19 June 2025
EMA/233111/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Imreplys

International non-proprietary name: sargramostim

Procedure No. EMEA/H/C/006411/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

Table of contents

List of abbreviations

ADA	Anti-drug antibody
AE	Adverse event
ALL	Acute lymphoblastic leukaemia
AML	Acute myelogenous leukaemia
ANC	Absolute neutrophil count
ANLL	Acute nonlymphocytic leukaemia
AQL	Acceptable quality level
Arg	Arginine
ARS	Acute radiation syndrome
AST	Aspartate aminotransferase
AUC	Area under the curve
AUClast	AUC up to the last measurable concentration
AWC	
BID	Twice per day
BLA	US Biologics License Application
BM	Bone marrow
BMT	Bone marrow transplantation
BSA	Body surface area
BWFI	Bacteriostatic Water for Injection
CBC	Complete blood count
cGy	Centi Gray
CL/F	Apparent body clearance
CMAX	Maximum (observed) serum concentration
CMH	Cochran-Mantel-Haenszel
CML	Chronic Myeloid Leukemia
CQA	Critical quality attribute
CR	Complete remission
CSR	Clinical study report
CV	Coefficient of variation
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
EDTA	Ethylenediaminetetraacetic acid
FEP	Fluorinated ethylene propylene
GC/MS	Gas chromatography-mass spectrometry
GLP	Good Laboratory Practice
H-ARS	Haematopoietic Syndrome of Acute Radiation Syndrome
HD	Hodgkin disease
HED	Human equivalent doses
HLA	Human leukocyte antigen
IBD	International birth date
ICP-OES	Inductively coupled plasma optical emission spectroscopy

ICP-MS	Inductively Coupled Plasma Mass Spectrometry
IFC	Imaging flow cytometry
IH	Inhalational administration
IPS	In-process specifications
ISR	Injection site reaction
IV	Intravenous
LC/MS/MS	Liquid chromatography tandem mass spectrometry
LC/UV/MS	Liquid chromatography with ultraviolet and mass spectrometry detection
LD	Lethal dose
$LD_{xx/60}$	Lethal dose for a defined percentage of the population of control animals within 60-days after TBI
$LD_{50\text{-}60/60}$	Lethal dose for 50-60% of the population within the first 60-days after total body irradiation
LDH	Lactate dehydeogenase
LP-CEX	Low-pressure cation exchange chromatography
MALDI-TOF MS	Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry
MOD/MOF	Multi-organ dysfunction and failure
MSC	Minimal supportive care
MW	Molecular weight
N	Number
NAb	Neutralizing antibodies
NE	Not estimable
NF	National formulary
NHANES	National Health and Nutrition Examination Survey
NHL	Non-Hodgkin’s lymphoma
NHP	Non-human primate
OS	Overall survival
PACMP	Post-approval change management protocol
PADER	Periodic Adverse Drug Experience Report
PBPC	Peripheral blood progenitor cell
PD	Pharmacodynamic(s)
PDE	Permitted daily exposure
PI	Prescribing Information or Product Information
PK	Pharmacokinetic(s)
P&L	Paesel & Lorei GmbH & Co
PLA	Product License Application
PLT	Platelets
popPK	Population pharmacokinetic(s)
PSC(T)	Peripheral stem cell (transplantation)
PSIA	Pounds per square inch absolute
PSIG	Pounds per square gauge
PT	Preferred term
PVDF	Polyvinylidene difluoride
RAMM	Risk assessment mitigation matrix
RBC	Red blood cells
RP-HPLC	Reverse phase high pressure liquid chromatography

RMM	Resonant mass measurement
RPN	Risk priority number
RTI	Ready to inject
SAE	Serious adverse event
SCS	Summary of clinical safety
SOC	System organ class
SWFI	Sterile Water for Injection
TAMC	Total aerobic microbial count
TBI	Total body irradiation
TEAE	Treatment emergent adverse event
TYMC	Total yeast and mold count
	Unloading accumulation buffer
USPI	United States Prescribers Information
V/F	Apparent volume
vpm	Vials/min

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Partner Therapeutics Limited submitted on 5 April 2024 an application for marketing authorisation to the European Medicines Agency (EMA) for Imreplys, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 14 September 2023

The applicant applied for the following indication:

- Treatment for exposure to myelosuppressive doses of radiation (Haematopoietic Syndrome of Acute Radiation Syndrome [H-ARS]) *(adults and paediatric population)*

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0089/2024 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP P/0089/2024 was not yet completed as some measures were deferred

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request for consideration

1.5.1. Marketing authorisation under exceptional circumstances and accelerated assessment.

The applicant requested consideration of its application for a marketing authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation.

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

1.6. Scientific advice

The applicant did not seek scientific advice from the CHMP.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Maria Grazia Evandri Co-Rapporteur: Peter Mol

The application was received by the EMA on	5 April 2024
Accelerated Assessment procedure was agreed-upon by CHMP on	21/03/2024
The procedure started on	25 April 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	28 June 2024
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	2 July 2024
In accordance with Article 6(3) of Regulation (EC) No 726/2004, the CHMP Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	28 June 2024

The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	11 July 2024
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on The procedure was reverted to normal timetable	23 July 2024
The applicant submitted the responses to the CHMP consolidated List of Questions on	14 October 2024
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	20 November 2024
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	28 November 2024
The CHMP agreed on a list of outstanding issues in writing and to be sent to the applicant on	12 December 2024
The applicant submitted the responses to the CHMP List of Outstanding Issues on	25 February 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	12 March 2025
The CHMP agreed on a 2nd list of outstanding issues in writing and to be sent to the applicant on	27 March 2025
The applicant submitted the responses to the CHMP 2nd List of Outstanding Issues on	15 May 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the 2nd List of Outstanding Issues to all CHMP and PRAC members on	05 June 2025
The CHMP Rapporteurs circulated the updated CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the 2nd List of Outstanding Issues to all CHMP and PRAC members on	12 June 2025
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Imreplys on	19 June 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The agreed therapeutic indication for Imreplys is

"Imreplys is indicated for treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic sub-syndrome of Acute Radiation Syndrome (H-ARS).

Imreplys should be used in accordance with official radiological/nuclear emergency recommendations."

Acute Radiation Syndrome (ARS), also known as radiation sickness or radiation toxicity, occurs when individuals are acutely exposed to high doses of whole body or significant partial-body irradiation at doses greater than 1 Gy over a relatively short period of time. H-ARS occurs after whole-body or partial-body (>60%) irradiation to doses >0.7 Gy, causing damage to rapidly dividing tissues, including bone marrow. Exposure to doses >2 Gy causes moderate to severe pancytopenia that may lead to infection, sepsis, bleeding, and death.

Children are more radiosensitive than adults, which means that a lower lethal dose for 50% of the paediatric population within 60-days of exposure would be expected.

2.1.2. Epidemiology

The Health Emergency Preparedness and Response Authority (HERA) was launched as a new European Commission (EC) Directorate-General in September 2021 to strengthen coordination at the European Union (EU) level when facing cross-border health threats. In June 2022, HERA presented a list of the top-3 cross-border health threats that require coordination at the EU level in the context of medical countermeasure procurement (European Commission, 2022). Included in the top three threats was a radiological, and/or nuclear catastrophe (e.g., nuclear weapons attack, etc.) with the potential to spread across Member States.

It is estimated that tens of thousands to hundreds of thousands of individuals may be at risk for life-threatening H-ARS in a radiological and/or nuclear mass casualty incident due to an accident or a deliberate attack on a nuclear power plant, detonation of a radiological dispersal device ("dirty bomb"), or use of a tactical or strategic nuclear weapon.

Since 1945, approximately 400 radiological incidents have been reported involving about 3,000 substantial radiation exposures and 127 reported fatalities. These cases primarily involved industrial or medical exposure of small numbers of people, except for Chernobyl, where exposures were larger (Lazarus 2021). The lower reported mortality in these cases compared to Hiroshima and Nagasaki reflects both that personalised care was provided in these cases and that many accident victims experienced only partial body exposure. Fortunately, the number of cases of reported H-ARS have been low since 1945. However, the threat of radiological/nuclear incidents is increasing. Development of tactical nuclear weapons that might be used in the battlefield make a nuclear exchange more plausible compared to the past when there were only strategic nuclear weapons. Additionally, the threat of nuclear terrorism and of damage to nuclear power facilities has increased substantially (Kendall 2023, Lazarus 2023, Ryan 2023).

In Hiroshima, out of a population of 255,000 people there were 136,000 estimated casualties, including 45,000 victims who died within 24 hours, leaving a total of 91,000 survivors who would have benefitted from treatment. Unfortunately, treatment was severely limited, and 16,340 people died between day 2 and day 21 and an additional 2,660 victims died between day 21 and day 120 (Oughterson 1956, Woodruff 2014).

The impact of a nuclear detonation will increase significantly in a major city with a high population density and/or with the use of a larger weapon, in terms of the number of casualties, the size of "ground zero" and the distance from ground zero where there will be survivors who will benefit from treatment (Woodruff 2014). Based on US Government modelling, the detonation of a 10 KT nuclear device in a typical metropolitan area would result in approximately 500,000 persons requiring medical intervention to survive the expected effects of radiation or radiation combined injury.

A model of a nuclear blast of 10 KT in New York City has also been provided (Buddemeier 2018). In case of a highly populated area like NYC, the model esteems about 150,000-250,000 cases of H-ARS; as already specified, the high level of unpredictability of a nuclear accident together with demographic variations could largely influence the number of people affected by H-ARS. Therefore, the real scenario of emergency could largely vary, and its magnitude cannot be precisely defined. The evaluation of expected effects on special populations like infants, elderly, immune compromised patients considered to be more sensitive to radiation effects is hampered by the limited amount of evidence thus it cannot be excluded for them differences in clinical approach and outcome.

2.1.3. Biologic features, aetiology and pathogenesis

Acute radiation syndrome (ARS) results from exposure to high doses of ionising radiation, typically above 0.7 Gy, within a short period. Causes include nuclear explosions, reactor accidents, improper handling of radioactive materials, and radiological terrorism. Space radiation or accidental medical overexposures can also contribute, with severity depending on dose and exposure type

More specifically, H-ARS occurs after whole-body or partial-body (>60%) irradiation to doses >0.7 Gy, causing damage to rapidly dividing tissues, including bone marrow. Exposure to doses >2 Gy causes moderate to severe pancytopenia that may lead to infection, sepsis, bleeding, and death. Patients with H-ARS will be at risk for death within days to weeks of the event in the absence of medical intervention. According to literature, the level of irradiation of 2 Gy is commonly associated with the occurrence of H-ARS, but it should be noted that lower level of radiations can cause H-ARS in subjects particularly prone to the radiation damage (elderly, infants, immunocompromised patients); therefore, the risk of H-ARS occurrence could not be established *a priori* on the radiation dose but clinical and laboratory signs should be evaluated case by case.

Ionising radiation causes molecular and cellular damage, especially in rapidly dividing tissues like the bone marrow and gastrointestinal tract. Free radicals generated by radiation attack DNA, proteins, and lipids, with DNA double-strand breaks being the most critical. Lower doses primarily impair the hematopoietic system, leading to immune suppression and bleeding, while higher doses destroy intestinal mucosa, causing diarrhea and infections. Extremely high doses damage the brain and blood vessels, leading to cerebral edema and death. Systemic inflammation and oxidative stress amplify the damage, often resulting in multi-organ failure1.

[1] Christy, B. A., Herzig, M. C., Wu, X., Mohammadipoor, A., McDaniel, J. S., & Bynum, J. A. (2024). Cell Therapies for Acute Radiation Syndrome. *International Journal of Molecular Sciences*, *25*(13), 6973. https://doi.org/10.3390/ijms25136973

2.1.4. Clinical presentation, diagnosis and stage/prognosis

Lower doses of radiation primarily impair the hematopoietic system (H-ARS), leading to immune suppression and bleeding, while higher doses destroy intestinal mucosa, causing diarrhea and infections. Extremely high doses damage the brain and blood vessels, leading to cerebral edema and death. Systemic inflammation and oxidative stress amplify the damage, often resulting in multi-organ failure. (Christy, B. A., *et.al* 2024).

The signs and symptoms of H-ARS from the prodromal through final phase vary based upon the type and amount of radiation exposure and portion of the body exposed (partial vs. total body irradiation). Individual sensitivity to radiation, ongoing or recent use of immunosuppressive therapies and pre-existing medical conditions can also impact the risk and exposure level at which a person develops H-ARS (Baranov 1989, Mettler 2001, Staras 2006, Farrell 2008, Stricklin 2012, Jones 2014). Typical symptoms, latency periods, illness manifestations and probable based on estimated absorbed dose are shown in Table below.

Table 1 Time Course and Severity of Clinical Signs and Symptoms across Four Phases of H-ARS at Radiation Exposures (Garau 2011, Jones 2014)

Absorbed Dose (Gy)	Prodromal Phase	Latent Phase	Manifest Illness	Recovery / Final Phase
0.5 to 1.5	No symptoms, or nausea and vomiting for one day, temporary hair loss	1 day – several weeks	No symptoms or weakness, nausea and vomiting	Recovery
1.5 to 4	Nausea, vomiting, fatigue, weakness, diarrhoea for up to two days, hair loss	1 – 3 weeks	H-ARS: Leukopenia and thrombocytopenia	Recovery possible with supportive care
4 to 6	Nausea, vomiting, weakness, diarrhoea for up to two days	< 1-3 weeks	H-ARS: Leukopenia and thrombocytopenia, immune-suppression and sepsis, bleeding	Death without treatment
6 to 15	Severe nausea and vomiting, diarrhoea	Several days	H-ARS: Pancytopenia, immune-suppression and sepsis, bleeding, GI: bleeding, diarrhoea, fluid loss and electrolyte imbalance	Variable with supportive care and treatment

Note: patients exposed to more than 15 Gy would be expected to die within days of exposure from neurovascular effects of ARS and no treatments are currently available to address injuries in this population.

The risk of death is strongly correlated with the levels of myelosuppression and pancytopenia that present in patients. Myelosuppression and pancytopenia present in a radiation exposure-dependent fashion are impacted by the radiation dose, volume of body irradiated, and time of exposure and the presence of combined injuries (wound or burn). Patient age and gender, as well as comorbidities and concomitant injuries, are believed to impact susceptibility to H-ARS, clinical outcomes and ultimately risk of death (Goans 1989, Dainiak 2018, Adams 2017, FDA MDRE 2018, WHO 2022, SNS SSJ 2023).

2.1.5. Management

H-ARS is a life-threatening condition in adults and paediatric patients for which there is no approved treatment in the European Union (EU). At present, in the EU, treatment of radiation-exposed subjects relies on supportive therapies (i.e., transfusion support, fluids, antiemetic, antifungal and antibiotic therapy, anticonvulsants).

Table 2 Management of hematopoietic toxicity is stratified according to the degree of myelosuppression as determined by complete blood count with differential and signs of bleeding.

Degree	ALC	ANC	Platelet count	Bleeding and anemia
0*	1400 to 3500	4000 to 9000	140 to 400,000	None
1	≥1500	≥2000	≥100,000	Petechiae, bruising
				Normal Hb level
2	1000 to 1500	1000 to 2000	50 to 100,000	Mild blood loss
				<10 percent decrease in Hb
3	500 to 1000	500 to 1000	20 to 50,000	Gross blood loss
				10 to 20 percent decrease in Hb
4	<500	<500	<20,000	Spontaneous bleeding
				>20 percent decrease in Hb

Management of radiation injury, UpToDate 2024

Eltrombopag is authorised in adult patients with acquired severe aplastic anaemia (SAA) who were either refractory to prior immunosuppressive therapy or heavily pretreated and are unsuitable for haematopoietic stem cell transplantation. Medicines authorised for myelodysplastic syndrome (MDS) by EMA include: epoetin, filgrastim, pegfilgrastim, efbemalenograstim, azacitidine, luspatercept, lenalidomide, imetelstat. Iron overload may develop in MDS as a result of repeated RBC transfusions, which are a major part of the supportive care for anaemic MDS patients.

Medicines authorised in the treatment of immune thrombocytopenia (ITP) include: romiplostim, Immunoglobulins, avatrombopag and fostamatinib.

Treatment options also include allogeneic stem cell transplantation (allo-SCT). GM-CSF has been used over many years following HSCT and chemotherapy to help white blood cell levels recover.

However, for grade ≤1 with other toxicity/adverse feature and grade 2 radiation-induced myelosuppression, the World Health Organisation (WHO), the US Center for Disease control and prevention, and experts recommend the use of growth factors, as it is considered key: i) to improve survival of adults and children exposed to myelosuppressive doses of radiation, ii) to shorten the duration of severe neutropenia, and iii) to minimise the severity of neutropenia-associated complications, including infection.

Several growth factors, including sargramostim (2018), are FDA-approved for H-ARS. On April 2023, Ukraine issued an Emergency State Registration for sargramostim given the ongoing threat of a radiation incident.

Early treatment with growth factors increases the LD50/60 to 6-8 Gy, but the therapeutic window of use of these drugs is limited and does not exceed the 24 hours from the radiation exposure. Moreover, given the selectivity of each growth factor (filgrastim and peg-filgrastim for granulocyte and romiplostim for platelets) their use is intended to be in association. Noteworthy, at present none of the available erythropoietin growth factors are recommended by WHO for management of radiation induced anaemia, which could be treated only with red blood cell transfusions.

For grade 3 to 4, subjects with severe bone marrow aplasia are considered unable to reach a spontaneous autologous recovery; hospitalisation is usually required and allogeneic hematopoietic stem cell transplant (HSCT) should be considered after an observation period of 14-21 days has elapsed. However, the reported attempts at the time of Chernobyl and Hiroshima blasts in patients exposed to high dose of radiations (8-10 Gy) were not successful. To note, the efficiency of bone marrow transplantations procedure has been largely improved during in the last 20 years, so it is reasonable to assume that the current transplant-related mortality is far less than what reported. Nevertheless, transplantation access is hampered by several factors like the process of identification of suitable donors that may require 3-6 months and the need of specialized centres with trained transplant units. For those reasons, the procedure could not be considered a possible alternative therapy to sargramostim in an emergency setting. Further, feasibility of this approach is strongly limited in large mass casualty.

Sargramostim could be important for treating H-ARS, a serious condition with no approved treatment in the EU. Its broad action on bone marrow precursors and large therapeutic window support its potential. In a nuclear or radiological emergency, an effective, well-tolerated, and easy-to-administer treatment could enhance emergency response and reduce the impact on individuals and populations. Improved medical countermeasures would also boost the EU's preparedness and response capabilities. However, obtaining comprehensive data under normal conditions remains challenging due to the nature of the condition.

2.2. About the product

Sargramostim, a recombinant human granulocyte-macrophage colony-stimulating factor [rhu GM CSF], is a 127 amino acid glycoprotein produced by rDNA technology in a yeast (*S. cerevisiae*) expression system. Sargramostim differs from native human GM-CSF by substitution of leucine (Leu) for arginine (Arg) at position 23.

Sargramostim is a recombinant human GM-CSF. The binding to GM-CSF receptors expressed on the surface of target cells (haematopoietic progenitors and mature immune cells), initiates an intracellular signalling cascade which induces the cellular responses (i.e., division, maturation, activation). GM-CSF is a multilineage factor and, in addition to dose-dependent effects on the myelomonocytic lineage, it can promote the proliferation and maturation of megakaryocytic and erythroid progenitors. It drives host immunity by boosting innate and adaptive host defence and targets epithelial repair and restoration. GM-CSF is necessary for the repair and maintenance of barrier tissues, such as the gastrointestinal tract and lung.

The applied indication was "Treatment for exposure to myelosuppressive doses of radiation (Haematopoietic Syndrome of Acute Radiation Syndrome [H-ARS])."

The agreed indication is the following: 'Imreplys is indicated for treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic Sub-syndrome of Acute Radiation Syndrome (H-ARS). Imreplys should be used in accordance with official radiological/nuclear emergency recommendations.'

Imreplys powder for solution for injection, 250 µg/vial, is provided as a sterile, preservative-free lyophilised powder drug product for single use in an 8 mL Type I glass vial. The excipients are: sucrose, mannitol, and trometamol. The vial contains an overfill of 14 µg sargramostim allowing the labeled amount of 250 µg to be withdrawn after reconstitution with sterile water for injections.

Imreplys should be administered once daily as a subcutaneous injection and dosing is based on body weight as follows:

- 7 micrograms/kg in children and adolescents weighing greater than 40 kg and in adults and 10 micrograms/kg in children and adolescents weighing 15 kg to 40 kg
- 12 micrograms/kg in neonates, infants or children weighing less than 15 kg.

The first clinical use of sargramostim was in 1986 in the aftermath of the Chernobyl nuclear power facility accident.

In 1991, US FDA approved sargramostim for its first indication and throughout the 1990s the product received approval for 5 haematological indications. In 2018, sargramostim received marketing authorisation in the United States for H-ARS to increase survival in adult and paediatric patients from birth to 17 years of age acutely exposed to myelosuppressive doses of radiation.

On April 2023, Ukraine issued an Emergency State Registration for sargramostim given the ongoing threat of a radiation incident.

In the EU, sargramostim is only approved as an ancillary substance within Origio *In Vitro* Fertilisation device, origio A.R.T media (opinion 20/07/2023), with a strength of 2 ng/ml to provide an *in vitro* environment to better simulate the conditions *in vivo* for the embryo before the transfer into the woman's uterus."

2.3. Type of application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on:

- The fact that a radiological, and/or nuclear catastrophe (e.g., nuclear weapons attack, etc.) with the potential to spread across Member States was included in the top-3 cross-border health threats that require coordination at the EU level presented by the Health Emergency Preparedness and Response Authority (HERA). Acute radiation syndrome (ARS), also known as radiation sickness or radiation toxicity, occurs when individuals are exposed to high doses of total body irradiation (TBI) that causes multiorgan injury. The haematopoietic syndrome of ARS (H-ARS) occurs after whole-body or partial-body (>60%) irradiation to doses >0.7 Gray (Gy), causing damage to rapidly dividing tissues, including bone marrow, resulting in pancytopenia that may lead to infection, bleeding, and death. H-ARS is a life-threatening condition in adults and paediatric patients for which there is no approved treatment in the EU.
- The recognised unmet medical need for treatment of H-ARS, a life-threatening condition for which there is no approved treatment in the EU, along with a broad mechanism of action on bone marrow precursors and a large therapeutic window could support the claim that sargramostim represents a major therapeutic improvement for the management of radiation-exposed subjects.
- From a public health point of view, in case of nuclear/radiological events the availability of an effective treatment that is well tolerated and easy to administer could strongly improve mobilisation of emergency response without delay and mitigate the impact of the radiation accident on the individual and population level. Also, improvement in medical countermeasures would increase EU common preparedness and response capabilities to radiation/nuclear threats.

However, during assessment the CHMP concluded that it was no longer appropriate to pursue accelerated assessment due to the number of major objections and other concerns raised in the List of Questions.

