diagram

Description automatically generated**

Figure : 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](#T6).

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 | |
| 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](#T7), [Table 8](#T8), and [Table 9](#T9).

Table : Raw Materials Used in Working Cell Bank Production

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

Table : Raw Materials Used in Cell Expansion and Fermentation

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

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

| Product Name | Grade |
| --- | --- |
| Acetonitrile Acetic Acid, Glacial  ß-Alanine Bakerbond: Silica, C4, 15 μm  Bakerbond: Silica, C18, 15 μm Hydrochloric Acid Methanol Cycle-tainer Methylparaben N-Propanol Nitrogen Pyridine S-Sepharose, Fast Flow  Sodium Hydroxide, 50 % Trifluoroacetic Acid TRIS-Hydrochloride  Tromethamine | ACS USP Non-compendial Non-compendial Non-compendial NF ACS NF Non-compendial NF ACS Non-compendial Non-compendial Non-compendial Non-compendial 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](#T10) summarizes these materials:

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

| Biological Raw Material | Biological Source | Country of Origin | Manufacturer | Step | Suitability for Use |
| --- | --- | --- | --- | --- | --- |
| Bacto-Peptone | 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. |
| 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](#T11).

Table : 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 Wet Cell Weight Product Concentration Calculated Yield Non-Host Contamination | CPP CPP PC PC PC PC PC PC IPC IPC IPC  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 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    Calculated Step Yield | CPP CPP CPP CPP CPP CPP PC PC PC IPC 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 % 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](#T12).

Table : 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  Reduced Tryptic Map/MS/MS  Acid pH Glu-C Map/MS/MS | | Confirmed  Confirmed  Confirmed | Confirmed  Confirmed  Confirmed | Confirmed  Confirmed  Confirmed | Confirmed  Confirmed  Confirmed | Confirmed  Confirmed  Confirmed |
| Amino Acid Composition | Amino Acid Analysis | | Results of a typical analysis provided | | | | |
| Molecular Formula | Primary Sequence | | C639H1002N168O196S8 | | | | |
| Theoretical Molecular Weight | Primary Sequence | | 14430 g/mole | | | | |
| Extinction Coefficient | Calculated based on Trp, Tyr, Cys. | | Theoretical: e = 14180 cm-1moles-1liters, E0.1%280 nm = 0.983 cm2/mg | | | | |
| Spectrophotometry/Protein Conc | | Not required | Not required | 1.12 cm2/mg | 1.10 cm2/mg | 1.16 cm2/mg |
| Carboxy-terminal Analysis | Neutral pH Glu-C Map/MS/MS  Reduced Tryptic Map/MS/MS | | C-terminus intact  Not done | C-terminus intact  no result -assay problems | C-terminus intact  no result -assay problems | C-terminus intact  no result -assay problems | C-terminus intact  no result -assay problems |
| Assignment of Disulfide Bonds | Acidic pH HPLC Glu-C Map/MS | | Cys54-Cys96 Cys88-Cysl21 | Cys54-Cys96 Cys88-Cysl21 | Cys54-Cys96 Cys88-Cysl21 | Cys54-Cys96 Cys88-Cysl21 | Cys54-Cys96 Cys88-Cysl21 |
| Methionine Oxidation | Neutral pH Glu-C Map  Acid pH Glu-C Map/MS/MS  Tryptic Map/MS/MS | | Met79 ~ 5-7%  Met36,Met46 no ox  Not done | Met79 4.79%  Not required  Met79 5%, Met46 ~1% | Met79 2.70%  Not required  Met79 3%, Met46 ~1% | Met79 2.64%  Not required  Met79 3%, Met46 ~1% | Met79 2.55%  Not required  Met79 3%, Met46 ~1% |
| Glycosylation Site Occupancy  O-glycosylation  N-glycosylation | Neutral pH Glu-C Map/MS  Acid pH Glu-C Map/MS/MS  Reduced Tryptic Map/MS/MS | | Consistent with Ser9  with Asn27  Asn37 none detected | Ser9 46%  Asn27 26%  Asn37 none detected | Ser9 49%  Asn27 25%  Not available | Ser9 49%  Asn27 25%  Not available | Ser9 49%  Asn27 24%  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 | N17, Q20, N27 <1% | N17, Q20, N27 <1% | N17, Q20, N27 <1% | N17, Q20, N27 <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 N37-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 MAS1  MALDI TOF MS | Phosphorous confirmation  -PO3 glycoforms, similar masses | Not required  -PO3 glycoforms, similar masses | Not required  -PO3 glycoforms, similar masses | Not required  -PO3 glycoforms, similar masses | Not required  -PO3 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  Inflexion 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 ELISA2 | | 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](#T13). Data demonstrated no need to control the impurities in the final drug substance.