- The applicant requested consideration of its application for a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of the above-mentioned Regulation

based on:

The indication for which the product in question is intended are encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence:

- Sargramostim is seeking approval for the treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic Sub-syndrome of Acute Radiation Syndrome (H-ARS). Imreplys should be used in accordance with official radiological/nuclear emergency recommendations). H-ARS is a life-threatening condition in adults and paediatric patients for which there is no approved treatment in the EU. Acute radiation syndrome (ARS), also known as radiation sickness or radiation toxicity, occurs when individuals are exposed to high doses of total body irradiation (TBI). The number of cases of reported H-ARS have been low since 1945. However, the threat of radiological/nuclear incidents is increasing.

It would be contrary to generally accepted principles of medical ethics to collect such information:

- Sargramostim efficacy studies cannot be conducted in humans with H-ARS. Such studies to collect clinical safety and efficacy data would be contrary to generally accepted principles of medical ethics due to the harmful levels of radiation required to induce H-ARS. The scenarios in which individuals may be exposed to radiation overdose include accidental exposure due to misused, lost, or stolen industrial or medical sources containing radionuclides, nuclear and/or radiological terrorism or attacks, or accidental and/or deliberate release from nuclear reactors. It is estimated that tens of thousands to hundreds of thousands of individuals may be at risk for lifethreatening H-ARS in a large-scale radiological and/or nuclear incident. Patients with H-ARS will be at risk for death within days to weeks of the event in the absence of medical intervention. It would therefore be contrary to acceptable medical ethics to conduct a clinical trial in these patients or withhold potentially life-saving treatment in low resource environments – often at or near the point of injury. This will place a significant strain on the healthcare system.

A comprehensive non-clinical data package, as well as clinical efficacy and safety data from other indications is included in this application. The clinical efficacy data that cannot be comprehensively provided are clinicals trials to collect efficacy and/or safety data from humans with H-ARS.

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as a sterile lyophilised powder for solution for injection containing 250 µg of sargramostim as active substance.

Other ingredients are: mannitol, sucrose, trometamol.

The product is available in 8 mL type I clear glass vials.

2.4.2. Active substance

2.4.2.1. General information

The active substance is sargramostim (INN), chemical name: recombinant human granulocyte-macrophage colony-stimulating factor [rhu GMCSF].

Sargramostim (rhu GM-CSF) is a 127 amino acid glycoprotein produced by rDNA technology in a yeast (*S. cerevisiae*) expression system. While sargramostim is in the colony stimulating factor (CSF) class of substance it differs from native human GM-CSF by substitution of leucine (Leu) for arginine (Arg) at position 23, in order to protect the product from degradation by the protease KEX2.

Sargramostim contains four different molecular forms: the non-glycosylated form, and three glycosylated forms (O-glycosylated, N-glycosylated, and N- plus O-glycosylated). The relative potency of the different glycoforms is presented. The molecular weight of non-glycosylated sargramostim is 14.43 kD. Relative molecular weights of the three glycosylated forms of sargramostim are approximately 19.5, 16.8, and 15.5 kiloDaltons (kD).

2.4.2.2. Manufacture, characterisation and process controls

Sargramostim active substance is manufactured at Partner Therapeutics, Inc. Lynnwood Washington, USA (PTx/Northpointe facility), referred to as 'NP'. Partner Therapeutics, Inc. is also in charge of the generation, the release and the stability testing of the working cell bank, and the in-process, release and stability testing of the bulk active substance.

A valid proof of GMP compliance covering activities and facilities was provided for all involved active substance manufacturers.

Description of manufacturing process and process controls

The sargramostim active substance manufacturing process has been adequately described. Main steps are fermentation, harvest and recovery, purification, final buffer exchange and filtration. The ranges of critical process parameters (CPPs) and the routine in-process controls along with acceptance criteria, including controls for microbial purity and endotoxin, are described for each step. The active substance manufacturing process description in the dossier is considered acceptable.

Table 3: Manufacturing unit operations of the sargramostim bulk active substance

Manufacturing Process	Unit Operation
Fermentation	1.5 L Shake Flask
	15 L Seed Fermentation
	100 L Production Fermentation
Harvest and Recovery	Microfiltration Harvest
	Ultrafiltration Concentration
Purification	C4 Capture
	C4 Purification
	C18 Purification
Final Buffer Exchange and Filtration	Final Cation Exchange
	Bulk Drug Substance Filtration

The Working cell bank (WCB) is cultured through two expansion steps before transferring to aproduction fermenter, followed by harvest and recovery unit operations (microfiltration and ultrafiltration). Downstream processing consists of reverse phase high pressure liquid chromatography columns, low-pressure cation exchange chromatography column, and filtration to yield sargramostim bulk active substance.

Production fermentation is a fed-batch process using a glucose solution feed. The description of the bulk drug filtration unit, together with relevant details such as volume of the pre-filtration flush buffer, the mixing parameters, mass of BDS to be filled into each BDS bottle is adequate.

Imreplys Powder for Injection has originally been licensed by the US FDA in 1991 (ref. Leukine) and, therefore, as part of this marketing authorization application (MAA) procedure, some historical data were leveraged from the use of Leukine as approved by the US FDA.

However, significant changes were introduced to the manufacturing process of sargramostim bulk active substance (BDS) since the initial US FDA approval of Leukine in 1991.Comparative data between the old and the new active substance and assessment of the subsequent changes introduced to the manufacturing process were provided. The comparison is adequate and both active substances were demonstrated to be comparable. Based on adequate demonstration of comparability, data obtained from the old active substance manufactured with the old manufacturing process are accepted as supportive for the current active substance manufactured with the presented new manufacturing process.

Control of materials

The cell bank system is based on *S. cerevisiae*. A two-tiered cell banking system is used, and sufficient information is provided regarding testing of master cell bank (MCB) and WCB and release of future WCBs. Genetic stability has been demonstrated for cells at and beyond the limit of cell age.

Out-of-specification (OOS) results for non-host assay occurred and resulted in an investigation to identify the root cause. The details of this OOS, the investigation, the root-cause and the adequate CAPA were provided in a clear and comprehensive manner, thus solving the raised Major Objection.

Sufficient information on raw materials used in the active substance manufacturing process has been submitted. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented.

The list of raw material used in the manufacturing process of sargramostim, including filter and chromatography gels, has been provided. Raw materials used in the manufacture of sargramostim are tested by suppliers and accepted on a certificate of analysis (CoA) or tested in-house.

The sargramostim fermentation process utilises two animal derived raw materials. There are no animal derived raw materials in the downstream isolation, purification, and filtration steps or the finished product manufacturing process. The Certificate of Suitability from the EDQM is provided.

Control of critical steps and intermediates

Within the upstream process unit operations, only the production fermentation has CPPs that have the potential to impact critical quality attributes (CQAs) including glycoform distribution and N-terminal clipping. The CPPs identified in production fermentation that have a significant impact on sargramostim are temperature and pH.

Within the downstream process unit operations, the purification chromatography steps have CPPs that have the potential to impact CQAs including the separation of product glycoforms from process-related impurities, the separation of hyperglycosylated material from sargramostim including removal of process-related impurities, and a further reduction in process-related impurities.

A comprehensive overview of critical in-process controls (IPCs) and critical in-process tests performed throughout the sargramostim active substance manufacturing process is given. Acceptable information has been provided on the control system in place to monitor and control the active substance manufacturing process with regard to critical, as well as non-critical operational parameters and in-process tests. Actions taken if limits are exceeded are specified. No reprocessing is foreseen within the manufacturing process of sargramostim active substance.

Process validation

Continued Process Verification (CPV) is configured to ensure that the active substance manufacturing process remains in a validated state of control. At least one active substance batch is produced on an annual basis as a CPV batch, thus complying with regulatory guidelines. Moreover, supplementary data are collected as part of this program in order to monitor the consistency of the production process and detect unwanted process variability.

Manufacturing process development

The applicant provided a description of the activities conducted in order to fully develop the manufacturing process to be run at NP site at the time of transfer from 51U site.

Changes introduced to the manufacturing process were evaluated for potential impact to product quality using comparability acceptance criteria derived from bulk active substance (BDS) commercial lots produced at the original facility. Additionally, historical process and development experience, investigations, and development studies determined the significance of each change. Development of the acceptance limits/controls/ranges for performance parameters set for the commercial process are sufficiently described.

Characterisation

Extensive data about the physicochemical and functional characteristics of sargramostim was provided, including primary sequence confirmation by means of mass spectrometry data combined with three different peptide-mapping procedures, molecular weight and extinction coefficients determination, C-terminal analysis, disulfide bond assignments, as well as key information about higher order structure and biological properties.

The characterisation exercise was performed with commercial and validation batches in order to address the comparability exercise. Sufficient information has also been provided for relevant post-translational modifications (PTMs) such as Methionine Oxidation, Aspartate Isomerisation, Deamidation.

Individual glycoforms were also isolated exploiting a reverse phase analytical method and then analyzed together with their parental batches for characterization and comparability across the lots obtained at old and current facilities.

In general, the active substance sargramostim is considered to be sufficiently characterised. However, to complement the characterisation, the applicant is requested to clarify few remaining points through post-authorisation recommendations. Impurities were correctly identified, and their impact on the quality and biological activities of sargramostim active substance adequately evaluated.

2.4.2.3. Specification

The active substance release specification includes tests for Physical Appearance (Ph. Eur.); Identification (isoelectric focusing, peptide mapping); Quantity (protein concentration); Biological activity (potency); Purity (SDS-PAGE, glycosylated variants, HMWC, protein purity); Other tests (monosaccharide composition, pH); Microbial (endotoxin, microbial content).

The release specifications proposed for sargramostim active substance are overall acceptable.

Justification was provided for not testing host cell proteins (HCPs) at release or as an IPC test, but as part of the annual continued process verification.

Analytical methods

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines with some exceptions that have been raised as post-approval recommendations.

Batch analysis

The Batch analysis section includes the list and the release data of all the representative NP commercial batches, including those used for initial and last process validation, non-clinical/clinical studies, comparability exercise and reference standards.

With respect to justification of specifications, it is noted that specifications are determined on a set of historical data, from previous process since comparability with previous process versions is demonstrated.

Reference materials

Information on reference standard system is presented.

Confirmation on the suitability of reference standards was requested as a Major Objection. In the course of the procedure, sufficient information was provided resolving the Major Objection.

The history of all the used reference standards is also provided, with the associated BDS batches used. Currently, the WHO International Standard 88/646 is used to qualify the PTx bioassay SRS and all the relevant information is included in 3.2.S.5. In this regard, the applicant committed to introduce a two-tiered Reference Standard Program in response to a post-approval recommendation.

Container closure

The bulk active substance (BDS) container closure system consists of a Nalgene narrow-mouth 2000 mL bottle with a Nalgene 28 mm screw-cap closure. CCS is correctly described in terms of composition, volume, temperature stability and (low) propensity to interact with chemicals and solvents. Products made from Teflon FEP 100 resin comply with the Commission Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact with food.

Qualification and safety tests include a trace metal analysis and extractables/leachables studies, with no elements and non-volatile or semi-volatile compounds detected above the limit of quantification, respectively. The bottles and caps are also tested separately to verify the integrity during molding at periodic intervals.

Since compliance of container closure referred to USP only, a comparison of the testing certificate for the active substance container closure to the relevant Ph. Eur. monographs has been conducted and a number of gaps have been identified, which the applicant will address in response to a post-approval Recommendation.

2.4.2.4. Stability

All stability results at the long-term storage conditions meet specifications of stability indicating critical quality attributes. Stability data at accelerated storage conditions (5 °C, 15 °C, 40 °C) 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.

In addition, photostability was assessed to determine potential effects of light exposure on bulk active substance demonstrating that vials stored protected from light met critical quality attributes. However, a photo induced degradation was observed in bulk active substance vials exposed to light. In this regard, the applicant confirmed that appropriate measures are in place following bulk active substance (BDS) filtration and subsequent sampling to protect the active substance from light exposure.

Shipping temperature must be -80 °C to -60 °C with allowable temperature excursions of up to -20 °C for not more than 48 hours. Data are provided to support BDS temperature excursions as claimed during shipping.

The post approval stability protocol and commitment have been presented and is considered acceptable. The applicant has committed to inform the competent authorities in case of OOS result occurring on long-term stability for any bulk active substance.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

The finished product (FP) is a sterile lyophilised powder for solution for injection. The qualitative and quantitative composition of the finished product is presented in the table below.

Table 4: Composition of the finished product

Ingredients			**Pharmaceutical Function**	**Quality Standards** [3]
Active Ingredient				
Sargramostim			Active ingredient	In-house [4]

Ingredients			Pharmaceutical Function	Quality Standards [3]
Inactive Ingredients				
Mannitol			Bulking agent	Ph.Eur.
Sucrose			Stabilizer	Ph.Eur.
Trometamol [5,6]			Buffer component	Ph.Eur.
1 N Hydrochloric Acid			pH adjustment	Footnote 8
Water for Injections			Solvent	Ph.Eur.
Nitrogen			Vacuum neutralization	Ph.Eur.

Ph. Eur. = European Pharmacopoeia; q.s. = Quantity sufficient; NA = Not applicable

[3] *The excipients comply with Ph. Eur. and United States Pharmacopeia (USP)/National Formulary (NF).*

[4] *Refer to Section 3.2.S.5.*

Each vial of the finished product contains 264 µg of the active substance sargramostim, which includes an overfill of 14 µg of sargramostim to allow withdrawal of the labeled amount of active substance and obtain a concentrated solution for injection of 250 µg of sargramostim per mL after reconstitution with 1 mL Water for Injections (WFI) Ph. Eur. After reconstitution, the volume of the finished product is approximately 1.05 mL allowing withdrawal and administration of 1 mL.

All excipients are well known pharmaceutical ingredients, and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. No ingredients of animal or human origin are used.

The primary packaging is 8 mL (Type I clear glass) vials. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product.

Imreplys Powder for Injection is a legacy product approved by the US FDA in 1991 under the marketing name Leukine and the formulation development was not included in the initial US FDA MAA. Therefore, the initial development information that is now typically included in a new MAA is not available from previous sponsors.

2.4.3.2. Manufacture of the product and process controls

Imreplys finished product manufacturing and filling operations are performed in accordance with current EU GMP Annex 1: Manufacture of Sterile Medicinal Products at the following facility at *Patheon Manufacturing Services LLC, 5900 Martin Luther King Jr. Highway, Greenville, NC 27834-8628, United States*.

Release and stability testing of Imreplys finished product are performed at *Partner Therapeutics, Inc. 2625 162nd Street SW, Lynnwood, WA 98087-3263, United States* for all tests excepts particulate matter that are tested at *Nitto Avecia Pharma Services, Inc. 10 Vanderbilt Irvine, CA 92618-2010, United States.*

The finished product is released in the EU by *Paesel & Lorei GmbH & Co. KG Biochemika Diagnostika Und Pharmazeutika (P&L) Nordring 11, D-47495 Rheinberg, North Rhine -Westphalia (Germany).* A major

objection was raised during the procedure requesting a Manufacturing and Import Authorisation (MIA) for the EU batch release site, which was provided.

The process consists in thawing of the frozen sargramostim bulk active substance (BDS), finished product compounding and bioburden reduction filtration, sterile filtration), filling into 8 mL glass vials, partial stoppering, transfer and lyophilisation, capping, visual inspection, labelling and packaging.

Adequate in-process specifications were defined during the manufacture of the finished product.

Results relative to manufacturing process validation (PPQ), sterile filter validation, aseptic process simulation (media-fills), sterilization and/or depyrogenation of the containers, closures, equipment and components, cleaning validation have been provided.

Shipping validation data to support the finished product transportation procedures from the US to EU, were provided. However, by 30 October 2025, the applicant should provide summary results of the execution of the summer verification protocol to fulfil a post-approval recommendation. PPQ was designed to demonstrate that the Patheon manufacturing process is capable of consistently delivering a finished product of the intended quality.

The validation of the Imreplys finished product manufacturing process at Patheon Greenville was confirmed with three nonconsecutive lots including two PPQ batches and one continued process verification batch.

In conclusion, the applicant has provided satisfactory proof that the manufacturing process has been appropriately validated. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate.

2.4.3.3. Product specification

The finished product release specification includes tests for Appearance (Ph. Eur.); Identity and Purity (SDS-PAGE, peptide mapping); Quantity (protein concentration); Potency (bioassay); Purity and Related Substances (glycosylated variants, HMWC); General tests (reconstitution time, pH of the reconstituted solution, water, particulate matter, uniformity of dosage units); Safety (bacterial endotoxins, sterility).

In general, the release specification set for Imreplys Powder for Injection finished product result is in accordance with the principles defined in ICH Q6B.

Following a request from a Major Objection, the potency specification limit has been tightened. The Major Objection was therefore resolved.

Analytical methods

The analytical methods used have in general been adequately described and non-compendial methods appropriately validated in accordance with ICH guidelines with some exceptions that have been raised as post-approval recommendations.

Batch analysis

Batch analyses data are provided for one Imreplys finished product engineering batch and three validation batches. The results are within the specifications and confirm consistency of the manufacturing process.

Characterisation of impurities

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment it can be concluded that it is not necessary to include any elemental impurity controls. The information on the control of elemental impurities is satisfactory.

A risk evaluation concerning the presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). Based on the information provided it is accepted that no risk was identified on the possible presence of nitrosamine impurities in the active substance or the related finished product. Therefore, no additional control measures are deemed necessary.

Reference materials

The reference standard used for Imreplys finished product is the same as the reference standard used for active substance.

2.4.3.4. Stability of the product

Based on available stability data, the proposed shelf-life and storage conditions of 48 months under refrigerated conditions (2 °C – 8 °C), protected from light, as stated in the SmPC are acceptable.

After removing the carton from the refrigerator, the shelf-life claim is 12 months at 25 °C and 1 month at 40 °C, storage conditions applicable to emergency-use conditions only such as a severe nuclear reactor accident or in the event of a nuclear detonation.

Primary and supportive stability studies were conducted at the long-term conditions at 5° C, accelerated conditions at 25ºC, 25 °C /60 %RH and 40 °C /75 %RH and under stress conditions at 40°C.

A photostability study conducted on one sargramostim active substance batch and on two Imreplys finished product batches has been provided and concluded that the active substance is photosensitive and the finished product must be protected from light.

In addition, the stability data at stress (40±2 °C) storage conditions demonstrate that Imreplys Powder for Injection can be stored for 1 month at 40±2 °C.

It is noted that the storage conditions out of the refrigerator (25 °C/40 °C) are applicable to emergency-use conditions only, such as a severe nuclear reactor accident or in the event of a nuclear detonation that might require exceptional storage out of refrigerator.

Considering that the administration of Imreplys finished product might occur within the radioactive zone, data supporting the stability profile of Imreplys after low-level gamma irradiation were provided. The protocol was designed to mimic the dispensation of the finished product in a triage situation after nuclear disaster. The irradiation stability results demonstrated that Imreplys finished product critical quality attributes are able to withstand nuclear incident conditions for up to 15 days with radiation levels up to 1 Gy and temperatures of up to 40 °C.

Since comparability was adequately demonstrated between the historical US version of Imreplys, Leukine,and current Imreplys, both at active substance and finished product level, stability data available for the historical Leukine finished product batches are accepted in support of the proposed

shelf-life for current Imreplys finished product. The shelf-life of 48 months is therefore sufficiently supported for Imreplys finished product considering all stability data available with historical US Leukine batches and Imreplys finished product batches manufactured with the commercial manufacturing process presented in this application. However, as post-approval recommendations, the applicant is requested to complete stability studies on current Imreplys and revise shelf-life specification if necessary.

2.4.3.5. Adventitious agents

Sargramostim is expressed in yeast, which will not propagate mammalian viruses, and the animal-derived raw materials used in the fermentation process are of low risk of viral contamination. Through careful selection of raw materials, implementation of in-process controls, and process monitoring, there is minimal risk of adventitious agent contamination in the manufacture of sargramostim active substance.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Several deficiencies were identified leading to Major Objections (MO) on Quality aspects during the marketing authorisation procedure pertaining to:

- Manufacture of the active substance: The applicant proposed a new active substance manufacturing process leveraging historical data from the use of the initial version of Imreplys approved by the US FDA in 1991 under the name Leukine. This approach was not endorsed mainly due to the lack of comparative, validation, and stability data on the new version of the active substance manufacturing process presented in this MAA. Comparative data between the old and the new active substance and assessment of the subsequent changes introduced to the manufacturing process were provided. The presented data was deemed acceptable to sufficiently address the Major Objection.

- Control of materials: The release of a contaminated WCB was not accepted. Investigations on the root cause of the contamination and implementation of adequate CAPAs were requested in addition to clarification on the strategy for WCB release testing were requested and sufficiently addressed by the applicant.

- Reference standard: Confirmation of the suitability of reference standards was requested as a Major Objection. Sufficient information was provided resolving the MO.

- Manufacturers of the finished product: A manufacturing authorization was requested and provided for the EU batch release site.

- Batch uniformity in aggregates content and control of HMWC on the finished product: Further demonstration of the uniformity between finished product batches along with tightening of the specification limit was requested.

- Validation of the potency method: The proposed potency method and specification was not considered acceptable due to incomplete validation, unalignment of proposed specification limit with batch analysis result from the commercial process, and uncertainty on the sensitivity of the method in detecting differences in biological activity of the different glycoforms.

These major objections could be resolved and there is no remaining concern on the potential impact of quality issues on the Benefit/Risk of the product.

At the time of the CHMP opinion, there were fourteen minor unresolved quality issues having no impact on the benefit/risk ratio of the product, which mainly pertain to the control and stability of the active substance and finished product. These points are put forward and agreed as recommendations for future quality development.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

2.4.6. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

1. By December 2025, the applicant should provide summary results of the new binding study using current technology.
2. Within 30 days after granting the marketing authorisation, the applicant should introduce, through a suitable variation procedure, the new validated TF-1 bioassay. An appropriate specification should be set, substantiated with data.
3. By Q3 2026, the applicant should introduce, through a suitable variation procedure, the new validated capillary IEF method, currently under development, and should consequently include icIEF control of impurities in DS release specification. An appropriate specification should be set, substantiated with data.
4. By Q1 2027, the applicant should introduce, through a suitable variation procedure, the new validated CE-SDS method, currently under development. An appropriate specification should be set, substantiated with data.
5. Within 60 days after granting the marketing authorisation, the applicant should implement, through a suitable variation procedure, a two-tiered Reference Standard Program.
6. By 30 October 2025, the applicant should provide summary results of the execution of the summer verification protocol evaluating the performance of the Credo Containers under high external temperatures.
7. By Q2 2026 the applicant should introduce, by a suitable variation procedure, the new UPLC methods aimed to adequately resolve either size variants and monomer of sargramostim. An appropriate specification should be set, substantiated with data.
8. At completion of the ongoing stability studies, the applicant should duly update section 3.2.P.8. by including Stability data (summary Tables and Plots) relative to DP batches currently placed in stability for all the planned timepoints.
9. By Q3 2026, the applicant should introduce, by suitable variation procedure, the HCP assay fully validated as per ICHQ2(R2) and Ph Eur 2.6.34. An appropriate specification should be set, substantiated with data.

10. By Q1 2026, the applicant should provide summary results relative to the validation of the scanning densitometry method.
11. By Q4 2025, the applicant should provide characterisation data by HILIC-HPLC on at least three BDS batches to evaluate the consistency of the DS process and to demonstrate that the same proportion of each isoform (non-glycosylated, N, N/O, O-glycosylated and phosphorylated isoforms) is obtained.
12. By Q3 2026, in line with REC 3 (IEF Q3 2026), REC 7 (RP-UPLC Q2 2026) and REC 11 (HILIC-HPLC, Q4 2025), the applicant should provide, by compiling all data obtained on at least three BDS batches, a discussion on batches consistency of the DS process with regards to same identity and proportion of each isoform (non-glycosylated, N, N/O, O-glycosylated and phosphorylated isoforms).
13. By Q4 2025, the applicant should provide summary results of the study planned to show complete compliance of DS container closure to Ph.Eur. monographs.
14. Once 48-month stability data become available for the PPQ and initial CPV batches manufactured at Patheon, the applicant should conduct a full statistical analysis on all available stability data and revise, if necessary, the HMWC shelf-life specifications through a suitable variation procedure.

2.5. Non-clinical aspects

2.5.1. Introduction

To support the marketing authorisation application (MAA) of sargramostim for the H-ARS indication, pharmacology, pharmacokinetic (PK), and toxicology studies were performed (see Table below for complete list of studies). All studies, except for the pilot efficacy study, were compliant with Good Laboratory Practice (GLP).

Table 5. Studies with sargramostim

Study Number	Study Type and Duration	Route	Species	Compound Administered	GLP Compliance
Pharmacology					
TSK0143	Pilot efficacy sargramostim administered daily for 14 days, starting 48 hours after irradiation	SC	Irradiated Rhesus monkeys 670 cGy ($LD_{50-60/60}$)	sargramostim[a]	No
FY14-045 FY14-045-amend1	Adequate and Well Controlled (AWC) efficacy sargramostim administered daily through Day 18 or until ANC >1000/µL, starting 24 or 48 hours after irradiation	SC	Irradiated Rhesus monkeys 680 cGy ($LD_{50/60}$)	sargramostim[a]	Yes
TSK0144 TSK0144-amend1	Confirmatory AWC efficacy sargramostim administered daily until ANC ≥1000/µL for 3 consecutive days or ANC ≥ 10,000/µL, starting 48 hours after irradiation	SC	Irradiated Rhesus monkeys 655 ($LD_{50-60/60}$) 713 ($LD_{70-80/60}$)	sargramostim[a]	Yes
1017-3493	Time to treat AWC efficacy sargramostim administered daily until ANC ≥1000/µL for 3 consecutive days or ANC ≥ 10,000/µL, starting 48, 72, 96 or 120 hours after irradiation	SC	Irradiated Rhesus monkeys 713 ($LD_{70-80/60}$)	sargramostim[a]	Yes
Pharmacokinetics					
DDK0110	14-day PK	SC	Rhesus monkeys	sargramostim[a]	Yes
DDK0111	14-day PK sargramostim administered daily for 14 days, starting 24 hours after irradiation	SC	Irradiated Rhesus monkeys 646 cGy ($LD_{30/60}$)	sargramostim[a]	Yes
Toxicology					
Single-dose toxicity					
2423-103	1-day	IV	*Cynomolgus* monkey	sargramostim[a]	Yes
Repeat-dose toxicity					

Study Number	Study Type and Duration	Route	Species	Compound Administered	GLP Compliance
2423-105	14-day toxicity	IV	*Cynomolgus* monkey	sargramostim[a]	Yes
2423-111	30-day toxicity	SC	*Cynomolgus* monkey	sargramostim[a]	Yes
A24993	42-day toxicity	SC	*Cynomolgus* monkey	sargramostim[a]	No
A27294	42-day toxicity	SC	*Cynomolgus* monkey	sargramostim[b]	Yes
Reproductive and Developmental toxicity					
A28816	14-day toxicity	SC	New Zealand white Rabbits (nonpregnant)	sargramostim[b]	Yes
A31774	14-day toxicity	SC	New Zealand white Rabbits (nonpregnant)	sargramostim[b]	No
A39389	Fertility and early embryonic	SC	New Zealand white Rabbits	sargramostim[b]	No
A38192	Fertility and early embryonic	SC	New Zealand white Rabbits	sargramostim[b]	Yes
A38918	Embryo-foetal	SC	New Zealand white Rabbits	sargramostim[b]	No
A38193	Embryo-foetal	SC	New Zealand white Rabbits	sargramostim[b]	Yes
A43883	Pre- and post-natal	SC	New Zealand white Rabbits	sargramostim[b]	Yes

Abbreviations: AWC: adequate and well controlled; cGy: centiGray; LD: lethal dose; PK: pharmacokinetics; SC: subcutaneous

[a] Released clinical material containing 40 mg/mL mannitol, 10 mg/mL sucrose and 1.2 mg/mL tromethamine as excipients.

[b] Formulation contained EDTA, which is no longer being manufactured (40 mg/mL mannitol, 10 mg/mL sucrose, 1.2 mg/mL trometamol, 11.5 mg/mL benzyl alcohol and 1.9 mg/mL EDTA

Analytical methods

Validated solid phase enzyme linked immunosorbent assay (ELISA) to quantify sargramostim in monkey plasma and a validated bridging ELISA assay to detect anti-drug antibodies (ADA) in monkey serum, were used in the PK studies.

Similar validated ELISA assays to those for the Rhesus monkey studies were used to quantitate serum concentrations of sargramostim and to detect ADA in the 42-days *Cynomolgus* monkey toxicity studies and the rabbit reproductive and developmental toxicology studies. Additionally, a validated bioassay was used to detect sargramostim neutralising antibodies in the *Cynomolgus* monkey and rabbit studies.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

The pharmacology program consists of 4 non-clinical efficacy studies (one pilot, one AWC, one confirmatory AWC, and one time-to-treat AWC) utilising total body irradiated rhesus monkeys (referred to as non-human primate (NHP) H-ARS model). The 3 pivotal AWC studies are also the basis for demonstration of sargramostim clinical efficacy in children (from birth) and adult humans in the sought indication H-ARS and are presented in the clinical efficacy section of this report.

2.5.2.2. Secondary pharmacodynamic studies

No secondary pharmacodynamic study was performed.

2.5.2.3. Safety pharmacology programme

No independent safety pharmacology studies were performed. Cardiovascular function endpoints (blood pressure and electrocardiograms) were evaluated in the context of *Cynomolgus* 42-day repeated dose toxicology study (A27294, EDTA liquid formulation). Overall, daily subcutaneous administration of sargramostim at 20, 63 or 200 μg/kg/day for 6 weeks had no effect on blood pressure or electrocardiograms.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interactions study was performed.

2.5.3. Pharmacokinetics

DDK0110 study: haematologic parameters, pharmacokinetics and immunogenicity were evaluated in naïve, non-irradiated male Rhesus monkeys with daily SC injections of sargramostim for 14 consecutive days at dosages of 7 µg/kg/day or 20.8 µg/kg/day (5 males per group). The time to reach maximum plasma concentration (T_{max}) was 1 to 2 hours (median values) and the terminal elimination half-life ($t_{1/2z}$) was 1.19 to 2.27 hours (mean values), regardless of dose or dosing frequency. On Day 1, the increases in C_{max} and AUC_{last} were slightly greater than dose proportional between the 7 µg/kg/day and 20.8 µg/kg/day dose levels while the increases of both parameters were generally dose proportional on Day 14. There was no evidence of accumulation of sargramostim following 14 days of daily SC injections, with lower exposure observed at both dose levels after two-weeks of administration. This was consistent with the presence of ADAs in all animals at the Day 15 time point.

DDK0111 study: In this study, rhesus monkeys (3 per sex per group) received a total body irradiation dose of approximately 646 cGy (targeted $LD_{30/60}$). A radiation dose to achieve $LD_{30/60}$ was used to minimise animal mortality, thereby allowing for a robust analysis of sargramostim pharmacokinetic parameters. Starting 24 hours post-irradiation, sargramostim at 7 µg/kg/day or 20.8 µg/kg/day was administered subcutaneously for 14 consecutive days followed by a 16-day observation period.

All irradiated monkeys in both sargramostim dose groups presented with changes in body weight, body temperature and clinical signs that are commonly associated with H-ARS [DDK0111]. Two animals in the 20.8 µg/kg/day group were euthanised on Day 14 because they both met euthanasia criteria. In the irradiated animals that survived, PK parameters were similar to that observed in non- irradiated

monkeys [DDK0110]. The T_{max} was 1 to 2 hours (median values) after SC injection and the $t_{1/2z}$ ranged from 1.24 to 3.46 hours (mean values) after a single or repeat dosing. On Day 1, the increase in sargramostim systemic exposure was slightly greater than dose proportional between the 7 µg/kg and 20.8 µg/kg dose levels. C_{max} and AUC_{last} increased by approximately 4-fold in males and approximately 5-fold in females following a single SC injection. On Day 14, increases in C_{max} and AUC_{last} were generally dose proportional for both sexes with no evidence of accumulation; exposure was lower on Day 14 than on Day 1 for the female animals in the 20.8 µg/kg/day group. No ADAs were detected in any of the samples.

No DDI PK studies were carried out: however, in PK DDK0111 study (irradiated, male and female animals), the following supportive care was provided based on the clinical judgment of the veterinarian: Buprenorphine, Marcaine, Parenteral fluids, Sucralfate, Enrofloxacin, Ondansetron.

2.5.4. Toxicology

The safety of sargramostim was evaluated in 4 GLP compliant studies in *Cynomolgus* monkeys following a single IV administration ([2423-103]) and repeat IV daily dosing up to 14 days in duration ([2423-105]) and repeat SC daily dosing up to 42-days in duration ([2423-111], [A27294]).

2.5.4.1. Single dose toxicity

A single IV administration of 300 µg/kg sargramostim to *Cynomolgus* monkeys [2423-103] resulted in enlarged spleen of 1 of 4 animals, otherwise all parameters were similar to the saline control group.

2.5.4.2. Repeat dose toxicity

Study 2423-105 tested a single dose of Sargramostim, lyophilised powder 300 ug/kg/day for 14-day followed by 14 recovery day in *Cynomolgus* macaque.. All animals survived until their scheduled sacrifice, and no test article related clinical signs were noted throughout the observational period.

In study **2423-111** (completed in 1988) 2 groups of three male and three female *Cynomolgus* monkeys each received sargramostim lyophilised powder via subcutaneous administration once daily for 30 days at dose levels of 0 (vehicle), 20 and 200 µg/kg. One animal per sex per group was retained for a 14-day recovery period. All animals survived to termination. Administration of 200 µg/kg/day of recombinant human GM-CSF (rGM-CSF) via subcutaneous injection for 30 consecutive days was associated with dramatic increases in total leukocyte, segmented neutrophils, monocytes, eosinophils, basophils and lymphocytes. This increase was seen by approximately 8 days after initiation of the treatment. This increase in circulating cells reached its maximal level by 10-17 days after treatment was started. The elevated level of circulating cells began to fall before the 30-day treatment course was completed. In all cell lineages a rapid fall to pretreatment levels was seen within 6 days of completing treatment. Thus, there is no evidence whatever to suggest a potential for continued stimulation of these cell populations after treatment has been completed. Absolute and relative spleen weights were increased in terminal animals. Histopathologic evaluation confirmed a moderate lo moderately severe bone marrow hypercellularity (interpreted as myeloid hyperplasia) with infiltrates of mononuclear cells- and eosinophils into other organs. Foci of mononuclear cell infiltrates were noted in the heart of 3 of the 4 animals (200 ug/kg/day) at terminal sacrifice but not at the end of the recovery period. Moderate to moderately severe thymic atrophy (despite increased lymphocyte counts) in both terminal and recovery animals (200 µg/kg/day): whether this is a primary or secondary effect of sargramostim could not be determined.

The dramatic increase in leukocyte counts and spleen weights demonstrate and that recombinant human GM-CSF is a potent stimulator of haematopoiesis in normal primates.

No adverse effects on male and female reproductive tracts were evident.

Although a NOAEL was not determined by the study director, this could be set at 20 ug/kg/day.

No toxicokinetics was assessed.

Study A27294 (completed in 2010) was carried out in F/M sexually mature *Cynomolgus* monkeys, being treated SC daily for 42 day (6 week). The doses tested were 20, 63 and 200 µg/kg sargramostim EDTA formulation. The same study was referred for safety pharmacology assessment of cardiovascular function: no effect on blood pressure or electrocardiograms was observed at any dose levels.

Sargramostim was generally well-tolerated except for three high dose animals which were prematurely sacrificed due to severe abscess formation and / or skin necrosis at the injection site and skin and inflammatory cell infiltration to multiple organs (parenchymatous organs, pericarditis, synovitis).

A pronounced pharmacological response occurred with sargramostim treatment at dosages of $\geq$ 20 µg/kg/day, as indicated by the marked increase in WBC count particularly, neutrophils, monocytes and eosinophils by Day 15; however, the pharmacodynamic effects were almost completely reversed by the end of the treatment period. The lympho-hematopoietic system was identified as the primary target.

Hypercellularity/hyperplasia and inflammation of bone marrow, spleen and lymph nodes, inflammatory cell infiltrates in numerous organs (e.g., liver, heart, lung, brain, injection site and skin) and increases in spleen and lymph node organ weights with dosages $\geq$ 20 μg/kg/day were observed at the end of the 6-week treatment and were considered to be due to the exaggerated pharmacological effects of sargramostim and correspond to increased numbers of activated circulating white blood cells. Cellular infiltrations in different organs were not completely reversible after the 12-week recovery period, however showed tendency to reversibility.

No adverse effects on male and female reproductive tracts were evident.

Although a NOAEL was not determined by the study director, this could be set at $\geq$ 20 ug/kg/day.

Toxicokinetic evaluation revealed that after single and repeated subcutaneous administration of sargramostim, the mean systemic exposure (AUC [0-t_{last}]) increased with increasing dose in the animals. The generally observed decrease of the dose normalised AUC[0-t_{last}] during the course of the study (maximum reduction of 30% following the last injection on Day 42 compared to Day 1, occurred at 200 µg/kg dose level), was attributed to the formation of binding and/or neutralising antibodies against sargramostim reflecting the immunogenicity of the recombinant human protein in monkeys. Exposure multiples above the expected clinical exposure at 7 µg/kg were relatively maintained across the dosing period. However, caution should be taken when using these exposure multiples since sargramostim was immunogenic in all but one monkey, with the detection of neutralising anti-drug antibodies starting at Week 2 and continuing throughout the study. This was the reason why it was not feasible to extend the treatment period of sargramostim longer than 42 days.

2.5.4.3. Genotoxicity

No genotoxicity study was performed.

2.5.4.4. Carcinogenicity

No carcinogenicity study was performed.

2.5.4.5. Reproductive and developmental toxicity

All developmental and reproductive toxicology (DART) studies were carried out in rabbit. In all DART studies sargramostim EDTA liquid formulation was used.

Fertility and early embryonic development

In the pivotal study A38192, sargramostim was administered once daily by subcutaneous injection to 3 groups of 20 New Zealand white [Hra:(NZW) SPF] rabbits beginning 6 days prior to artificial insemination and continuing through gestation day 7, inclusively. Dosage levels were 25, 70 and 200 µg/kg/day.

One female in the 200 µg/kg/day group was found dead on GD 2 (prior to implantation) after exhibiting low food consumption, severe body weight loss and decreased defecation for approximately 1 week prior to death; this death was determined to be test article related. The only internal finding was red contents in the vagina. All other animals survived until their scheduled termination.

There were no test article-related effects on placental weights, corpora lutea number, or post-implantation loss in any sargramostim dose group or on intrauterine survival in the 70 and 200 µg/kg/day groups. A dose-dependent reduction in mean numbers of implantation sites and decreased number of viable embryos (6.5, 6.4, and 6.0 implantation sites per dose (i.e., female) and 6.3, 5.9, and 5.5 viable embryos per doe, respectively) was observed.

The ability of females to conceive was unaffected by test article administration at all dosage levels. However, based on lower embryonic survival (primarily preimplantation loss) in the 200 µg/kg/day group, a dosage level of 70 ug/kg/day was considered to be the NOAEL for female reproductive and early embryonic toxicity when administered by subcutaneous injection to New Zealand white rabbits. Signs of maternal toxicity were recorded at the mid-dose level and higher and consisted of body weight losses and/or reduced body weight gains, reduced food consumption and associated clinical signs (decreased defecation) as well as mortality in the 200 µg/kg/day group. The NOAEL for systemic maternal toxicity was considered to be 25 ug/kg/day.

Toxicokinetic analyses revealed that subcutaneous administration of sargramostim to rabbits resulted in systemic exposure. Across dose groups, the dose-normalised AUC(0-t_{last}) values were in the same range, indicating a generally linear and dose-proportional increase of systemic exposure with increasing dose over the 25 to 200 µg/kg/day range. Exposure to sargramostim decreased about 10-fold from study day 0 (6 days prior to artificial insemination) to gestation day 7. This is assumed to be, at least in part, due to the formation of ADA.

Fertility in sexually mature male and female *Cynomolgus* Monkeys was observed in the pivotal 42-day toxicity study [A27294]. Overall, 6 weeks of subcutaneous administration of sargramostim at doses up to 200 µg/kg/day resulted in no sargramostim-related effects on any of the parameters, including sperm investigations and menstrual cyclicity.

Effects on Embryo-Foetal Development in Rabbits

In the Pivotal study A38193, sargramostim was administered once daily by subcutaneous injection to 20 time-mated female New Zealand white rabbits/group during gestation days 6-19 (Collective A, 25 time-mated females in the 200 ug/kg/day group) or gestation days 19-28 (Collective B). Dosage levels were 25, 70 and 200 ug/kg/day. Based on lower embryo/foetal survival at 70 (Collective B) and/or 200 ug/kg/day (Collectives A and B), a dosage level of 25 ug/kg/day was considered to be the NOAEL for embryo/foetal developmental toxicity when administered by subcutaneous injection to New Zealand white rabbits. No test article-related foetal malformations were noted up to 200 µg/kg/day. Based on

reduced food consumption, body weight losses and/or reduced body weight gains in the 25 ug/kg/day group, the NOAEL for systemic toxicity was considered to be <25 ug/kg/day.

Subcutaneous administration of sargramostim to rabbits resulted in systemic exposure to sargramostim on the first and last days of dose administration for Collective A TK phase females. Across dose groups, the dose-normalized AUC(0-t_{last}) values for sargramostim on gestation day 6 were in the same range, indicating a generally linear and dose-proportional increase of systemic exposure with increasing dosage over the 25 to 200 µg/kg/day range.

Exposure to sargramostim decreased substantially from gestation day 6 to gestation day 19, potentially due, in part, to the formation of anti-drug antibodies during the course of dose administration. With the exception of 1 animal in the 25 µg/kg/day group, anti-drug antibodies were detected in all toxicokinetic animals. Formation of neutralizing antibodies was noted for 1 of 4 Collective A TK phase females each in the 25 and 70 µg/kg/day groups when evaluated on gestation day 19 (the last day of the 14-day dose administration period).

Prenatal and postnatal development, including maternal function

In pivotal study A43883, sargramostim was administered by daily subcutaneous injection to 3 collectives of time-mated female New Zealand white rabbits (10-15 animals per group). The dose administration period was GDs 6 through 19 for Collective A, GD 19 through the day of parturition for Collective B, and LDs 1 through 14 for Collective C.

Abortion, complete litter resorption and total litter loss were limited to the 200 µg/kg/day group in Collectives A or B; however, total litter loss with corresponding decreased F1 postnatal survival was noted at 25 µg/kg/day and above in Collective C and occurred in conjunction with F0 maternal toxicity. Lower mean numbers of kits born and live litter size on PND 0 were limited to the 200 µg/kg/day group in Collective A. Lower F1 kit body weights (Collective B) or body weights and gains (Subset C) occurred at 200 µg/kg/day. F0 maternal toxicity was evidenced by mortality, body weight losses and/or reduced body weight gains, reduced food consumption and associated clinical signs (decreased defecation) at ≥25 µg/kg/day (Collectives B and C) and 200 µg/kg/day (Collective A). Based on these results, the NOAEL for F0 maternal systemic toxicity and F1 neonatal toxicity was considered to be <25 µg/kg/day.

When animals received sargramostim only during the lactation period, LD1 to LD14, all dose levels (25 to 200 µg/kg/day) caused a reduction of postnatal kit survival during the entire postnatal period and total litter loss with corresponding decreased F1 postnatal survival for at least one animal at all dose levels and in conjunction with F0 maternal toxicity (i.e., reduced body weight, body weight gain and food consumption and decreased defecation). Post-weaning, there were no effects on F1 kit survival, F1 reproductive or developmental parameters or F2 foetal parameters, and no external malformations in F1 or F2 foetus were detected.

Intrauterine growth and survival of the F2 foetuses was unaffected, and there were no external malformations in the F2 foetuses that were attributed to F0 maternal exposure to the test article. Therefore, the NOAEL for F2 embryo/foetal development was considered to be 200 µg/kg/day, the highest dosage evaluated.

No toxicokinetics was assessed within study A43883. Indirect TK data coming from other DART studies submitted in the sargramostim applicant was attempted by the applicant (see table below).

Table 6: Exposure of sargramostim in Reproductive and Developmental Studies in Rabbits

Study Study number	Dosing period	NOAEL (µg/kg/day)	C_{max} (ng/mL)		AUC (ng•hr /mL)	
Rabbit fertility and early embryonic development study [A38192]	Beginning 6 days prior to artificial insemination and continuing through GD 7	Reproductive and early embryonic development toxicity: 70	SD0:[a] 51.1	GD7:[a] 6.8	SD0:[a] 148.5	GD7:[a] 20.7
		Maternal toxicity: 25	SD0:[a] 21.0	GD7:[a] 2.3	SD0:[a] 54.3	GD7:[a] 3.7
Rabbit Embryo-foetal study [A38193]	GD 6-GD 19 GD 19-GD 28	Embryo-foetal development: 25 Maternal toxicity: <25	GD6:[b] 22.1	GD19:[b] 3.6	GD6:[b] 74.4	GD19:[b] 16.6
			GD19:[c] 17.5	LD0:[c] 1.9	GD19:[c] 60.7	LD0:[c] 3.5
Rabbit pre & post-natal study [A43883]	GD6-GD19 GD19-Partuition LD1-LD14	F_1 reproductive and F_2 embryo-foetal toxicity: 200	GD6:[d] 106.9	GD19:[d] 8.5	GD6:[d] 629.9	GD19:[d] 26.2
			GD19:[e] 161.0	LD0:[e] 12.2	GD19:[e] 487.7	LD0:[e] 113.8
			SD0:[f] 114.5	GD7:[f] 11.4	SD0:[f] 527.2	GD7:[f] 42.2
		F_1 neonatal toxicity and maternal toxicity: <25	GD6:[d] 22.1	GD19:[d] 3.6	GD6:[d] 74.4	GD19:[d] 16.6
			GD19:[e] 17.5	LD0:[e] 1.9	GD19:[e] 60.7	LD0:[e] 3.5
			SD0:[f] 21.0	GD7:[f] 2.3	SD0:[f] 54.2	GD7:[f] 3.7

[a] C_{max} and AUC_{0-24} from Study A38192.
[b] C_{max} and AUC_{0-24} from Study A38193 (pivotal embryo-foetal study).
[c] C_{max} and AUC_{0-24} from Study A33918 (Dose range-finding embryo-foetal and early postnatal study)-chosen for GD19-GD28 dosing period since closest range with 25 µg/kg/day dose level.
[d] C_{max} and AUC_{0-24} from Study A38193 (pivotal embryo-foetal study).
[e] C_{max} and AUC_{0-24} from Study A33918 (Dose range-finding embryo-foetal development and early postnatal survival study).
[f] C_{max} and AUC_{0-24} from Study A38192 (Pivotal fertility and early embryonic development study)-chosen to represent LD1-LD14 since initial dosing occurred in unpregnant animals.