Table : 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](#T14).

Table : 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 | 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. |
| **Quantity** | | |
| Protein Concentration | T-0315 | 5.00 – 8.30 mg/mL |
| **Biological Activity** | | |
| Potency | T-0091 | 4.0 – 6.9 x 106 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 %; 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](#T15).

Table : 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. |
| Biological Activity | | |
| Potency | T-0091 | 4.0 – 6.9 x 106IU/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](#T16).

Table : 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](#T17).

Table : 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 |
| 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](#T18). The batches met the sargramostim bulk drug substance release specifications.

Table : 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](#F6) |
| B26063 | 19 November 2020 | November 2025 | [Figure 7](#F7) |

Figure : Certificate of Analysis – Drug Substance Batch B25981

Table

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Figure : Certificate of Analysis – Drug Substance Batch B26063

Table

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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](#T19) summarizes the results of testing and qualification of the current reference standard RS-121-4.

Table : 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 Hydrolysis/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 Component   (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]  214 nm elution profile compares to Ref. Std. | 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 | |
| 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 ≤ 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 106 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 106 IU/mg | Mean Log10 IU/mg = 6.73  Reported Specific Activity  = 5.3 x 106 IU/mg | | | Mean Log10 IU/mg = 6.78  Reported Specific Activity = 6.1 x 106 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 °C ± 10 °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 °C ± 10 °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](#T20). The stability specifications are presented in [Table 15](#T15).

Table : 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](#T21) 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 : 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](#T22) |
| B24483 | Long-term (-70 °C) | 36 months | 2019 Annual stability | [Table 23](#T23) |
| B25981 | Long-term (-70 °C) | 12 months | 2020 Annual Stability | [Table 24](#T24) |

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

Table

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

Table

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

Table

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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](#T25).

Table : 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 7QAUnited 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 : Testing Facilities and Tests Performed

| Testing Facility | Release Testing | Stability Testing |
| --- | --- | --- |
| Partner Therapeutics, Inc. 2625 162nd 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 |
| 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](#T27). 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 ± 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 : 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 ± 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](#F8).

Figure : Schematic of the Drug Product Manufacturing Process

Diagram, schematic

Description automatically generated

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 % ± 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](#T28)) is initiated via the lyophilizer control system.

Table : 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 |
| 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](#T29) 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](#T30)).

Table : 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 : 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](#T31).

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 : 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](#T32).

Table : 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 106 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 %  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) | ≤ 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](#T33).

Table : 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 106 IU/mg |
| 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](#T34). 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 : 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 Test2 |

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](#T35).

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](#T36).

Table : Analytical Procedure Method Validation Reports for the Drug Product

|  |  |  |
| --- | --- | --- |
| Analytical Procedure | Validation Report Number | Validation Report Title |
| T-0023 | QCMT-052813P1 | 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-052813P1 | 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-052813P1 | 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 : 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](#T37). 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 : 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  Solution is essentially free of visible particulates | Pass  Pass | Pass  Pass |
| 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 106 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 |
| 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](#T38).

Table : 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](#T39).

Table : 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](#T40).

Table : 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 m2 |
| 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 cm2  ≤ 3.5 particles/10 cm2  ≤ 0.9 particles/10 cm2 |
| 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](#T41).

Table : 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](#T42).

Table : 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](#T43) 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](#T44) |
| 25 °C/60 % RH | 12 months | [Table 45](#T45) |
| B26910 (clinical) | 2 – 8 °C | 12 months | [Table 46](#T46) |
| 25 °C/60 % RH | 12 months | [Table 47](#T47) |

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

Table

Description automatically generated with medium confidence

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

Table

Description automatically generated

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

A picture containing text, number, screenshot, parallel

Description automatically generated

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

A picture containing text, number, screenshot, parallel

Description automatically generated

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](#T48).

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](#T49).

Table : 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 : 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.