2.5.4.6. Toxicokinetic data - interspecies comparison and exposure margins to clinical exposure

Since there are no clinical data on the condition for which the MAA has been submitted, exposure margins were calculated versus the AUC/Cmax measured in a clinical study in healthy subjects (study 15367) administered SC with a single dose of different sargramostim formulations produced at Seattle facility: 250 ug/m^2 (7 ug/kg) (liquid EDTA) or 125 ug/m^2 (lyophilised).

Table 7: PK parameters of sargramostim following a single subcutaneous dose of 250µg/m^2 administered as the liquid formulation using drug produced at either Seattle or Northpointe (geometric mean/%CV)

	Seattle	Northpointe
AUC (µg·h/L)	20.8 /36%	19.2 / 39%
C_{max} (µg/L)	3.33 / 57%	2.86 / 57%
$t_{½}$ (h)	1.36 / 29%	1.45 / 31%
t_{max} (h)[a]	3.0 (1.2 to 7.0)	3.0 (1.6 to 7.0)

a) = median (range)

Since both toxicity studies (42-day general toxicity in *Cynomolgus* monkeys and the pivotal DART studies in rabbit) used the liquid EDTA formulation at the dosage proposed in human for H-ARS, comparison with clinical study 15367 is appropriate.

42-day tox study in *Cynomolgus monkeys*: minimal toxicity findings targeting lymphoid organs like spleen, observed at the lowest dose of 20 ug/kg/day (approx. 250 ug/m^2) at Day 1, correspond to clinical multiples of 3.0- to 4.1-fold for C_{max} and approximately 2-fold forAUC, respectively, at the proposed recommended clinical dose of 7 ug/kg.

Table 8: Multiples of sargramostim Clinical Exposure for GLP 42-Day Toxicology Study in Female *Cynomolgus* Monkeys [A27294]

Day	Dose level (µg/kg/day)	C_{max} (ng/ml)	C_{max} Multiples of Clinical Exposure	$AUC_{0\text{-tlast}}$ (ng•hr/mL)	AUC Multiples of Clinical Exposure
1	20	13.7	4.1	49.0	2.4
	200	64.2	19.3	388	18.7
42	20	10.0	3.0	46.5	2.2
	200	46.6	14.0	265	12.7

Recommended human dose 7 µg/kg/day [15367]: C_{max} =3.33± 1.898 ng/mL; AUC = 20.8 ± 7.488 ng•hr/mL

As regards the reprotoxicity, when administered during gestation or lactation periods in rabbits (pre and post natal A43883 study), embryo-foetal toxicity observed at 200 ug/kg/day corresponds to a safety margin of 11.0-fold at GD6 but at GD19 the safety margin is much lower (1.3 fold) the recommended clinical dose of 7 ug/kg/day in adults, based on AUC from clinical study in healthy subjects 15367. Exposure to sargramostim during lactation resulted in a reduction in kit survival at doses ≥25 ug/kg/day.

Table 9: Multiples of sargramostim Clinical Exposure for Reproductive and Developmental Studies in Rabbits

			Multiples of Clinical Exposure			
		NOAEL (µg/kg/day)	C_{max} (ng/mL)		AUC (ng•hr /mL)	
			first	last	first	last
Fertility and early embryonic study [A38192]	Reproductive and early embryonic development toxicity	70	SD0: 15.3	GD7: 2.0	SD0: 7.2	GD7: 1.0
	Maternal toxicity	25	SD0: 6.3	GD7: 0.7	SD0: 2.6	GD7: 0.2
Embryo-foetal study [A38193]	Embryo-foetal development	25	GD6: 6.6	GD19: 1.1	GD6: 3.6	GD19: 0.8
	Maternal toxicity	<25	GD19: 5.3	GD28: 0.6	GD19: 2.9	GD28: 0.2
Pre & post-natal study [A43883]	F_1 reproductive and F_2 embryo-foetal toxicity	200	GD6: 32.1	GD19: 2.5	GD6: 30.3	GD19: 1.3
			GD19: 48.3	LD0: 3.7	GD19: 23.4	LD0: 5.5
			SD0: 34.4	GD7: 3.4	SD0: 25.3	GD7: 2.0
	F_1 neonatal toxicity and maternal toxicity	<25	GD6: <6.6	GD19: <1.1	GD6: <3.6	GD19: <0.8
			GD19: <5.3	LD0: <0.6	GD19: <2.9	LD0: <0.2
			SD0: <6.3	GD7: <0.7	SD0: <2.6	GD7: <0.2
Human [15367]	Recommended human dose: 7 µg/kg/day	C_{max} = 3.33 ± 1.898 AUC[a] = 20.8 ± 7.488				

[a] AUC value corresponds to AUC_{inf}, which is the same value as AUC_{last} reported in the study. Thus, $AUC_{0\text{-}24}$ is also 20.8 ng*hr/mL

It should be noted that the above safety margins refer to sargramostim liquid formulation different from that proposed to be marketed (lyophilised powder). Moreover, the dramatic reduction in exposure over time in the reprotoxicity studies and the presence of neutralising antibodies in both rabbit and monkey toxicity studies, make risk statements based on exposure multiples to the expected human exposure at 7 mcg/kg difficult. Part of the reprotoxicity studies is therefore conducted at presumably too low exposures and at what time point in the study the exposure in the rabbits was below the human exposure, remains unknown.

2.5.4.7. Local tolerance

Consistent with ICH S6(R1), local tolerance assessments were incorporated into the general repeated-dose toxicity studies in *Cynomolgous* monkey: studies 2423-111 4 week and A27294 6 week. In both studies sargramostim was administered as subcutaneous injection.

In the 4 week toxicity study, daily administration of sargramostim at dosages up to 200 µg/kg/day induced only transient swelling noted at the injection site. Additionally, in 1 low-dose (20 µg/kg/day) male and 1 high-dose (200 µg/kg/day) male and female, dark areas around the injection site were noted at terminal sacrifice, which correlated with microscopic evidence of chronic inflammation and/or foreign body granuloma. Sargramostim liophylised powder was used.

In the 6 week toxicity studies, injection site reactions were observed upon repeat administration with all sargramostim dosages ranging from 20 to 200 µg/kg/day. Pyogranulomatous inflammation (graded as slight to marked) characterised by the presence of lymphocytes, granulocytes, monocytes, epitheloid cells and fibroblasts was present at the left and/or right injection site in treated animals with a slight increase in incidence and severity with increasing dose when considering both injection sites. The lesion was predominantly located perivascular in the deep subcutis. Sargramostim EDTA liquid formulation was used.

2.5.4.8. Other toxicity studies

None.

2.5.5. Ecotoxicity/environmental risk assessment

As part of their Environmental Risk Assessment (ERA), the applicant submitted a justification for not performing ERA studies that was based on the nature of the active substance and the low risks of impact on the environment. Sargramostim is a 127 amino acid glycoprotein produced by rDNA technology. The amino acid sequence of sargramostim differs from the natural human version by a substitution of leucine for arginine at position 23, and the carbohydrate moiety may be different from the native protein. According to CHMP Guideline on the environmental risk assessment of medicinal products for human use (CHMP/SWP/4447/00 corr. 2) naturally occurring substances such as proteins can be exempted from the need to submit ERA studies when they are unlikely to pose a risk to the environment. It is accepted that based on the nature of the active substance sargramostim is not expected to have undesirable effects on the environment.

2.5.6. Discussion on non-clinical aspects

Sargramostim non-clinical dossier and particularly the toxicological evaluation comprises of studies carried out in 1990's to support the US MAA in haematological conditions. Additionally three pivotal AWC efficacy studies and the 2 pivotal PK studies were carried out in a rhesus monkey total body irradiation (TBI) model to support the relevant application in the US in 2018. One of the 3 AWC studies (1017-3493 in H-ARS Delayed Treatment), has been completed in 2019 after the FDA approval of sargramostim for use in H-ARS. Two sargramostim formulations were used: lyophilised powder in AWC, PK and 30-day repeated dose toxicity studies, and EDTA liquid in 42-day repeated toxicity and DART studies. The sargramostim powder formulation is the one intended to be marketed.

Pharmacodynamics

Sargramostim efficacy studies cannot be conducted in humans with H-ARS. Such studies would be contrary to generally accepted principles of medical ethics due to the harmful levels of radiation required to induce H-ARS. Therefore, 3 pivotal AWC efficacy studies were conducted in a rhesus monkey total body irradiation (TBI) model which is considered the most appropriate approach for studying H-ARS and has been extensively characterised: characteristics such as haematopoietic stem cell biology, distribution of active bone marrow and radiation effects on the haematopoietic system are similar to human. In addition, NHP is the sole animal species relevant for human sargramostim (which only partially cross reacts also with rabbit).

This approach is acceptable in the context of an approval under exceptional circumstances.

Female and male (only male in study Y14-015) animals were treated with 7 µg/kg sargramostim daily SC. Animal age ranged from 2 to 5.8 years at the start of treatment, thus all animals were young adults. The primary evidence of efficacy was the increase in survival.

These studies were carried out during the years 2016 to 2019. Study 1017-3493, in H-ARS Delayed Treatment, was completed after the 2018 FDA approval of sargramostim for use in H-ARS, thus not included in the FDA's review.

Since these studies are considered pivotal to the demonstration of efficacy of sargramostim in the claimed indication, the assessment of results is reported in the Clinical section.

No *in vitro* studies nor secondary PD studies, were carried out. Off-label use is unlikely since sargramostim is an endogenous protein with high specificity to the GM-CSF receptor. Since in AWC study 1017-3493 azithromycin was given concomitantly with sargramostim, information on absence of significant pharmacodynamic interaction is reported in SmPC section 4.5. Further potential co-administration of sargramostim with supportive treatments, cannot be excluded.

Stand-alone safety pharmacology studies, were not performed. Cardiovascular function in terms of blood pressure and electrocardiograms was evaluated in the pivotal 42 days (6 weeks) toxicity study in *Cynomolgus* (study A27294) and no effect on blood pressure or electrocardiograms was observed at any dose levels. The lack of complete safety pharmacology characterisation could be accepted only considering that sargramostim is a known active substance whose safety profile consists in an exaggerated pharmacological effect and for which clinical data from the use in indications other than H-ARS, are available.

Pharmacokinetics

The aim of the two repeated-dose PK studies in non-irradiated males, and irradiated males and females R. macaque was to assess the PK behaviour of 2 different doses of sargramostim administered SC: 7 µg/kg/day (84 ug/m2) used in the AWC efficacy studies and 20.8 ug/kg/day (260 ug/m2) similar to that in use in humans for all of the US haematological indications. However, no formal dose-selection studies were carried out.

PK profiles for sargramostim in non-irradiated and irradiated monkeys were similar, generally following dose-dependent kinetics with no evidence of accumulation following once-daily SC injections of 7.0 µg/kg/day or 20.8 µg/kg/day for 14 days. The terminal elimination half-life of sargramostim ranged across the 2 PK studies with 7 or 20 ug/kg/day, at Day 1 and 14, from 1.19 to 3.46 hours.

Although the SC administration respect the IV route may increase the development of ADA, in the intended context of use of sargramostim, the radiation impairs the ability of the body to develop ADA, as observed in the irradiated PK study on NHP; thus, immunogenicity is not expected to be an issue in the treatment of H-ARS.

In the **non-irradiated** animals, lower exposures were apparent on Day 14 compared to Day 1 at both dose levels correlating with the presence of ADAs in all (male) animals after repeat dosing. Similarly, lower sargramostim exposure on Day 14 versus Day 1 was observed in irradiated animals, reaching 55% reduction at 20.8 µg/kg/day in female monkeys, and 20% reduction at 7 µg/kg/day in female monkeys: no ADAs were detected in this study. Although female animals were only n=3, a trend towards a lower efficacy in female R. macaque was noted in the NHP H-ARS model (See efficacy section). While in the non-irradiated study, the presence of anti-sargramostim antibodies in all animals on Day 15 may be the reason for the lower Day 14 exposure compared to Day 1, no explanation was given for the lower exposure observed in irradiated female monkeys exposed to clinically relevant dose of 7 µg/kg/day. In the high dose irradiated female monkeys, the euthanization of one of the monkeys before the end of the study was suggested by the applicant to be the cause for the overall calculated reduction of AUC in this group. Since the group size in these pharmacokinetic studies was very small (3 NHP animal per group), definitive conclusions cannot be drawn. It is unfortunate that there is insufficient knowledge regarding possible sex differences in response to irradiation, where in general females appear to be more sensitive to the effects of irradiation than males. It was noted that a lower efficacy in female NHPs was observed in two NHP efficacy studies using radiation dose DL70-80: TSK044 where the mortality in female was higher (both in placebo and sargramostim treatment), and study 1017 where the subgroup analysis showed a statistically significant advantage in male NHP vs female.

Since the sargramostim exposure achieved in irradiated and non-irradiated monkeys after the first administration were similar, the data was combined and compared to the healthy male human exposure determined after a 6 µg/kg SC dose in Study 309904 (see efficacy section). This human study was chosen since it was obtained using the same formulation of sargramostim (lyophilised) and the same route of administration (SC) as that used in the NHP AWC studies. The data generated on Day 1 of each of the NHP PK studies was selected for this comparison due to the potential impact of ADAs on PK on Day 14.

The exposure (Cmax and AUC) achieved in these two pooled young adult NHP PK studies with dose 7 µg/kg/day used in the AWC efficacy NHP studies, was approx. 4-fold lower than the mean exposures achieved with a similar dose in human healthy male adults. The reason for this PK difference might be due to several factors among those different absorption through the skin, different sargramostim elimination pattern, even if R. macaque is considered a species similar to human. Interspecies considerations should therefore have been investigated before initiating the NHP efficacy studies, and this raises doubts on the adequacy of dose selection study for the extrapolation of the results to humans.

However, the sargramostim dose is (also) based on body surface area (BSA). The applicant did not include a comparison based on BSA, so this was explored by the assessor to assess why there appeared to be a difference in AUC values between humans and monkeys. A dose of 7 ug/kg in humans is equivalent to 250 ug/m2. In Rhesus monkeys, the high dose of 20.8 ug/kg equals a dose of approximately 260 ug/m^2 while the 7 ug/kg equals a dose of approximately 87.5 ug/m2. Basing the comparison of an equal dose/m^2 BSA, it is apparent there is no exposure difference between humans and NHP at therapeutic levels.

Table 10: Comparison of dose calculation based on weight and body surface

Species	Dose (µg/kg)	Dose (ug/m^2) (calculated)	n	Mean (SD) [min, max]	
				C_{max} (ng/mL)	AUC_{last} (h•ng/mL)
Rhesus macaque[a]	7	87.5	11	1.34 (0.252) [0.796, 1.73]	5.489 (1.459) [2.96, 8.38]
Rhesus macaque[a] (Calculated from mean values)	20.8	260	11	6.44	28.7
Human[b]	6	220	39	3.15 (1.11) [1.4, 5.5]	20.4 (5.86) [8.8, 32.1]
Human popPK[c]	7	250	500	3.03	21.3

[a]Studies [DDK0110] and [DDK0111], [b]Study [309904], [c]as reported in the SmPC

If exposure between NHP and healthy humans were compared, based on the dose in ug/m2 BSA, at approx. 250 ug/m2 similar PK values would have been observed (AUC ranging from 20 to 28 ng*h/mL). Indeed, these PK values are reached with the high dose of 20.8 ug/kg/day used in the NHP PK studies. The applicant explained that since the human dose is expressed as pro-kg (instead ug/m^2) for ease of administration in a mass casualty emergency setting (calculation assumed a 70 kg adult with a body surface area of 1.96 m^2), the monkey and human comparisons were based on the mcg/kg dose.

The applicant justified the choice of 7 ug/kg/day to be used in the NHP efficacy studies so that the expected exposure level (and pharmacodynamic effects) would not exceed exposure achieved with clinical dose used in haematological conditions which has been proved to be efficacious and safe. However, the use of an underweighted dosage did not show a meaningful impact on efficacy studies; instead, it is shown in PK studies that a dose of 20.8 µg/kg is within the range of clinical AUC values. As this higher dose might have resulted in higher efficacy and therefore less mortality in the treated groups, this would have been a more appropriate dose in light of animal welfare.

In the PK on non-irradiated NHP, an increase in leukocytes, mostly due to changes in neutrophil counts, was observed at both dose levels as well as a strong progressive increase in platelet count more pronounced at the high dose.

In line with ICH S6 guideline, no complete ADME study was performed. Being a protein, sargramostim does not interact with cytochrome P450 enzymes and therefore no significant PK drug interactions are expected. In DDK0111 PK study (irradiated, male and female animals), the following supportive care was provided based on the clinical judgment of the veterinarian: Buprenorphine, Marcaine, Parenteral fluids, Sucralfate, Enrofloxacin, Ondansetron.

Toxicology

The toxicity of sargramostim was evaluated in single-dose and repeat-dose toxicity studies up to 42 days in *Cynomolgus* monkeys, and a complete series of GLP-compliant reproductive and developmental studies (and supportive pilot studies) that includes assessments of fertility, embryo-foetal development, and pre- and postnatal development were conducted in New Zealand white rabbits.

Both *Cynomolgus* and rabbit are relevant species for toxicity characterisation of sargramostim, although the sequence identity of rabbit GM-CSF to human GM-CSF is less conserved at ~70%. Moreover, considering that primarily monocytes and granulocytes were only increased at higher doses (FEED and EFD study) it is questionable whether sargramostim has the same mechanism of action in rabbits as in NHP/humans.

Although the target tissues are comparable between studies using the lyophilised (2423-111) and liquid (A27294) formulations, the 30-day lyophilised formulation study could be considered more

valuable, using the formulation to be used clinically but no TK and immunogenicity assessment were performed in study 2423-111.

The lympho-haematopoietic system is identified as the primary target of toxicity, which is not unexpected based on the pharmacology of the product. These toxicity findings were apparent at dose levels as low as 20 ug/kg/day with a liquid formulation containing EDTA: whether the EDTA can contribute to the toxicity remains unclear. EDTA is a well-known cell permeability enhancer. The applicant speculated that the systemic exposure achieved in NHP with the EDTA formulation may be greater than what would have been seen with lyophilised formulation, and this would account for the higher toxicity observed at 20 ug/kg/day in 42-day study A24993 performed with the EDTA liquid formulation vs 30-day study 2423-111 in which lyophilised powder sargramostim. However, no increased AUC was observed with EDTA formulation when comparing different NHP studies (A27294 and DDK0110) in which similar doses of EDTA and lyophilised formulations, respectively, were given.

It should also be noted that in human study 309404, sargramostim EDTA and lyophilised formulations resulted bioequivalent in terms of AUC and Cmax.

The applicant also speculated that the faster absorption observed in human PopPK study POH547 using SC EDTA formulation vs. lyophilised one, could be the reason for the exaggerated toxicity seen in NHP study A27294; however, all clinical safety available data in haematological indications different from the ARS, come from EDTA formulation. Even if this PK feature of EDTA formulation would occur in NHP, it hardly could be considered a plausible justification for the observed NHP toxicity findings.

One of the main limitations for the extrapolability of repeated-dose toxicity and reprotoxicity results to humans is the fact that most of toxicity studies were carried out using the no more marketed EDTA liquid formulation instead of the lyophilised one. Available data do not allow to conclude that the clinical safety profile of liophylised sargramostim, is expected to be milder that that observed with EDTA formulation in NHP.

It is noted that while AWC efficacy and PK studies used R. macaque species as NHP, *Cynomolgus* macaque was the species used in all repeated-dose toxicity studies. In the pre-submission meeting with the Rapporteur, the applicant explained that the *Cynomolgus* monkey toxicity studies were performed to support other clinical indications before development of the H-ARS program. The applicant also commented that there is no substantial difference with respect to PK aspects between rhesus and *Cynomolgus* macaques. Additionally, *Cynomolgus* macaques GM-CSF protein sequence is approximately 96% identical to human GM-CSF. The applicant also confirmed that there was high homology between the two monkey species. This is noted.

Based on the rhesus monkey PK data and considering that PK of *Cynomolgus* monkeys would not be considerably different (as both monkey species are in the same weight range), a dose of around 20 ug/kg/day would be needed to obtain a comparable exposure (AUC) and dose per m^2 in monkeys as in humans treated with 7 ug/kg (250 ug/m2) sargramostim. Most toxicity studies contain doses equal to and above this 20 ug/kg/day, which is considered appropriate for toxicity studies.

ADA that reduced systemic exposure and/or neutralised the activity of sargramostim were observed in the 42-day *Cynomolgus* monkey study and in rabbit DART studies. In both sets of studies, a PD effect, increase in WBCs, particularly neutrophils, was observed, with maximum levels occurring within 2 weeks of repeated daily subcutaneous injections. Thereafter, the levels started to decline and, in some cases, returned to baseline levels by the end of the treatment period.

Due to the production of ADA, study designs for the DART studies were modified to try to maintain exposure over the critical evaluation period, limiting the dosing periods to approximately 14 days.

In the PK study with healthy rhesus monkeys, considerable decrease in exposure was noted around day 14, likely due to the formation of ADAs. Antibody induction was not investigated in the lyophilised formulation toxicity study 2423-111 but considering that increased blood cell counts already diminished before the recovery period, it is very likely that (neutralising) ADAs have also been formed in this monkey study. As such, the exposure margin of the used doses would be substantially lower and the results less relevant for clinical. It remains therefore unknown what exact toxicity would be expected (in patients) when the exposure would stay sufficiently high over time. This is a limitation of all toxicity studies in monkeys.

In the rhesus monkey PD studies, an increase in both granulocytes and monocytes was observed following total body irradiation (and sargramostim treatment). In the lyophilised formulation toxicity studies, a considerable increase in granulocytes is observed without a corresponding high increase in monocytes/macrophages, while sargramostim would stimulate both haematopoietic lineages. The applicant did not further discuss this. However, it should be outlined that monocyte count is not considered clinically relevant in the assessment of response to G-CSF.

The temporary haematological effects and tissue findings on macroscopic and microscopic level (in e.g. bone marrow, spleen and thymus) can be regarded a (known) consequence of the pharmacological effect of sargramostim and no unexpected toxicity was observed. Nevertheless, thymic atrophy was observed, while an increase in lymphocyte counts were found. These findings seem not to be compatible. Thymic atrophy may result in decreased T cell lymphopoiesis/maturation, which may have an impact on the lymphocyte reconstitution following irradiation in (especially young) patients. However, data from clinical studies and post-marketing surveillance do not suggest thymic atrophy nor the cardiac cell infiltrates and inflammation induced with sargramostim.

No signs of toxicity related to fertility were observed in the male and female *Cynomolgus* monkeys. It should thereby be mentioned that it is considered very likely that total body irradiation by itself will already have a significant effect on fertility.

All DART studies were conducted with the liquid formulation (with EDTA) of sargramostim, which is not the product intended to be used clinically. Relevant decrease in exposure over time, which was below human exposure at least at the end of the study, was correlated to the neutralising ADA formation: however, embryo-foetal development toxicities were still observed. Moreover, considering the expected impact of TBI on fertility and pregnancy and on ADA formation, the clinical relevance of the DART results is considered limited in the proposed indication.

The applicant has posed several NOAELs for DART study endpoints. Relevance of calculated safety margins based on AUC Day 1 is limited, especially when considering the reduction in systemic exposure over time. For transparency, the exposure multiples from the reproductive toxicity studies in rabbits at begin and end of dosing period, are included in section 5.3 of the SmPC.

No TK was evaluated in PPND study and only indirect comparisons to other studies were possible in order to calculate safety margins.

Regarding the EFD study, while sargramostim caused embryo-foetal toxicity in the form of spontaneous abortions and increased post-implantation losses at a high dose of 200 ug/kg/day, at a dose of 70 ug/kg/day (GD19-GD28) there was a sargramostim-related increase in late resorptions, indicative of lower embryo/foetal survival, and a reduction of fetal weight, corresponding to safety margins of 2.9 and 0.2 (first and last dose).

These results are consistent with reports in the literature suggesting that increased prepartum concentration of GM-CSF is associated with spontaneous preterm birth.

In the PPND study, in collective C a sargramostim-related increase in total litter loss and F1 postnatal survival was already evident from ≥25 µg/kg/day. The applicant was asked to to elaborate on these findings and discussed that the postnatal effects (decrease in pup survival) are dose responsive and correlative with the dose-dependent increase in maternal toxicity (body weight loss and reduction in body weight gain and food consumption at all doses, reduced defecation at ≥70 µg/kg and mortality at 200 µg/kg), suggesting that maternal health was a contributor to the findings.

In the absence of a significant correlation to sargramostim dose with the number of pups with no milk in the stomach, the absence of specific signs of reduced maternal care, the absence of sargramostim-related foetal skeletal or morphological abnormalities or effects on functional or developmental endpoints, and the absence of specific age-related safety concerns from the experience with paediatric patients receiving sargramostim, it is agreed that the decrease in nursed pup survival is likely related to the maternal toxicity (indirect effect of sargramostim).

However, the contribution to pups' toxicity by exposure of the offspring through breastfeeding, cannot be excluded. However, due to the instability at low pH, sargramostim is expected to degrade within the gastrointestinal tract of the suckling pups following ingestion, thereby limiting potential systemic exposure in breast-fed neonates and infants.

Therefore, the applicant overall concludes that the direct contribution of sargramostim to the mortality of nursed pups observed in Collective C is inconclusive: it can be agreed with the applicant that the maternal toxicity that was observed (body weight loss, food consumption) and the adverse findings in paediatric populations in the clinic (leukocytosis) can be monitored and managed clinically, which would be an argument to derisk the potential adverse effects on the breast-fed neonate/infant (see warning in section 4.4).

It is acknowledged that in the context of a potential radiation incident, alternative nutritional supplements may not be readily available, and breastfeeding may be the only readily available nutrition source for neonates and infants. Taking all the arguments into account and considering that the indication is for patients of all ages acutely exposed to myelosuppressive doses of radiation, it seems not in line to discontinue breast-feeding to prevent sargramostim exposure in the neonates/infants. Therefore, it is even proposed that breast-feeding may be considered during treatment with sargramostim, keeping in mind that the newborn also needs treatment (see SmPC section 4.6).

Taking into account: i) that sargramostim is a biotech product for which genotoxicity potential has not been assessed, ii) the context of use, iii) that sargramostim is indicated from birth, iv) that the adverse effect in rabbit was seen at 7.2 multiple the human adult dose, v) that the terminal half-life in healthy subjects is 1.4 hours, no recommendations on the duration of contraception following the end of treatment for WOCBP is made.

In addition, the following warning has been added in section 4.4 of the SmPC: 'Acute exposure to myelosuppressive doses of radiation has per se' a toxic effect on fertility and embryo/foetal development. This should be considered for clinical judgement on the use of Imreplys in pregnant and/or lactating women. There are no or limited data on the use of sargramostim in pregnant women. Studies in animals have shown reproductive toxicity (see section 5.3). Imreplys can be used in pregnant women with H-ARS if clinically needed.'

Sargramostim is a recombinant protein that exerts pharmacological activity through a membrane bound receptor. It is not expected to reach the nucleus nor directly interact with DNA or other chromosomal material.

Although the carcinogenic potential of sargramostim is expected to be low on the basis of its mode of action and the intended short-term use, in the pivotal 42-day toxicity study in *Cynomolgus*, the

following findings were observed at the low dose of 20 ug/kg/day and were attributed to the pharmacological response to sargramostim: enlargement and granulocytosis of lymph nodes, increased spleen weight associated with lymphoid hyperplasia of red and white pulp, lymphoid hyperplasia of tissue associated lymphatic tissue, not completely reversible; bone marrow hyperplasia, reversible; focal/multifocal inflammatory or lymphoid cell infiltrates in multiple organs including liver, heart, lung, adrenal gland, kidney, testes, epididymides, skin, cerebrum and application site, tendency to reversibility. Section 4.2 of the SmPC which recommends that: 'Dose modification: For grade 3 or 4 adverse reactions (see section 4.8), Imreplys dose should be reduced to 50%, or interrupted until the adverse reaction abates and then resumed at 50% of the dose. Other measures to manage the adverse reaction should be instituted and continued as necessary. If a grade 3 or 4 adverse reaction persists or recurs following dose adjustment/resumption, Imreplys should be permanently discontinued. For grade 1 or 2 adverse reactions (see section 4.8), sargramostim should be continued with close patient monitoring and management of the adverse reaction.'

Overall, the general toxicity profile of sargramostim appears characterised and manageable on the basis of its mode of action and intended use. As regards the potential embryotoxicity, the biological plausibility remains unclear: there are evidence in scientific literature regarding pivotal roles of cytokines like GM-CSF in embryo implantation and subsequent development being propitious for the success of pregnancy. Moreover, it should be considered that acute exposure to myelosuppressive doses of radiation has per se' a toxic effect on fertility, embryo/foetus development and that sargramostim is administered from birth. Considering the clinical need for treatment of the mother, the benefit will always be greater than the potential risk, thus it is agreed that sargramostim can be used during pregnancy and breast-feeding. Consequently, the following warning has been added in section 4.4 of the SmPC: 'Acute exposure to myelosuppressive doses of radiation has per se' a toxic effect on fertility and embryo/foetal development. This should be considered for clinical judgement on the use of Imreplys in pregnant and/or lactating women. There are no or limited data on the use of sargramostim in pregnant women. Studies in animals have shown reproductive toxicity (see section 5.3). Imreplys can be used in pregnant women with H-ARS if clinically needed.'

ERA

As part of their Environmental Risk Assessment (ERA), the applicant submitted a justification for not performing ERA studies that was based on the nature of the active substance and the low risks of impact on the environment. This justification is in line with the CHMP Guideline on the environmental risk assessment of medicinal products for human use (CHMP/SWP/4447/00 corr. 2). It is accepted that based on the nature of the active substance sargramostim is not expected to pose a risk to the environment.

Assessment of paediatric data on non-clinical aspects

The indication of sargramostim is from birth in line with the PIP decision (P/0089/2024), which states that juvenile animal studies are not required. Standard assessments on offspring were performed as part of DART studies A38193 and A43883.

It should be noted that a higher dosage vs. the adult one is foreseen for paediatric subjects as reflected in SmPC section 4.2. The recommended posology is as follows:

- 7 micrograms/kg in children and adolescents weighing greater than 40 kg and in adults and 10 micrograms/kg in children and adolescents weighing 15 kg to 40 kg.
- 12 micrograms/kg in neonates, infants or children weighing less than 15 kg.

2.5.7. Conclusion on the non-clinical aspects

Overall, the non-clinical data package is considered acceptable, in view of the intended clinical use of sargramostim, and in the context of a MAA under exceptional circumstances.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The main evidence of Imreplys efficacy of the present MAA in H-ARS is based on studies performed in non human primates. However, clinical studies were also performed with sargramostim in haematological conditions different from H-ARS in the USA in the period 1989 to 1993. Nevertheless these studies were conducted by a sponsor different from the current applicant: therefore, the applicant is unable to verify the modality of conduct and compliance with good clinical practice of the trials. The presence of limitations regarding the robustness of supportive data is acknowledged, but it is accepted that indirect evidence of efficacy also come from the established clinical use of sargramostim in other haematological indications in which the supportive clinical trials were conducted.

- **Tabular overview of clinical studies**

Type of study	Study identifier	Location of study report	Objective(s) of the Study	Study Design and Type of Control	Test product(s): Dosage regimen Route of administration	Number of Subjects	Healthy Subjects or Diagnosis of Patients	Duration of Treatment	Study Status Type of Report
PK, Safety	309404	5.3.1.1	To evaluate the absolute BA of sargramostim solution with EDTA and BA relative to that of lyophilised sargramostim when administered SC in healthy subjects.	Part 1: Double-blind, placebo-controlled, three-way cross-over Part 2: Open-label two-way cross-over Single center	Liquid DP with EDTA versus lyophilised DP (reconstituted with 1.0 mL BSWFI) Part 1: Single 6 µg/kg SC dose Part 2: Two single doses of 500 µg SC and IV	55	Healthy	Part 1: 3 single doses on Days 1, 15, and 29 (full 14-day washout) Part 2: 2 single doses on Days 1 and 15 (full 14-day washout)	Complete Full
PK, Safety	001.0004	5.3.1.2	To determine PK and safety profiles of single 250 µg/m^2 doses of lyophilised and liquid sargramostim administered IV and SC to normal healthy male volunteers	Randomized, cross-over, open-label Single center	Lyophilised (reconstituted with 1.0 mL BSWFI) and liquid DP 250 µg/m^2 IV and SC Group A Day 1: lyophilised IV Day 3: liquid IV Day 5: lyophilised SC Day 7: liquid SC	25	Healthy	7 days (4 doses)	Completed Full

					Group B Day 1: liquid IV Day 3: lyophilised IV Day 5: liquid SC Day 7: lyophilised SC				
PK, Safety	001.0019	5.3.1.2	To compare the PK profile of single 250 µg/m^2 dose of liquid sargramostim to a 250 µg/m^2 dose of liquid sargramostim with EDTA (1.9 mg/mL) in RTI syringes when administered SC to healthy male volunteers	Randomized, cross-over, open-label Single center	Liquid DP and liquid RTI DP 250 µg/m^2 SC Group A Day 1: RTI Day 8: liquid DP Group B Day 1: liquid DP Day 8: RTI	25	Healthy	8 days (2 doses)	Completed Full
PK, Safety	15367 (BAY 86-5326, PH36647)	5.3.1.2	To compare the PK of sargramostim manufactured at two different facilities; determine if	Randomized, cross-over, open-label Single center	Dose consisted of drug produced at NP facility: 125 µg/m^2 (lyophilised;	60	Healthy	Single dose	Completed Full

Type of study	**Study identifier**	**Location of study report**	**Objective(s) of the Study**	**Study Design and Type of Control**	**Test product(s): Dosage regimen Route of administration**	**Number of Subjects**	**Healthy Subjects or Diagnosis of Patients**	**Duration of Treatment**	**Study Status Type of Report**
			lyophilised or liquid sargramostim exhibit changes *in vivo* PK that could be attributed to different manufacturing facilities		reconstituted with 1.0 mL BSWFI) or 250 µg/m^2 (liquid) – SC Dose consisted of drug produced at Seattle U51 facility: 125 µg/m^2 (lyophilised) or 250 µg/m^2 (liquid) – SC				
PK	309901	5.3.1.2	To evaluate BE of 1000 µg/mL to 500 µg/mL dose of sargramostim after SC administration to healthy male and female subjects.	Randomized, open-label, cross-over BE study Single center	Liquid DP 1000 µg/mL at 6 µg/kg SC Liquid DP 500 µg/mL at 6 µg/kg SC	48	Healthy	Single dose	Completed Full
PK, Safety	PTX-001-005 (PTX-P2-328)	5.3.1.2	To evaluate PK, PD, and safety of sargramostim administered SC, IV, or inhalation in healthy adult subjects	Single ascending dose cohorts and a repeat dose cohort Single center	Lyophilised DP (reconstituted with 1.0 mL BSWFI for IV, 1.0 mL SWFI for SC injection, 1.0 mL SWFI and 3.0 mL saline for IH) Single ascending doses of: 1. 250 µg IV 2. 25 µg SC 3. 125 µg SC	42	Healthy	Single dose or 2 doses over 8 days	Completed Full

					4. 250 µg SC 5. 500 µg SC 6. 250 µg IH Repeat dose: 1. 500 µg SC on Days 1 and 8				
PK, Safety	001.0001	5.3.3.2	To compare PK and safety profiles of liquid and lyophilised sargramostim administered IV to patients undergoing autologous cell transplantation	Randomized, cross-over, open-label PK study Single center	Lyophilised (reconstituted with 1.0 mL BSWFI) and liquid DP 250 µg/m^2 2-hour IV infusion	29	Patients with leukemia, Hodgkin's disease, non-Hodgkin's lymphoma or other solid tumors undergoing autologous BMT or PSCT	21 days (1 day on IV or SC and 20 days on SC or IV)	Completed Full
PK	308001	5.3.3.2	Safety and clinical effects of sargramostim treatment with and without concomitant corticosteroids in pediatric patients with active Crohn's disease	Open-label, pilot study 6 centers	Liquid DP, 4 and 6 µg/kg once daily SC	22	Crohn's disease	8 weeks (56 doses)	Completed Full

Type of study	**Study identifier**	**Location of study report**	**Objective(s) of the Study**	**Study Design and Type of Control**	**Test product(s): Dosage regimen Route of administration**	**Number of Subjects**	**Healthy Subjects or Diagnosis of Patients**	**Duration of Treatment**	**Study Status Type of Report**
PK	706 (8705) 701-711	5.3.3.2 5.3.5.4	Safety of sargramostim in the setting of autologous BMT, define the optimum biologic dose	Dose escalation Single center	Lyophilised DP (reconstituted with 1.0 mL sterile saline or SWFI) 15, 30, 60, 120, and 240 µg/m^2 2-hour IV infusion	29	Patients with ALL, NHL, or HD	14- or 21-day course	Completed Full (Pediatric Safety Report and Phase 1 Study Report)
PK	9208	5.3.3.2	Safety and effect of various doses of sargramostim in preterm neonates	Open-label dose escalation study 3 centers	Lyophilised DP (reconstituted with SWFI) 0.05 µg/kg QD, 5 µg/kg QD, 5 µg/kg BID, 10 µg/kg QD or 10 µg/kg BID 2-hr IV infusion	21	Patients (preterm newborns)	7 days	Completed Full
PK, Safety	308626	5.3.3.3	To investigate safety profile and PK properties of sargramostim in Japanese and Caucasian subjects	Open-label, randomized, cross-over study Single center	Liquid DP 2, 6, or 8 µg/kg SC injection	62	Healthy	2 doses, with washout period of at least 14 days between dose	Completed Full
Efficacy	001.0005 (Part B1)	5.3.5.1	Effect of sargramostim on the	Double-blind, randomized,	Lyophilised 500 µg DP vials	61	Patients (preterm 28-day neonates)	28 days	Completed Full

			proportion of infants with confirmed nosocomial infection during Days 0 – 28	placebo-controlled study 11 centers	(reconstituted with 5.0 mL SWFI or saline) 8 µg/kg/day 2-hr IV infusion				
Efficacy	001.0005 (Part B2)	5.3.5.1	Effect of sargramostim on the proportion of infants with confirmed nosocomial infection during Days 0 to 27	Prospective, randomized, double-blind, placebo-controlled study 16 centers	Lyophilised 500 µg DP vials (reconstituted with 5.0 mL SWFI or saline) 8 µg/kg/day for 7 days, then every other day for 21 days 2-hr IV infusion	264	Patients (preterm newborns	28 days	Completed Full
Long-term Safety	001.0006	5.3.5.1	Evaluate the 1.5 to 2-yr neurodevelopmental status of very low birth weight infants previously treated with sargramostim or placebo (Protocol 001.0005)	Follow-up study 6 centers	No study drug administered	36	Patients (very low birth weight from Study 001.0005)	Not applicable	Completed Full
Efficacy	301 (8802)	5.3.5.1	Efficacy and safety of sargramostim versus placebo in promoting myeloid engraftment	Double-blind, randomized, placebo-controlled study	Lyophilised DP 250 µg/vial (reconstituted with 1.0 mL SWFI) 250 µg/m^2/day	44	Patients with acute lymphoblastic leukemia,	21 days	Completed Full

Type of study	**Study identifier**	**Location of study report**	**Objective(s) of the Study**	**Study Design and Type of Control**	**Test product(s): Dosage regimen Route of administration**	**Number of Subjects**	**Healthy Subjects or Diagnosis of Patients**	**Duration of Treatment**	**Study Status Type of Report**
			following autologous BMT in subjects with lymphoid malignancies	Single center	2-hr IV infusion		Hodgkin's disease, and non-Hodgkin's lymphoma		
Efficacy	302 (8803)	5.3.5.1	Efficacy and safety of sargramostim vs placebo in patients undergoing autologous BMT or PSCT following intensive chemotherapy or chemo-radiotherapy for lymphoid malignancy	Prospective, double-blind, randomized, placebo-controlled, parallel group study Single center	Lyophilised DP 250 µg/vial (reconstituted with 1.0 mL SWFI or saline) 250 µg/m^2/day 2-hr IV infusion	62	Patients with Hodgkin's disease or non- Hodgkin's lymphoma	21 days	Completed Full
Efficacy	303 (8810)	5.3.5.1	Efficacy and safety of sargramostim for promoting myeloid engraftment following autologous BMT in subjects with B-cell non-Hodgkin's lymphoma	Double-blind, randomized, placebo-controlled study Single center	Lyophilised DP 250 µg/vial (reconstituted with 1.0 mL SWFI) 250 µg/m^2/day 2-hr IV infusion	47	Patients with B-cell non-Hodgkin's lymphoma	21 days	Completed Full
Efficacy	305	5.3.5.1	Safety and efficacy of sargramostim as a daily 4-hour IV infusion following induction and	Randomized, double-blind, placebo-controlled study 25 centers	Lyophilised DP supplied as 500 µg vials (reconstituted with 1.0 mL SWFI) 250 µg/m^2/day	117	Patients with ANLL	Maximum: 42 days for induction and 42 days for	Completed Full

			consolidation chemotherapy in elderly patients with de novo ANLL		4-hour IV infusion once daily			consolidation	
Efficacy	501	5.3.5.1	Safety and efficacy of sargramostim in patients with failure or delay of engraftment after autologous and allogeneic BMT	Prospective, non-randomized, open-label, multicenter, historically controlled study 38 centers	Lyophilised DP 250 µg vial (reconstituted with 1.0 mL SWFI or saline) 60 to 1000 µg/m^2/day 2-hr IV infusion	243	Patients with leukemia, lymphoma, solid tumor, or other (myelodysplasia/ preleukemia, aplastic anemia, Fanconi's anemia, osteopetrosis, or non-HIV associated immune deficiency syndrome)	14 days on, 7 days off; maximum of 3 courses	Completed Update
Efficacy	9002	5.3.5.1	Effect of sargramostim and placebo following allogeneic BMT on neutrophil recovery and length of hospitalization	Prospective, randomized, double-blind, placebo-controlled study 7 centers	Lyophilised DP 250 µg vial (reconstituted with 1.0 mL SWFI) 250 µg/m2/day 4-hr IV infusion	109	Patients undergoing HLA identical sibling BMT	Maximum 27 days	Completed Full
Safety	001.0005 (Part A)	5.3.5.2	Safety of sargramostim in preterm neonates	Open-label, non-placebo controlled study	Lyophilised DP 500 µg vial (reconstituted	6	Patients (preterm neonates)	28 days	Completed Full

Type of study	**Study identifier**	**Location of study report**	**Objective(s) of the Study**	**Study Design and Type of Control**	**Test product(s): Dosage regimen Route of administration**	**Number of Subjects**	**Healthy Subjects or Diagnosis of Patients**	**Duration of Treatment**	**Study Status Type of Report**
				2 centers	with 5.0 mL SWFI or saline) 10 µg/kg/day 2-hr IV infusion				
Efficacy	202	5.3.5.2	Safety of sargramostim in pediatric patients with refractory malignancies and effectiveness in reducing depth and duration of chemotherapy-induced myelosuppression	Open, nonrandomized, unstratified, dose ranging study Single center	Lyophilised DP 250 µg vial (reconstituted with 1.0 mL SWFI) 60, 250, 500, and 750 µg/m^2/day 2-hr IV infusion	13	Patients with refractory malignancies	14 days	Completed Interim
Safety	502 (8903)	5.3.5.4	Compassionate use pediatric study	Uncontrolled open label study Multiple investigators	Formulation and reconstitution not specified. Recommended dose: 250 µg/m^2/day IV or 500 µg/m2/day SC IV (2 to 24 hr infusions) SC (1 to 3 injections/day)	26	Patients with idiopathic or iatrogenic bone marrow failure states	From 1 to 126 days	Completed Safety report

Safety	701 701-711	5.3.5.4	To assess safety and MTD of sargramostim in different clinical settings	Uncontrolled, open-label, dose range and dose escalation, single and multiple course studies Single center	DP 100 or 250 µg vial (reconstituted with saline) 15, 30, 60, 120, 240, or 480 µg/m^2 QD Continuous IV	58	Patients with disseminated cancer	Daily for 14-days with 2-week rest between cycles	Completed Final (Pediatric Safety Report)
Safety	703 701-711	5.3.5.4	Safety and effects of sargramostim after BMT in ALL	Uncontrolled, open-label, dose range, single and multiple course studies Single center	DP 100 or 250 µg (reconstituted with 1.0 mL saline) 16, 32, 64, 128, 256 µg/m^2 every 6-hrs IV bolus	27	Patients with acute lymphoblastic leukemia	14 to 21 days	Completed Final (Pediatric Safety Report)
Safety	701-711	5.3.5.4	To assess safety and MTD of sargramostim in different clinical settings and assess efficacy by evaluating total leukocyte and absolute granulocyte counts	Uncontrolled, open-label, dose range, single and multiple course studies 8 centers	DP 100 or 250 µg (reconstituted with 1.0 mL saline or SWFI) 15 to 3900 µg/m^2/day IV, SC, and IM	215	Patients with leukemia, lymphoma, MDS, solid tumor, and AIDS	7-day to 4-week course duration	Completed Interim
Populati on PK	POH0547	5.3.3.5	To develop & qualify a PopPK model for sargramostim using data from healthy adults; to scale the adult PopPK model for sargramostim to pediatric population; and to simulate the exposures in adult and pediatric population using the adult and scaled pediatric models	Sargramostim population pharmacokinetic analysis based on studies 308626, 309404, 309901, 15367	Liquid DP with and without EDTA, Lyophilised DP, Placebo	225	Healthy adults	Various; see individual studies	Completed

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Bioanalytical methods

The pharmacokinetic (PK) of sargramostim in humans has been studied using enzyme-linked immunosorbent assays (ELISAs) developed by Immunex, Berlex, Bayer, and Partner Therapeutics.

A Validation summary for Berlex, Bayer and PTX-W8-337 methods is reported in Table 4 below, while there is no validation report available for the Immunex method.

Table 11: Validation Date of ELISA Methods for sargramostim Quantification in Human Serum

	Berlex method		Bayer method		PTX-W8-337	
Test Site	Covance Laboratories Inc, Virginia, US		Syrinx Bioanalytics Oy, Finland		AltaSciences, Quebec, Canada	
Test Site Study No.	7548-100		2010043-01131		PTX-W8-337	
Matrix	Human serum		Human Serum		Human Plasma	
Used in clinical study	308626, 309404, 309901, 308001		15367		PTX-001-005	
Analyte	Sargramostim		Sargramostim (batch B11412)		Sargramostim	
Calibration curve	1.09, 1.84, 3.00, 4.99, 8.28, 13.7, 22.8, 37.8, 62.8, 104 pg/mL		2.00, 4.00, 8.00, 16.0, 64.0, 256, 512, 2048, 4096 pg/mL		5.00, 15.0, 25.0, 50.0, 75.0, 120, 200, 300, 400, 500 pg/mL	
LLOQ	2.17 pg/mL		2.00 pg/mL		15.0 pg/mL	
ULOQ	93.4 pg/mL		4096 pg/mL		500 pg/mL	
QC samples	LLOQ 2.17 pg/mL LoQC 3.26 pg/mL MeQC 32.6 pg/mL HiQC 81.5 pg/mL ULOQ 93.4 pg/mL UHQC 272 pg/mL		LLOQ 1 2.00 pg/mL LLOQ 2 4.00 pg/mL LoQC 8.00 pg/mL MeQC 1 128 pg/mL MeQC 2 512 pg/mL HiQC 2048 pg/mL ULOQ 4096 pg/mL		LLOQ 15.0 pg/mL LoQC 45.0 pg/mL MeQC 150 pg/mL HiQC 350 pg/mL ULOQ 500 pg/mL	
	LLOQ (mean)	Low, Mid, High, ULOQ (cumulative mean)	LLOQ 1 (range)	LoQC, MeQC1, MeQC2, HiQC, ULOQ (range)	LLOQ (range)	LoQC, MeQC, HiQC, ULOQ (range)

Evaluation and qualification of models

Population pharmacokinetic (PopPK) analyses were performed to characterise the disposition of sargramostim in healthy adults. The PopPK analyses were performed using the NONMEM 7.4 and parameter estimations were performed using first order conditional estimation method with the interaction option (FOCE-I). The initial data set consists of serum sargramostim concentrations from four phase 1 studies (Table below).

Table 12: Description of the phase 1 studies initially considered

Study	Dose and Regimen	Formulation(s)[a]	Number of Periods[b]	Population	Number of Subjects (N)	Study Design
308626	2, 6, 8 µg/kg SC single dose	liquid sargramostim EDTA (500 µg/mL)	2	Healthy male adults	62	Crossover
309404 Part 1	6 µg/kg SC single dose	liquid sargramostim EDTA (500 µg/mL), sargramostim LY, placebo EDTA	3	Healthy male adults	41	Crossover
309404 Part 2	500 µg SC and IV single dose	liquid sargramostim EDTA (500 µg/mL)	2	Healthy male adults	14	Crossover
309901	6 µg/kg SC single dose	liquid sargramostim EDTA (500 µg/mL and 1000 µg/mL)	2	Healthy male adults	48	Crossover
15367	125 µg/m² (LY) or 250 µg/m² (liquid) SC single dose	sargramostim LY, liquid sargramostim	2	Healthy adults (21 female, 39 male)	60	Crossover

EDTA, ethylenediaminetetraacetic acid; IV, intravenous; LY, lyophilized; SC, subcutaneous

[a] Liquid sargramostim, liquid formulation without EDTA; liquid sargramostim EDTA, liquid formulation with EDTA (no longer marketed); sargramostim LY, lyophilized sargramostim formulation

[b] Washout between periods at least 14 days

The preliminary examinations highlighted three different concentration-time profile types: a) one characteristic of intravenous administration of sargramostim; b) one characteristic of SC administration of a sargramostim in a formulation without EDTA; c) one characteristic of SC administration of a

sargramostim in a formulation with EDTA. The initial data set was subset to contain only data from individuals who received SC sargramostim as the liquid formulation without EDTA or the lyophilised formulation without EDTA from studies 309404 and 15367 (primary data set) for further model development. This primary data set was composed of 99 subjects with 2346 concentrations. In the primary data set, 178 concentration records (11.8% of the dataset) were excluded due to being below the BLQ. One hundred fourty-four of these were pre-dose (6.1% of the dataset) and one hundred thirty-four were post-dose (i.e., 16 h and 24 h sample; 5.7% of the dataset). A one-compartment model with first-order absorption (with lag time) and first-order elimination was found to be the most appropriate model to fit these PK data. Most fixed effect and random effect parameters were estimated with good precision (%RSE < 33%). Subsequently a covariate analysis was performed to explore the additional sources of variability in sargramostim PK profile. PopPK covariate model development was undertaken using screening of covariates, forward selection followed by a backward elimination procedure. Forward selection followed by a backward elimination procedure allows to include body weight in the popPK model. Therefore, the final popPK model included body weight effect on clearance [CL/F = 26.6 × (body weight / 76.8)1.11] and volume of distribution [V/F = 66.0 × (body weight / 76.8)1.90] of sargramostim. Final PopPK parameters are shown in Table below.

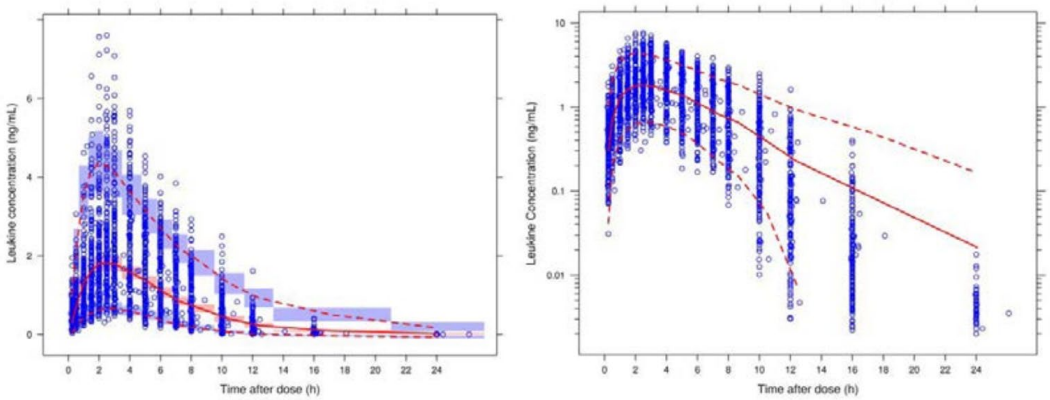
Table 13: Final popPK model parameters.

Parameter	Estimate (RSE%)	median [95% CI] (bootstrap)	Shrinkage (%)
CL/F (L/h)	26.6 (3.84%)	26.6 [25.2, 28.8]	1.68
V/F (L)	66.0 (3.87%)	65.9 [61.8, 72.1]	24.0
KA (/h)	0.395 (3.02%)	0.395 [0.372, 0.423]	34.0
ALAG1 (h)	0.083 (10.5%)	0.084 [0.067, 0.102]	23.8
$(WT/76.8)^{COV}$, CL/F	1.11 (15.4%)	1.14 [0.755, 1.50]	NA
$(WT/76.8)^{COV}$, V/F	1.90 (11.2%)	1.91 [1.44, 2.43]	NA
Interindividual variability (CV%)			
IIV (CL/F)	32.6% (14.4%)	32.2% [27.7%, 36.5%]	2.28
IIV (V/F)	41.4% (16.3%)	40.9% [34.1%, 47.8%]	23.4
IIV (KA)	34.3% (14.8%)	34.0% [28.8%, 39.5%]	34.2
IIV (ALAG1)	52.1% (30.4%)	50.9% [35.9%, 67.8%]	29.2
Residual variability			
σ1 (proportional)	27.2% (12.1%)	26.9% [24.1%, 30.6%]	5.07
σ2 (additive)	0.046 (46.5%)	0.046 [0.026, 0.074]	5.07

CL/F, apparent clearance. V/F, apparent volume of distribution. KA, absorption rate constant. ALAG1, absorption lag time. WT, weight. COV, covariate. IIV, interindividual variability. CV, coefficient of variation. RSE, relative standard error. CI, confidence interval. OFV, objective function value, NA, not applicable.

Most fixed and random effect parameters were estimated with good precision (RSE%<30%). As shown in the Figure below, the majority of observed sargramostim concentrations fell between 95% CI of the predicted concentrations, which indicates that the model captured the variability in observed data.

Figure 1: Final model visual predictive check after a single dose of sargramostim (left, linear scale; right, semi-log scale)



A dataset with 500 virtual adults was created via random sampling based on the weight distribution of subjects in all four phase 1 studies initially considered for this popPK analysis. The final popPK model was used to simulate sargramostim exposures for this virtual population at a 7 µg/kg (approximately 250 µg/m2) SC dose, that is approved for other indications. Predicted sargramostim PK parameters (AUC0-24 and Cmax) after single SC dose are provided in Table below.

Table 14: Predicted sargramostim exposure after single SC dose in adults.

Dose (µg/kg)	Median (5^{th} - 95^{th} Percentile) Weight (kg)	Number (N)	Mean (CV%) [5^{th} - 95^{th} percentile]	
			AUC_{0-24} (ng.h/mL)	C_{max} (ng/L)
7	78.4 (57.4 –98.4)	500	21.3 (32.6) [11.9-34.2]	3.03 (31.0) [1.74-4.84]

CV, coefficient of variation; AUC_{0-24}, area under the concentration-time curve over 24 hours; C_{max}, maximum concentration

In addition, the adult popPK model without covariate effects was applied to a paediatric population using the principle of allometry, with allometric scaling exponents of 0.75 for clearance and 1.0 for volume of distribution of the drug. Paediatric demographic data (age, weight, gender) were obtained from the 2013 National Health and Nutrition Examination Survey (NHANES) database as shown in Table 4A.

Table 15: Weight and age distribution (number of subjects) in NHANES database.

Age (years)	Weight (kg)		
	0 to <15	15 to 40	> 40
0 to <2	580	7	0
2 to <12	350	1481	350
12 to <18	0	37	895

Predicted sargramostim PK parameters from the simulations for pediatrics at 7, 10, 12 µg/kg doses for each weight and age group are shown in Tables below.

Table 16: Predicted sargramostim exposure after single SC dose in pediatric populations.

Weight or Age Category	Median (5^{th} - 95^{th} percentile) Weight (kg)	Number (N)	Dose (µg/kg)	Mean (CV%) [5^{th} - 95^{th} percentile]	
				AUC_{0-24} (ng-h/mL)	C_{max} (ng/mL)
Weight Cohorts					
0 to < 15 kg	10.8 (5.40 - 14.6)	1000	7	12.5 (34.6) [6.96-20.7]	2.11 (33.4) [1.13-3.46]
15 to 40 kg	25.1 (15.7 – 37.9)	1000		15.4 (33.3) [8.39-24.9]	2.37 (33.2) [1.30-3.90]
> 40 kg	60.6 (41.9 – 92.6)	1000		19.2 (32.5) [10.8-30.3]	2.65 (32.4) [1.49-42.6]
0 to < 15 kg	10.8 (5.40 - 14.6)	1000	10	17.7 (33.8) [9.52-28.4]	2.98 (35.0) [1.58-4.94]
15 to 40 kg	25.1 (15.7 – 37.9)	1000		22.3 (35.5) [11.8-37.7]	3.47 (34.0) [1.91-5.71]
> 40 kg	60.6 (41.9 – 92.6)	1000		27.5 (33.1) [15.2-44.2]	3.81 (31.5) [2.18-6.09]
0 to < 15 kg	10.8 (5.40 - 14.6)	1000	12	21.7 (35.1) [11.5-35.5]	3.69 (34.0) [1.94-5.96]
15 to 40 kg	25.1 (15.7 – 37.9)	1000		26.1 (33.4) [14.5-42.6]	4.02 (32.5) [2.22-6.57]
> 40 kg	60.6 (41.9 – 92.6)	1000		33.6 (34.4) [18.2-54.5]	4.61 (34.1) [2.49-7.59]

Table 17: Predicted sargramostim exposure after single SC dose in paediatric populations.

Age Cohorts					
0 to < 2 years	9.17 (5.10 – 13.6)	1000	7	12.0 (34.5) [6.60-19.7]	2.06 (33.1) [1.10-3.30]
2 to < 12 years	28.1 (12.7 – 58.0)	1000		15.6 (34.1) [8.48-25.8]	2.38 (33.2) [1.32-3.92]
12 to < 18 years	62.3 (40.4 – 93.8)	1000		19.3 (32.7) [10.8-30.5]	2.66 (32.5) [1.48-4.19]
0 to < 2 years	9.17 (5.10 – 13.6)	1000	10	17.0 (34.3) [9.10-27.7]	2.91 (35.2) [1.53-4.81]
2 to < 12 years	28.1 (12.7 – 58.0)	1000		22.6 (37.3) [11.9-39.5]	3.49 (34.4) [1.90-5.88]
12 to < 18 years	62.3 (40.4 – 93.8)	1000		27.7 (33.9) [15.4-44.4]	3.82 (31.7) [2.20-6.12]
0 to < 2 years	9.17 (5.10 – 13.6)	1000	12	20.9 (35.2) [11.2-33.8]	3.61 (34.6) [1.90-5.94]
2 to < 12 years	28.1 (12.7 – 58.0)	1000		26.5 (35.1) [13.8-44.0]	4.04 (32.5) [2.23-6.58]
12 to < 18 years	62.3 (40.4 – 93.8)	1000		33.8 (34.2) [18.1-54.8]	4.62 (33.8) [2.47-7.62]

CV, coefficient of variation; AUC_{0-24}, area under the concentration-time curve over 24 hours; C_{max}, maximum concentration

Absorption

Following single SC doses of 25 to 500 µg in Part 1 of Study PTX-001-005, sargramostim exposure as assessed by Cmax and AUC increased with increasing dose in a greater than dose proportional manner. Following a single and repeat SC administration of 500 µg sargramostim in Part 2 of the study, sargramostim was detected in the serum early (15 min) and peak plasma concentrations were reached by 7.0 hours and 3.0 hours on Day 1 and Day 8, respectively. Exposures, as assessed by Cmax and AUC were similar on Day 1 at 2860 pg·h/mL and 22300 pg·h/mL, respectively, and on Day 8 at 2570 pg·h/mL and 19500 pg·h/mL, respectively. There was no accumulation observed following repeat dose administration with ratios for both Cmax and AUC below 1.

Study 309404 was conducted to evaluate the absolute bioavailability of liquid EDTA sargramostim formulation and the bioavailability relative to that of the lyophilised sargramostim formulation when administered SC in healthy subjects. In Part 2 of the study, the absolute bioavailability of the liquid formulation administered SC was 76%, based on the ratio of the mean AUC0-t. Median tmax (minmax) was 0.25 (0.25-3.0) hours for SC administration, and 2.0 (2.0-2.0) hours (i.e., at the end of infusion) for the IV administration.

The intended formulation to be marketed is the lyophilised formulation containing drug substance manufactured at Northpointe, and 10 mg/vial sucrose, 40 mg/vial mannitol, and 1.2 mg/vial tris as excipients. Lyophilised drug product is to be prepared for SC injection by reconstitution in 1 mL sterile water for injection. Multiple bioequivalence studies were submitted: sufficient data are available to demonstrate bioequivalence between drug substance manufactured at Seattle and drug substance manufactured at Northpointe, and data from BE and BA studies are consistent in demonstrating bioequivalence among the different formulations used in clinical studies (liquid EDTA, liquid non EDTA, lyophilised).

Distribution

Following a single SC administration of 500 µg sargramostim in Study PTX-001-005, concentrations in the CSF were below the limit of quantitation for all three subjects and, therefore, the penetration potential of sargramostim in the CSF could not be assessed. In the same study, observed volume of distribution after IV administration was 14 L.

Elimination

The sargramostim elimination was not described by the applicant. However, considering it is a protein, it is expected that catabolic endogenous pathways are involved in sargramostim elimination. According to data from study PTX-001-005, the half-life after SC administration of 250 µg sargramostim was 1.32 h, while the clearance estimated by PopPK model is 26.6 L/h.

Dose proportionality and time dependencies

Dose proportionality after a single dose was investigated in study 308626. As the sargramostim dose increased from 2 to 6 to 8 µg/kg (1:3:4-fold increase), proportional increases in Cmax were evident (Japanese 1.0:3.0:4.0- fold increase, Caucasian 1.0:2.9:4.1-fold increase).

AUC, however, showed greater than proportional increases, suggesting an apparent nonproportional relationship with dose administered (Japanese 1.0:4.7:6.3-fold increase, Caucasian 1.0:4.7:7.8- fold increase).

Dose proportionality was investigated also in Study PTX-001-005. In both parts of the study, a single SC dose was administered on Day 1 (Part 1: 25 to 500 µg; Part 2: 500 µg). The analyses results are presented in Table 11-5. For all 3 exposure parameters, the estimate of slope was > 1.0 and the 90% CI excluded 1, indicating that these parameters increased in a more than dose proportional manner with increasing dose.

Considering the short half life of sargramostim, no accumulation is expected when administered once daily. Elimination of sargramostim after multiple dose administration in study 308001 was similar to what has been observed following single dose administration, however time dependency was not formally investigated.

Special populations

Dedicated PK studies on special populations were not conducted, with the exception of Study 308626 on Japanese and Caucasian subjects.

The primary objective of the study 308626 was to investigate the safety profile and PK properties of sargramostim in healthy Japanese (first generation residing in the US) and Caucasian adult male subjects. This was a randomised, crossover study with 3 sargramostim dose levels (actual doses, 2.2, 6.5, and 8.7 µg/kg), 2 treatment groups (Japanese and Caucasian), and 6 treatment sequences. Each subject received 2 single SC doses of liquid sargramostim formulation with EDTA, with a washout period of ≥14 days between each dose. Thirty Caucasian and 32 Japanese subjects received at least one treatment with 28 Caucasian and 29 Japanese subjects completing the treatment per protocol.

The maximum serum concentrations (Cmax) were similar in both Japanese and Caucasian subjects. Mean (geometric) Cmax ranged between 1.77 ng/mL (after 2 µg/kg) and 7.05 ng/mL (after 8 µg/kg) in Japanese men. In Caucasian men, mean Cmax ranged between 1.50 ng/mL (after 2 µg/kg) and 6.17 ng/mL (after 8 µg/kg).

After a single SC dose of sargramostim, the area under the curve (AUC) demonstrated an apparent nonproportional relationship with dose administered. Sargramostim exposure, as measured by AUC, was similar in both Japanese and Caucasian subjects. In Japanese men, the mean (geometric) AUC ranged from 4.99 ng*h/mL to 31.37 ng*h/mL for the lowest and highest administered dose, respectively. In Caucasian men, the mean AUC ranged from 4.18 ng*h/mL to 32.62 ng*h/mL for the lowest and highest administered dose, respectively.

The results of three studies on the PK in the paediatric population were presented in the sargramostim dossier.

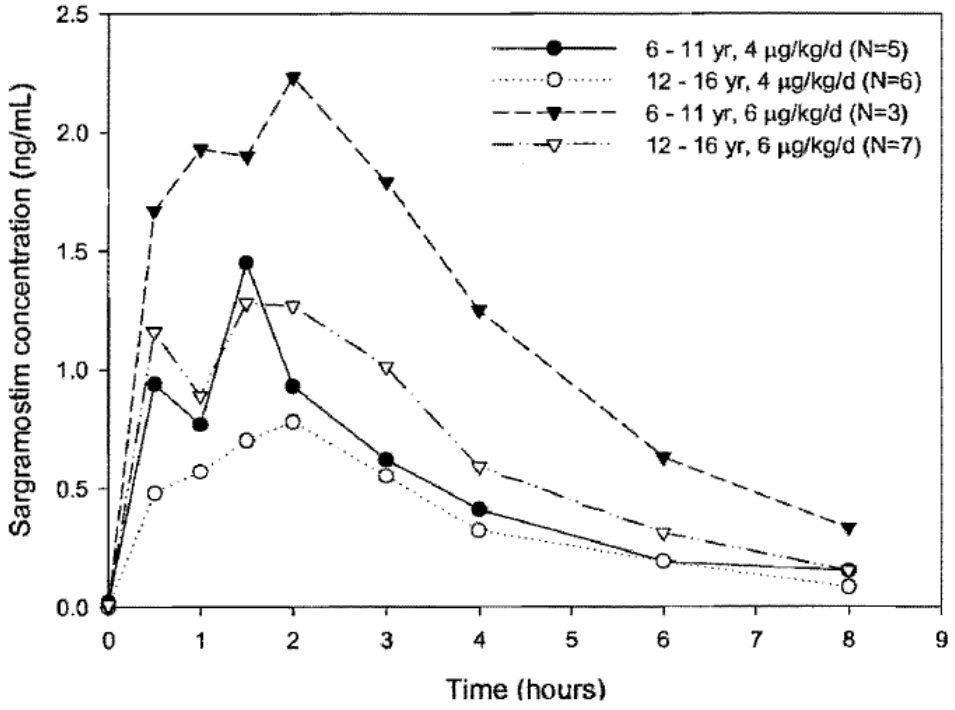
No clinical study report has been provided for study 001.8706; however, a summary from the publication of study results was provided (Stute, 1995). The pharmacokinetics of lyophilised sargramostim in children who had undergone intensive multiagent chemotherapy were evaluated. Eleven children with refractory solid tumours received 500 to 1500 µg/m2 of sargramostim as a daily 2-hour intravenous (IV) infusion, and 7 children received SC sargramostim at 1500 to 2000 µg/m2/day in 2 daily injections for 2 weeks. The median (range) age of the patients in the IV group was 4 (1.5 to 12) years and in the SC group was 7 (0.5 to 15) years. The PK data were subjected to compartmental analysis. Following 2-hour IV infusions, the concentration-time data were best described by a 2-compartment, first-order elimination model. The median (range) for sargramostim systemic total body clearance (CL) after IV administration was 49 mL/min/m2 (range, 15 to 118 mL/min/m2), terminal elimination half-life (t1/2z) was 1.6 hours (range, 0.9 to 2.5 hours), and the time for the sargramostim concentration to reach >1 ng/mL was 9 hours (range, 6 to 13 hours). The CL was not dose dependent or related to patient age. The SC concentration-time data were best described by a one-compartment model with first-order absorption and elimination. Median apparent total body clearance (CL/F) after SC administration was 72 mL/min/m2 (range, 27 to 231 mL/min/m2) and t1/2z was 2.3 hours (range, 0.3 to 3.8 hours). Absorption was prolonged, with peak concentrations obtained after 3 hours (range, 1.5 to 4 hours). These data established comparable PK characteristics of sargramostim in children and previously published results in adults.

Study 9208 was designed as a phase I/II safety and dose escalation trial of recombinant human granulocyte-macrophage, colony-stimulating factor (GM-CSF) in preterm neonates. 5 patients were enrolled at each of the following doses: 0.05 µg/kg once daily (QD), 5 µg/kg QD, 5 µg/kg twice daily (BID), 10 µg/kg QD, or 10 µg/kg BID given as a 2-hour IV infusion for 7 days. The study was stopped after 21 of the planned 25 patients were enrolled because significant improvement was noted in study endpoints at the lower doses. Blood samples for serum sargramostim concentration measurements were drawn over 24 hours following the first dose of sargramostim. PK parameters were determined using non compartimental analysis. The dose of 0.05 µg/kg resulted in sargramostim concentration levels below the LLOQ. Peak serum concentrations for the other doses occurred at the end of a 2-hour IV infusion, were dose dependent, and were undetectable by 24 hours post-dose. At the sargramostim dose of 5 µg/kg QD, the mean area under the concentration curve from time 0 to 24 hours (AUC0-24) in serum was 19.3 ng·h/mL, mean maximum observed concentration (Cmax) was 7.7 ng/mL, and mean t1/2z was 3.9 hours. At the sargramostim dose of 5 µg/kg BID, the mean serum AUC0-24 was 19.0 ng·h/mL, the mean Cmax was 11.6 ng/mL, and mean t1/2z was 1.0 hour. At the sargramostim dose of 10 µg/kg QD, the mean AUC0-24 was 61.2 ng·h/mL, the mean Cmax was 22.0 ng/mL, and mean t1/2z was 1.4 hour.

Study 308001 was a Phase 1/2, open-label pilot study of sargramostim treatment in pediatric subjects receiving induction therapy with corticosteroids for active Crohn's disease. Two doses of sargramostim, 4 µg/kg and 6 µg/kg administered SC once daily for 8 weeks (56 doses), were studied. Twenty-six patients with active Crohn’s disease were screened, and 22 paediatric patients aged 4 to 16 years were randomised to receive 4 µg/kg/day or 6 µg/kg/day of sargramostim SC daily for 8 weeks. The key PK variables (Cmax, time to reach maximum serum/plasma concentration [tmax], area under the curve at last measurable timepoint [AUClast], t1/2z, and CL/F) were determined by noncompartmental analysis. The PK evaluation demonstrated that maximum serum sargramostim concentration was reached within 2 hours after SC administration. Two peaks of absorption were seen, a fast-occurring peak at 30 minutes and then a gradual increase in concentration that peaked at 1.5 to 2 hours. Based on the evaluation of pre-dose PK samples collected on days 8 and 15, little to no accumulation was observed following repeated daily administration for 2 weeks. Higher exposures were achieved following administration of 6 µg/kg/day than with administration of 4 µg/kg/day for corresponding age groups. However, a clear conclusion of dose proportionality could not be reached due to the limited number of patients enrolled in the study. Elimination of sargramostim after multiple dose

administration was not affected by dose or age group and was similar to what has been observed following single dose administration in healthy adult subjects.

Figure 2: Mean concentrations (linear scale) of sargramostim in serum by age and treatment group on Day 15 after sc administration



Pharmacokinetic interaction studies

No DDI studies have been performed.

2.6.2.2. Pharmacodynamics

Mechanism of action

Sargramostim is a glycosylated recombinant human granulocyte-macrophage colony-stimulating factor (rhu GM-CSF). Sargramostim mechanism of action drives host immunity by boosting innate and adaptive host defence and targets epithelial repair and restoration. Specifically, sargramostim stimulates the differentiation, proliferation, and activation of haematopoietic cells of both granulocyte and monocyte lineages (e.g., neutrophils, monocytes/macrophages, and myeloid-derived dendritic cells, and megakaryocytes).

Primary and secondary pharmacology

Sargramostim stimulates white blood cell production, and in particular, neutrophil, macrophage, and dendritic cell production.

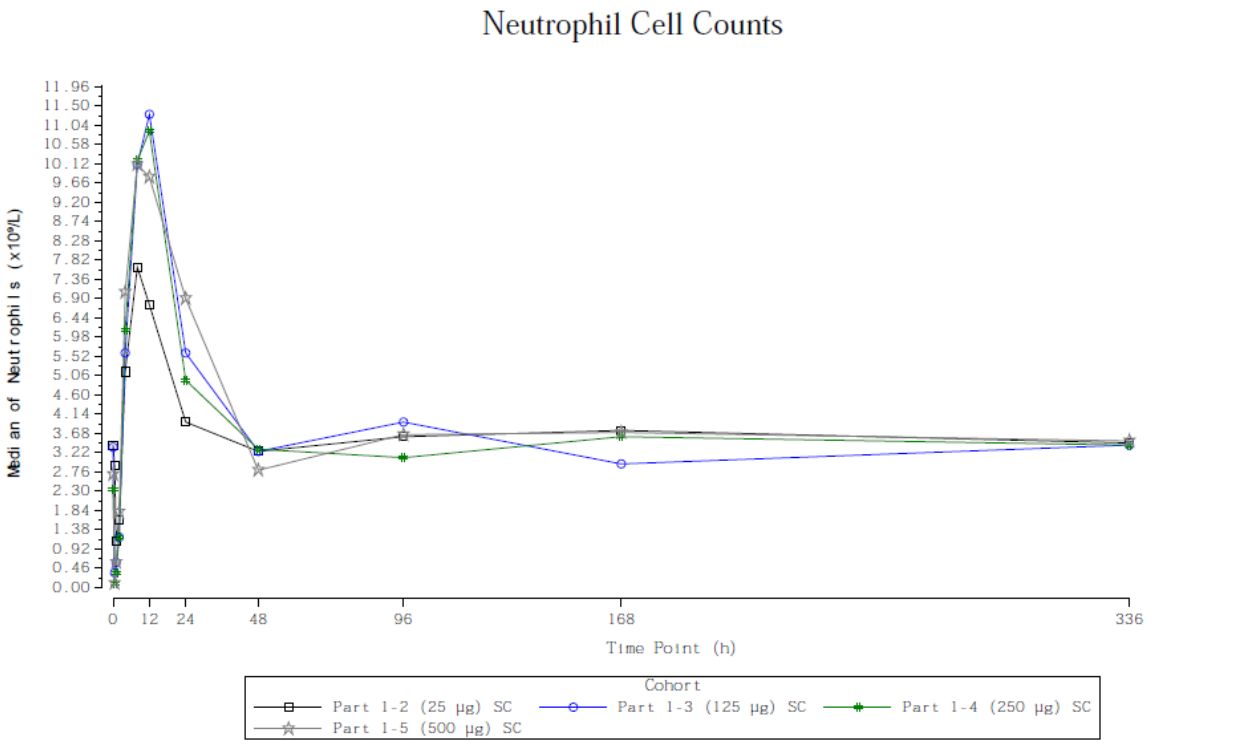
Following SC, IV, and IH sargramostim administration to healthy adult subjects in Study PTX-001-005, PD effects as seen on CBC parameters across all cohorts showed a consistent early nadir post sargramostim administration which was followed by a peak in median cell counts that were attained at variable timepoints. Phenotypic analysis of the peripheral blood cells showed a consistent increase in HLA-DR expression on monocytes in all cohorts and dosing routes.

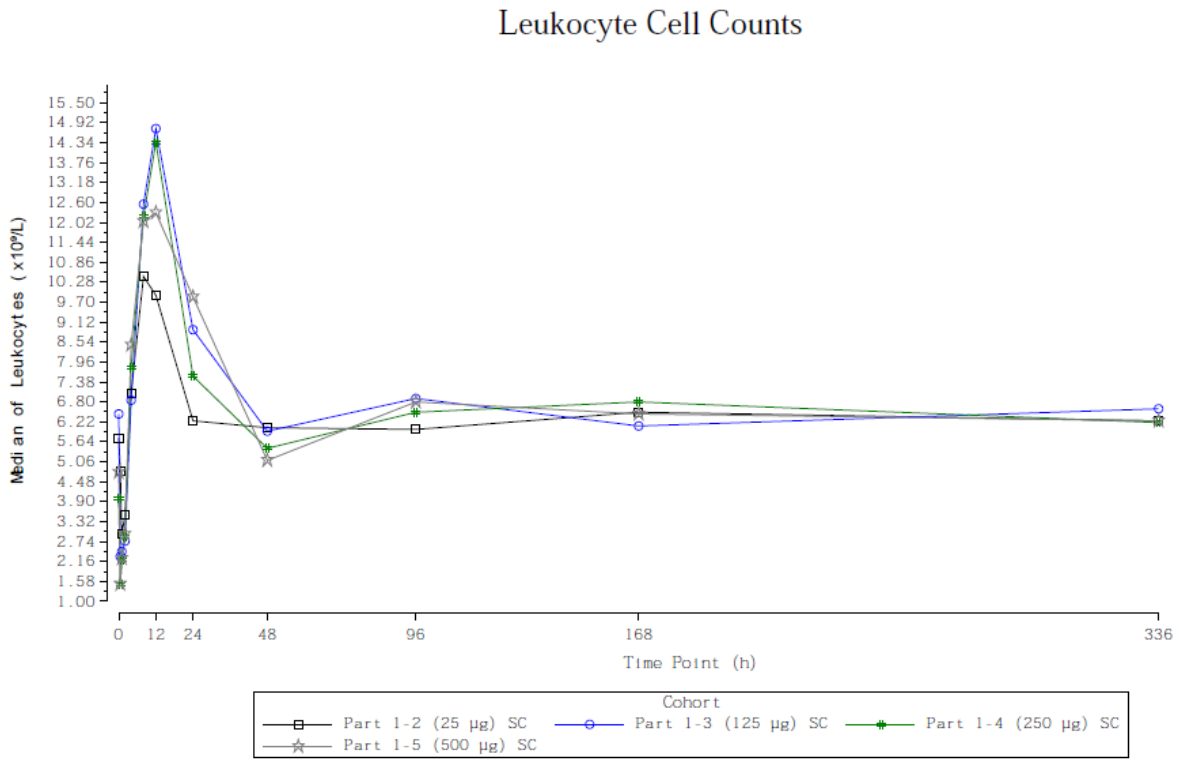
Overall, there was no impact of study drug on erythrocyte or platelet parameters.

Median neutrophil and leukocyte cell counts over time are presented in Figure below. Both neutrophils and leukocytes underwent rapid reductions to median nadir, which was observed at 0.5- or 1-hour postdose across all SC cohorts.

Subsequently, peak neutrophil or leukocyte cell counts were observed between 8 and 12 hours postdose, returning to approximate baseline values by 48 to 96 hours postdose. Changes in median ratios of neutrophils to leukocytes followed a similar pattern to neutrophil cell counts. No clear dose-response was observed for neutrophil or leukocytes with respect to median nadir or median peak cell counts. However, for the latter, relatively comparable magnitudes of median peak cell counts were observed for the 125 to 500 µg SC dose cohorts, whereas the impact of study drug for the 25 µg dose cohort on both neutrophil and leukocyte peak cell counts was less pronounced.

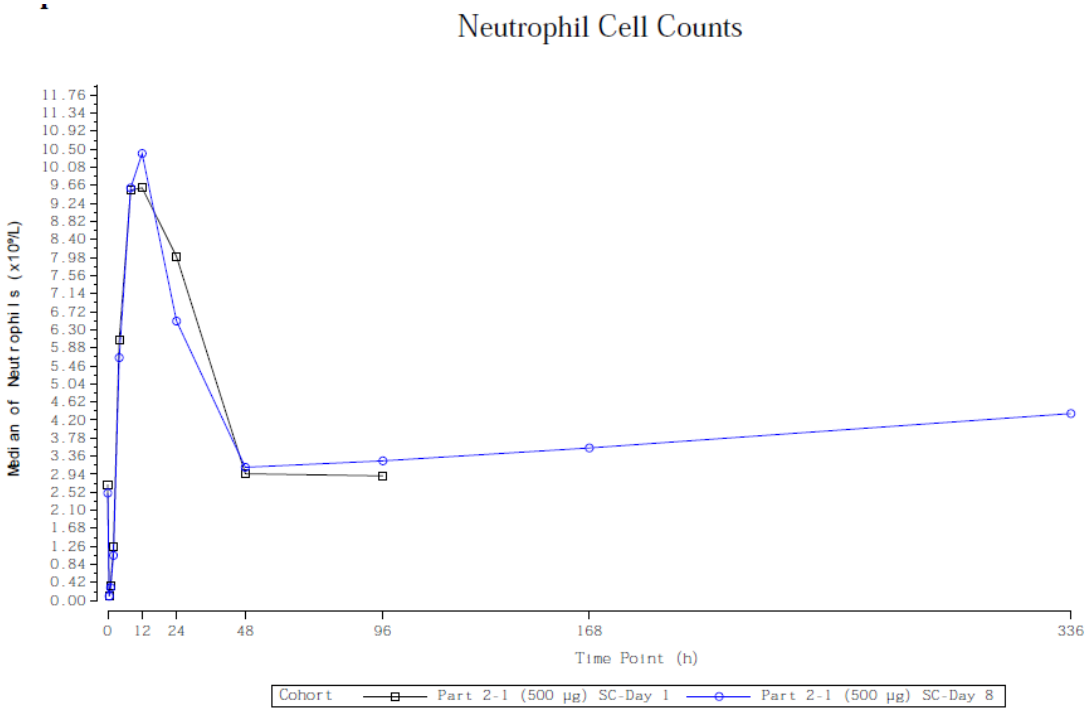
Figure 3: Median Neutrophile and Leukocyte cell counts vs time profiles for the SC cohorts





In general, median basophil, eosinophil, monocyte, lymphocyte, neutrophil, and leukocyte cell counts versus time profiles were qualitatively similar between Day 1 and Day 8 in Part 2 (Figure below) and between Part 1 and Part 2 following 500 µg SC doses.

Figure 4: Median Neutrophil cell counts vs time profiles for the repeat doses cohorts



2.6.3. Discussion on clinical pharmacology

Bioanalytical methods

The pharmacokinetic (PK) of sargramostim in humans has been studied using enzyme-linked immunosorbent assays (ELISAs) developed by Immunex, Berlex, Bayer, and Partner Therapeutics.

Method 2010043-01131 was generally acceptable and largely in line with ICH M10 guideline. Precision, accuracy, LLOQ, ULOQ, selectivity and specificity, prepossessing stability, freeze and thaw stability, intermediate stock solution stability, bench top stability, long term stability, dilution linearity, hook effects, parallelism, different formulations, robustness and ruggedness were evaluated and considered acceptable.

For method 7548-100 precision, accuracy, LLOQ, ULOQ, selectivity and specificity, prepossessing stability, freeze and thaw stability, bench top stability, long term stability, dilution linearity, hook effects were evaluated and acceptable.

As a general comment, a significant improvement in method validation and samples analysis is noted during bioanalytical method development.

Evaluation and qualification of models

Population pharmacokinetic (PopPK) analyses were performed to characterise the disposition of sargramostim in healthy adults. The initial data set was restricted to contain only data from individuals who received SC sargramostim as the liquid formulation without EDTA or the lyophilised formulation without EDTA from studies 309404 and 15367 for model development. The decision to exclude a large proportion of the initial data (as well as other PK data presented in the dossier) cannot be justified by questioning the inability of the model to explain the observed concentration profiles. Studies with multiple dosing were not used for model development. The data set was composed of plasma drug concentration data obtained from a limited number of healthy subjects enrolled in only two Phase 1 studies (99 subjects with 2346 concentrations). This means that intra-, and inter-individual variability cannot be fully quantified and explained. A one-compartment model with first-order absorption (with lag time) and first-order elimination was found to be the most appropriate model to fit these PK data. Most fixed effect and random effect parameters were estimated with good precision, but RSE values <33% and goodness of fit may depend more on the reduced variability in the data than on any real ability of the model to represent the system under study. Then, forward selection followed by a backward elimination procedure allows to include body weight in the popPK model. Therefore, the final popPK model included body weight effect on clearance [CL/F = 26.6 × (body weight / 76.8)1.11] and volume of distribution [V/F = 66.0 × (body weight / 76.8)1.90] of sargramostim. Most fixed and random effect parameters were estimated with good precision (RSE%<30%). The final popPK model was used to simulate the PK profiles of the drug in adults. A dataset with 500 virtual adults was created via random sampling based on the weight distribution of subjects in all four phase 1 studies initially considered for this popPK analysis. The final popPK model was used to simulate sargramostim exposures for this virtual population at a 7 µg/kg SC dose. In addition, the adult popPK model without covariate effects was applied to a pediatric population using the principle of allometry, with allometric scaling exponents of 0.75 for clearance and 1.0 for volume of distribution of the drug. Pediatric demographic data (age, weight, gender) were obtained from the 2013 National Health and Nutrition Examination Survey (NHANES) database. Predicted sargramostim PK parameters from the simulations for pediatrics at 7, 10, 12 µg/kg doses for each weight and age group were predicted.

As a conclusion, there are important limitations that prevent the applicability (simulations) of the model as suggested by the applicant, and the assumptions regarding simulations in both adult and paediatric populations are therefore not supported.

Pharmacokinetics

ADME was evaluated in multiple clinical pharmacology studies, but results are difficult to be interpreted, considering the different formulations, doses and patients populations studied. Exposures were greated following a single IV administration of 250 µg compared to SC administration of the same

dose. After subcutaneous administration, sargramostim was detected in the serum early (15 min) with tmax between 2 and 6 h. The absolute bioavailability of sagramostim liquid EDTA formulation administered subcutaneously compared to IV was 76%.

Multiple formulations were used during the development of sargramostim, which differed in excipients. Comparison between formulations in PK studies should be regarded with caution. Comparisons mostly were performed in PK studies for which bioanalytical method validation was not available and studies were relatively old/not conducted according to current guidelines. Nevertheless, exposures between different formulations were generally comparable regarding AUC0-t and Cmax. However, the 90%CI of the ratio between formulations fell outside 80%-125% limits in some of the studies, possibly due to a lack of power. There were no indications that formulations would not be bioequivalent, however, formal bioequivalence between all formulations could not always be established. Of note, although AUC0-t and Cmax generally were comparable for formulations with and without EDTA (e.g. in study 309404), formulations containing EDTA resulted in a distinct two-phase absorption profile. The sargramostim elimination was not described by the applicant. However, considering it is a protein, it is expected that catabolic endogenous pathways are involved in sargramostim elimination, via degradation into small peptides and individual amino acids. According to study PTX-001-005, the volume of distribution after IV administration of 250 µg sargramostim was 14 L, the half-life after SC administration of 250 µg sargramostim was 1.32 h, while the clearance estimated by PopPK model is 26.6 L/h.

After a single dose of sargramostim, AUC and Cmax increased linearly with dose; however, AUC demonstrated an apparent nonproportional relationship with dose administered in Study 308626, while both AUC and Cmax demonstrated a more than dose proportional increase in Study PTX-001-005. This finding suggests a nonlinear clearance.

Multiple doses were only administered in the paediatric studies. Although the evidence provided is somewhat conflicting, it is agreed that there are no signs of a clinically relevant time-dependency in PK.

PK was not investigated in the target population. Pivotal efficacy studies were conducted on NHPs. According to model simulated exposures at the same dose given to irradiated and not-irradiated NHPs (7 µg/kg/day, considered equivalent to 250 µg/m2 in humans), Day 1 AUC is 4-fold higher in adult human subjects than in NHPs.

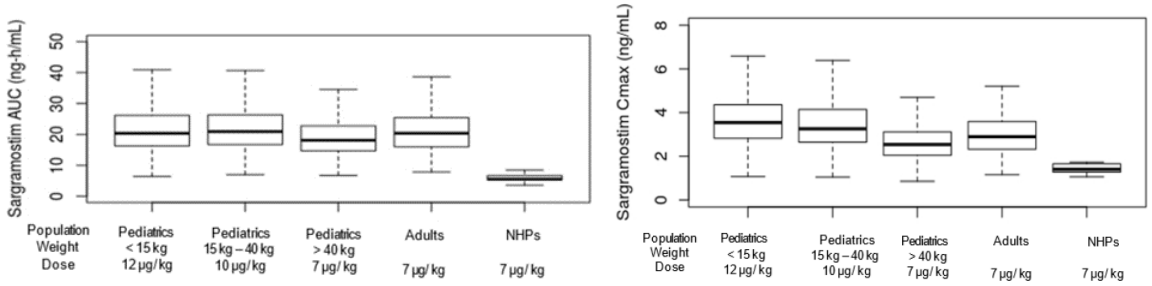
Dedicated PK studies in special populations were not conducted, with the exception of Study 308626 who enrolled Japanese and Caucasian subjects. Special patient populations were studied to a limited extent in the PopPK model but considering that PopPK model was built with PK data of Phase 1 studies 15367 and 309404 only, where a population with very low variability was enrolled, the impact of covariates on sargramostim PK cannot be considered reliable. Sex, age, body weight, body surface area and race (Caucasian, Black) were tested as covariates. Weight was a significant covariate on clearance and volume of distribution. Effects of hepatic impairment and renal impairment on PK were not studied, however, this is not expected to influence exposure given the proposed metabolism via protein degradation into small peptides and amino acids.

PK in paediatric patients was investigated in Study 001.8706 in children aged 6 months to 15 years, Study 9208 conducted in preterm neonates (within 72 hours from birth), and Study 308001 conducted in paediatric subjects receiving induction therapy with corticosteroids for active Crohn's disease aged 4 to 16 years. It is difficult to establish the relevance of the PK results of these studies for the present submission, since doses administered are different to the dose proposed for the intended indication or PK results are related to an IV administration.

The final adult PopPK model without covariate effects was applied to the paediatric population, with

allometric scaling exponents of 0.75 for clearance and 1.0 for volume of distribution of the drug. This model was then used to estimate exposure for the paediatric population for weight cohorts <15 kg, 15-40 kg and >40 kg (Figure below).

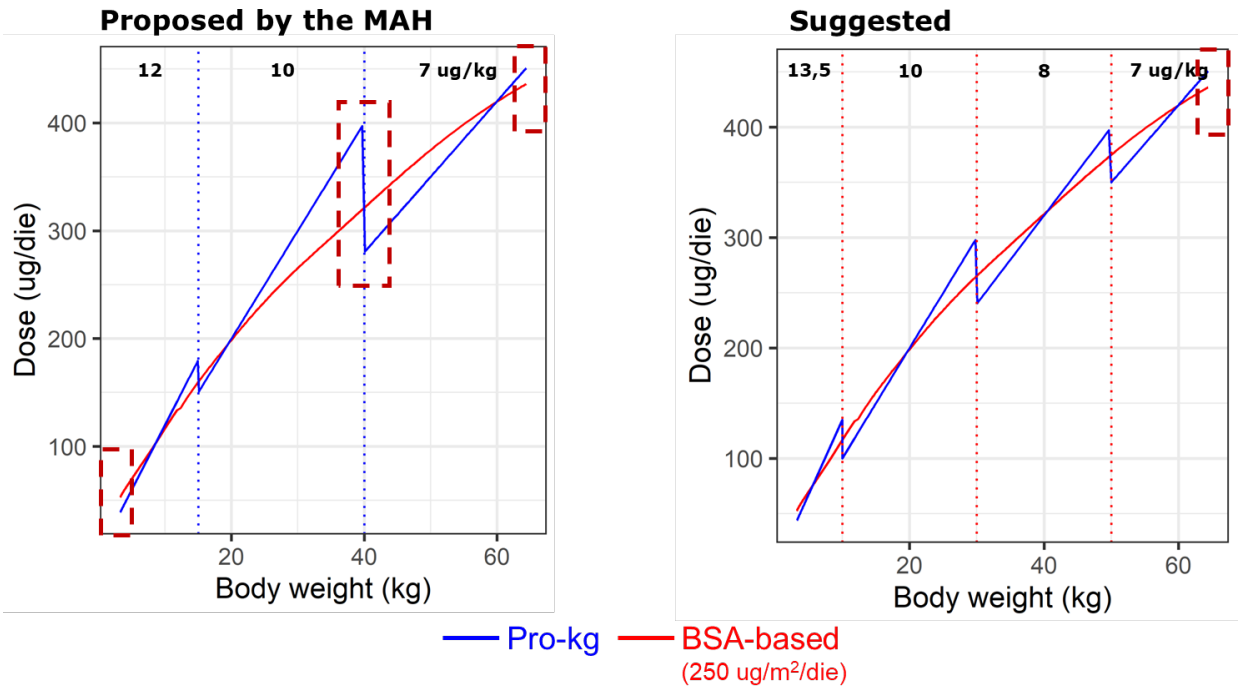
Figure 5: Box plots of (simulated) sargramostim AUC0-24 (left) and Cmax (right) Values in paediatrics, adults, and non-human primates.



In addition, simulations are performed for children 0-2 years, 2-12 years, and 12-18 years of age.

The dosing rationale for children below 18 years is only supported by simulations, not including data from PK studies. Since the model cannot be considered reliable for the paediatric population, the CHMP proposed to explore the option to optimize posology, i.e. to re-define subjects weight sub-categories to obtain more consistency between the BW-based dose and the BSA-based dose (supported by evidences in other indications). The proposal, described by the Figure below, was however not supported by the applicant.

Figure 6: The dosing rationale for children below 18 years proposed by applicant (left) and by the rapporteurs (right).



The body weight-based posology for paediatric patients, as proposed by the applicant, may not be optimal since it is not supported by simulations or evidence. However, considering the proposed emergency indication and the lack of a clearly established exposure threshold for efficacy in humans, it is considered acceptable.

Pharmacodynamics

PD was primarily investigated in Study PTX-001-005. The observed PD effects did not appear to be dose related at greater than 125 µg in the SC cohorts. With respect to CBC parameters (basophils, eosinophils, monocytes, lymphocytes, neutrophils, and leukocytes), sargramostim administration resulted in an initial fall from baseline and nadir values were reached within 0.5 to 2 hours post dose. This was followed by continued increase above baseline levels reaching peak values after 8 to 168 hours post-dose. In general, CBC parameters returned to approximate baseline levels within 336 hours.

The applicant justified the dose in the adult population based on the dose used in clinical practice in myelosuppressed patients. Sargramostim doses of 250 µg/m2/day have been shown to be safe and effective in adults and the sargramostim dose of 7 µg/kg/day would approximate to 250 µg/m2/day. However, in the paediatric population, the proposed pro-kilo dosing does not match with the dose calculated using body surface area.

2.6.4. Conclusions on clinical pharmacology

Several clinical pharmacology studies and a PopPK model were submitted to support the MAA of sargramostim. Although the submitted PopPK model cannot be considered reliable for establishing paediatric doses, it is deemed acceptable given the proposed emergency indication and the lack of a clearly established exposure threshold for efficacy in humans.

2.6.5. Efficacy

Sargramostim efficacy studies could not be conducted in humans with H-ARS since such studies would be contrary to generally accepted principles of medical ethics due to the harmful levels of radiation required to induce H-ARS. Therefore, 3 pivotal AWC efficacy studies were conducted in a rhesus monkey total body irradiation (TBI) model. These studies are the basis for demonstration of sargramostim clinical efficacy in children (from birth) and adult humans in the sought indication H-ARS. Study TSK0143 is only considered as a supportive study due to the absence of GLP compliance and the different administration schedule used.

Table 18: List of pivotal AWC efficacy studies conducted in a rhesus monkey total body irradiation (TBI) model

Study ID	Enrolment status Start date Total enrolment/ enrolment goal	Design Control type	Study & control drugs Dose, route of administration and duration Regimen	Population Main inclusion/ exclusion criteria
TSK0143(non GLP compliant)	Completed May 19 2015 24/24	Randomized, Controlled Study	**Group 1:** sargramostim (7 µg/kg/day), subcoutaneous route (s.c.) once daily for 14 consecutive days 48±1h post-TBI at 670 CGy (LD50-60/60) **Group 2:** Vehicle s.c. once daily for 14 consecutive days	**Group 1:** 6M/6F **Group 2:** 6M/6F (total 24) Healthy animals of adequate body size to receive TBI and tolerate the sequential, invasive monitoring techniques used to assess

			48±1h post-TBI at 670 CGy (LD50-60/60)	haematopoietic and immune reconstitution.
TSK0144	Completed October 29, 2017 108/108	Blinded randomized controlled study	**Group 1:** sargramostim (7 µg/kg/day) s.c. once daily until ANC criteria is reached, starting 48±1h post-TBI at 655 CGy (LD50-60/60) **Group 2:** Vehicle s.c. once daily until ANC criteria[a] is Reached, starting 48±1h post-TBI at 670 CGy (LD50-60/60) **Group 3:** sargramostim (7 µg/kg/day) s.c. once daily until ANC criteria[a] is reached, starting 48±1h post-TBI at 713 (LD70-80/60) **Group 4:** Vehicle s.c. once daily until ANC criteria[a] is Reached, starting 48±1h post-TBI at 713 (LD70-80/60)	Group 1: 18M/18F Group 2: 18M/18F Group 3: 9M/9F Group 4: 9M/9F Total 108 Healthy animals of adequate body size to receive TBI and tolerate the sequential, invasive monitoring techniques used to assess haematopoietic and immune reconstitution.
1017-3493	Completed March 05, 2018 308/308	Blinded randomized controlled study	**Group 1:** sargramostim (7 µg/kg/day) s.c. once daily until ANC criteria is reached, starting 48±1h, 72h ±1h, 96 ±1h, 120±1h post-TBI at 713 CGy (LD70-80/60) **Group 2:** Vehicle s.c. once daily until ANC criteria[a] is reached, starting 48±1h post-TBI at 713 CGy (LD70-80/60) **Group 3:** sargramostim (7 µg/kg/day) s.c. once daily + azithromycin until ANC criteria is reached, starting 48±1h post-TBI at 713 (LD70-80/60)	**Group 1:** 48±1h: 22M/22F 72h±1h: 22M/22F 96 ±1h: 22M/22F 120±1h: 22M/22F **Group 2:** 22M/22F **Group 3:** 22M/22F **Group 4;** 22M/22F Total 308 Healthy animals of adequate body size to receive TBI and tolerate the sequential, invasive monitoring techniques used to assess haematopoietic and immune reconstitution.

			Group 4: Vehicle s.c. once daily until ANC criteria is Reached, starting 48±1h post-TBI at 713 (LD70-80/60)	
FY14-045	Completed April 2014 105/105	Blinded randomized controlled study	**Group 1:** sargramostim (7 µg/kg/day) s.c. once daily through Day 18 or until ANC >1000 µL starting 1 day post TBI 680 cGy (LD50/60) **Group 2:** Vehicle s.c. once daily through Day 18 or until ANC >1000 µL starting 1 day post TBI 680 cGy (LD50/60) **Group 3:** sargramostim (7 µg/kg/day) s.c. once daily through Day 18 or until ANC >1x 10^9/L starting 2 day post TBI 680 cGy (LD50/60)	**Group 1:** 35M/0F **Group 2:** 35M/0F **Group 3:** 35M/0F Total 105 Healthy animals of adequate body size to receive TBI and tolerate the sequential, invasive monitoring techniques used to assess haematopoietic and immune reconstitution.

a:ANC criteria= dosing continued until absolute neutrophil count (ANC) returned to ≥ 1 x 109/L for 3 consecutive days or if the ANC was ≥ 10 x 10^9/L

Supportive clinical efficacy data also come from studies in patients undergoing autologous and allogeneic bone marrow transplants or with acute myelogenous leukemia (AML), that led to other approved indications in the US, and from narratives of accidental high-dose radiation exposure.

In particular data of efficacy of sargramostim in the paediatric population came from supportive studies in which the drug was studied in patients affected by haematological malignancies and from a trial in pre-term, low weighted newborns in which sargramostim was used as prophylaxis of infection.

2.6.5.1. Dose response studies

N/A

2.6.5.2. Main studies

Study TSK 0144 (Confirmatory AWC efficacy study)

Study TSK0144 was a confirmatory, blinded randomized study in which sargramostim or vehicle were administered daily starting 48h post-TBI at LD50-60/60; the survival benefit and efficacy of sargramostim on haematological parameters were also explored in separate cohorts of irradiated rhesus monkeys at a LD70-80/60 dose (713 cGy).

Methods

- **Study participants**

A total of 108 healthy animals were enrolled in TSK 0144; 72 animals were enrolled in the main cohort of LD50-60/60, while 36 were included in the exploratory arm at LD70-80/60. Randomization allowed to include an equal number of subjects for each group of treatment as well as the number of male and females.

> Given the nature of the study, no specific inclusion or exclusion criteria were provided, except for healthy, adult rhesus monkeys with an adequate body size to receive TBI and tolerate the sequential, invasive monitoring techniques used to assess haematopoietic and immune reconstitution.

- **Treatments**

Animals were randomized to receive sargramostim or vehicle; the test item and reference item/vehicle were administered subcutaneously daily until the absolute neutrophil count (ANC) returned to ≥ 1 x 10^9/L for 3 consecutive days.

The first injection was performed 48 ± 1 hour after irradiation. Treatment was stopped if the ANC reached ≥ 10 x 109/L; terminal necropsies were on Day 60. A total of 4 arms (2 for each level of irradiation) were planned.

Treatments were prepared according to protocol specific preparation of Dosing Formulations:

Reference Item/Vehicle (Groups 1 and 3): Sterile water for injection (USP) was used as provided by the manufacturer.

Test Item/sargramostim (Groups 2 and 4): Preparation of the stock solution:

The sargramostim (0.25 mg/vial) was reconstituted fresh on each dosing occasion in SWFI to obtain a nominal concentration of 250 ug/mL. The solution was gently swirled at room temperature until complete dissolution of the test item and was placed on wet ice pending use.

The arrangement of the dosing formulations was performed according to internal procedures, assuring the stability of the dosing formulations.

All animals received a minimal supportive care that is supposed to mimic the minimum level of assistance that can be provided in an emergency setting. The following table indicates the different products that were provided as prophylactic supportive care:

Table 19: Prophylactic Supportive care

Text Table #5 Prophylactic Supportive Care		
Drug Class	**Allowed Medication or Supportive Care Agents**	**Indication and/or Criterion for Administration**
Analgesics	Buprenorphine 0.01 mg/kg/dose BID, SC from Day 3 to Day 30	Pain Management:
Anti-ulcer	Sucralfate 1g/day, (0.5 g BID) daily from days 5-30	For treatment of possible ulcers of the stomach or proximal small intestine.
Antibiotics	Enrofloxacin (Baytril®) 10 ± 0.5 mg/kg, subcutaneous, SC, SID from Day 5 to Day 27	Prophylactic treatment of antibiotics during predicted period of neutropenia.
Anti-emetics	Ondansetron (1 mg/kg) IM 30-90 minutes prior to irradiation and 30-45 minutes following irradiation to suppress emesis.	Administered pre and post radiation to suppress emesis.

A supportive care with analgesics, parenteral fluids, anti-emetics and nutritional support was also permitted, based on clinical judgment of the veterinarian.

- **Objectives**

The primary objective of TSK0144 was to demonstrate the superior benefit of sargramostim as measured by mortality rate at 60 days (MR60) versus control vehicle rhesus monkeys after irradiation at $LD_{50\text{-}60/60}$; the statistical hypothesis for primary endpoint was set on a superiority assumption. The survival benefit and efficacy of sargramostim on haematological parameters were also explored in separate cohorts of irradiated rhesus monkeys at a $LD_{70\text{-}80/60}$ dose (713 cGy).

This trial does not contemplate key secondary endpoints; secondary and exploratory endpoints investigate the role of sargramostim in terms of haematological recovery (ANC and platelet count), overall survival, incidence of infection and H-ARS related clinical signs.

- **Outcomes/endpoints**

Primary endpoint: to demonstrate the superior benefit of sargramostim as measured by mortality rate at 60 days (MR60) versus control vehicle in irradiated rhesus monkeys at $LD_{50\text{-}60/60}$.

- **Sample size**

Main cohort $LD_{50\text{-}60/60}$

The sample size of 36 rhesus monkeys per group in the LD50-60/60 part of the trial, provides 90% power at a 1- sided alpha level of 5% to **demonstrate** a mortality rate at Day 60 of 25% in the sargramostim arm and 60% in the vehicle arm with a one-sided type one error of 5%.

Exploratory cohort $LD_{70\text{-}80/60}$

The sample size of 18 rhesus monkeys per group in this cohort provides approximately 75% power at a 1-sided alpha level of 10% to demonstrate a mortality rate at Day 60 of 25% in the sargramostim arm and 60% in the vehicle arm with a one-sided type one error of 10%.

For both cohorts, the mortality rate at Day 60 in the control arm was selected based on available historical data (e.g. Farese AM. et al. Radiation Research 2013; 179, 89-100) as well as previous sargramostim studies.

- **Randomisation and blinding (masking)**

The blinding procedure followed research laboratory SOPs, ensuring personnel were blinded to experimental groups, except for those involved in test item preparation, dosing formulation analysis, the study team leader, and the Unblinded Supporting Scientist. To prevent bias during subjective observations and data collection, memos documented unblinded animal IDs and dose formulation preparations.

Amendment 3 clarified the requirements for unblinding the study:

- Finalization of the statistical analysis plan (SAP)
- Recording of findings by the study pathologist from the initial blinded assessment of tissue slides
- Completion of the final data review, except for the peer review of pathology measures, with all critical queries resolved

Once these criteria were met, the Sponsor would request via email that the Study Director unblind the study.

- **Statistical methods**

The analysis set of TSK0144 is represented by 108 animals which was divided into two cohorts or

groups, based on the administered total irradiation dose; the $LD5_{0\text{-}60/60}$ and $LD_{70\text{-}80/60}$ dose was analyzed separately. All analyses will be performed using the intent-to-treat (ITT) population; the ITT population will include all randomized with TBI.

The primary endpoint of MR60 in $LD_{50\text{-}60/60}$ was defined as proportion of animals that died prior to the D60 scheduled euthanasia. MR60 will be summarized with descriptive statistics. 95% confidence interval will also be provided.

MR60 will be compared between the two treatment groups using a one-sided Fisher exact test at the 5% level. The sample size of 36 rhesus monkeys per group in the LD50-60/60 part of the trial, provides 90% power at a 1- sided alpha level of 5% to demonstrate a mortality rate at Day 60 of 25% in the sargramostim arm and 60% in the vehicle arm with a one-sided type one error of 5%.

The evaluation of $MR_{70\text{-}80/60}$ has been analyzed separately as exploratory endpoint; for the primary endpoint of mortality, a pre-specified statistical analysis plan was contemplated, while for the evaluation of the secondary endpoints in that cohort, results were reported as descriptive. The sample size of 18 rhesus monkeys per group in this cohort provides approximately 75% power at a 1-sided alpha level of 10% to demonstrate a mortality rate at Day 60 of 25% in the sargramostim arm and 60% in the vehicle arm with a one-sided type one error of 10%.

Results

Participant flow

A total of 108 animals (54 male and 54 female) were included in this trial; 72 were irradiated at $LD_{50\text{-}60/60,}$ while 36 with $LD_{70\text{-}80/60}$, according to the SAP of the protocol. No exclusion of previously selected animals was reported, as well as early or late discontinuation during the short follow up period of the protocol.

Animal replacement was required in 5 cases, for the presence of health issues that were considered able to have an impact on the results; in all cases all replacement happened before the irradiation. Spare animals were from the same shipment of animals and maintained under the same environmental conditions. Following the end of the replacement period, the spare animals were released from the study.

Recruitment

The study initiation date was October 29, 2015 (date of signature of the study plan by the Study Director) and the experimental start date was October 30, 2015 (date of animal transfer).

Animals were randomly assigned to the study on October 30, 2015. Irradiation (Day 0) was performed on November 24, 25 and 26, 2015 (for replicates A, B and C) and on December 1, 2 and 3 for replicates D, E and F).

Dosing was initiated (Day 2) on November 26, 27 and 28, 2015 (for replicates A, B and C) and on December 3, 4 and 5, 2015 (for replicates D, E and F). The last necropsy was performed on February 01, 2016.

The experimental completion date was June 13, 2017 with the signature of the Pathology Report; given the nature of the protocol, no follow-up was contemplated after the end of the observation phase (day 60).

Conduct of the study

Three amendments were implemented during the conduct of this study; no substantial changes able to affect the integrity of the trial were introduced.

Inspections were conducted in accordance with the requirements of the Principles of Good Laboratory Practice: a total of 58 inspections were conducted during the conduction of the study but none of them resulted in critical outcomes able to affect the integrity of the trial.

No changes in the statistical analysis plan were applied and its integrity was maintained until the end of the protocol. Occasional procedural deviations were reported but they were considered minor and not able to impact the integrity or outcome of the study.

Baseline data

Each group included the same numbers of male and female animals (18 for the $LD_{50\text{-}60/60}$ and 9 for the $LD_{70\text{-}80/60}$): at the onset of dosing, the age of the animals ranged from 3 years 1 month to 5 years 4 months; the body weights ranged from 2.9 to 6.2 kg and no substantial differences were reported in terms of basal characteristics of the enrolled animals. In order to ensure the unique identification process, each animal was uniquely identified by means of a tattoo.

Numbers analysed

All animals were eligible for the evaluation; five animals were replaced before irradiation according to a specific procedure reported in the protocol.

Therefore, for the primary endpoint, 36/36 animals (18/18 in sargramostim and 18/18 in vehicle groups) were considered suitable for the Intention To Treat analysis; in the exploratory cohort, 18 animals (9/9 in sargramostim and 9/9 in vehicle).

Outcomes and estimation

Primary endpoint

Analysis description	**Primary Analysis**		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Day 60 post-TBI		
Descriptive statistics and estimate variability	Treatment group	655 cGy Reference item/vehicle	655 cGy sargramostim
	Number of subjects	36	36
	Mortality Rate at Day 60 - $LD_{50\text{-}60/60}$ dose (proportion of animals that died prior to Day 60 scheduled euthanasia [No. of decedents/No. in group])	58% (21/36)	22% (8/36)
	Variability statistic	Not reported	Not reported
Effect estimate per comparison	Mortality Rate at Day 60 - $LD_{50\text{-}60/60}$ dose	Comparison groups	655 cGy reference item/vehicle vs. 655 cGy sargramostim

		Differences between groups in Mortality Rate at Day 60	36%
		95% Confidence Interval	15, 57%
		Fisher's exact test one-sided p-value	P=0.0018
Notes	The study was unblinded when the following requirements were met: • The statistical analysis plan (SAP) was finalised. • The study pathologist recorded their findings from the initial blinded assessment of tissue slides. • The final data review was completed, with the exception of the peer review of the pathology measures, and all critical queries were resolved. • The table with the Most Probable Cause of Death for each preterminally fated animal has been prepared and signed by the Study Director. The analysis of the primary endpoint showed a significantly lower mortality rate at day 60 for the sargramostim treated group compared to the reference item/vehicle group (22% versus 58%).		

Secondary endpoints

Analysis description	**Secondary Analysis - Overall Survival ($LD_{50-60/60}$ dose)** Secondary analysis was pre-specified in the Statistical Analysis Plan (SAP)		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Day 15, 30, 45, and 60 post-TBI		
Descriptive statistics and estimate variability	Treatment group	655 cGy Reference item/vehicle	655 cGy sargramostim
	Number of subjects	36	36
	Overall Survival - Day 15 (survival probability)	0.89	0.92
	95% Confidence Interval	0.73, 0.96	0.76, 0.97
	Overall Survival - Day 30 (survival probability	0.42	0.83
	95% Confidence Interval	0.26, 0.57	0.67, 0.92
	Overall Survival - Day 45 (survival probability)	0.42	0.81
	95% Confidence Interval	0.26, 0.57	0.63, 0.90
Effect estimate per comparison	Overall Survival - $LD_{50-60/60}$ dose	Comparison groups	655 cGy reference item/vehicle vs. 655 cGy sargramostim
		Kaplan-Meier median (days) - estimate of survival rates by treatment over time	20 days vs. Not Estimable (NE) in the 655 cGy reference item/vehicle versus sargramostim group
		Variability statistic	Not reported

		Log-Rank Test p-value (Time-to-Death Comparison)	P=0.0023
Notes	Survival analysis indicated a significant difference in the two survival curves in favour of the sargramostim group. The sargramostim treated group had a hazard ratio of 0.31 (95% CI: 0.14, 0.70).		
Analysis description	**Secondary Analysis - Neutrophil Related Parameters ($LD_{50-60/60}$ dose)** Secondary analysis was pre-specified in the Statistical Analysis Plan (SAP)		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Twice prior to irradiation and days 1 to 30, 35, 40, 45, 50, 55, and 60 after TBI and prior to unscheduled euthanasia, when possible.		
Descriptive statistics and estimate variability	Treatment group	655 cGy Reference item/Vehicle	655 cGy sargramostim
	Number of subjects	36	36
	ANC nadir (cells/µL) (mean)	20.3	34.4
	Standard Error (±SE)	2.8	6.0
	Duration (days) ANC < 0.5 x 10^9/L (mean)	11.5	9.9
	± SE (range)	1.0 (2-17)	0.76 (2-16)
	Duration (days) ANC < 0.1 x 10^9/L (mean)	5.9	4.7
	± SE (range)	0.61 (2-11)	0.42 (2-12)
	Time (days) to ANC recovery ≥ 0.5 x 10^9/L (median)	19	17
	95% Confidence Interval	18, 20	16, 18
	Time (days) to ANC recovery ≥ 1 x 10^9/L (median)	20	18
	95% Confidence Interval	19, 20	17, 18
Effect estimate per comparison	Time (days) to ANC recovery ≥ 0.5 x10^9/L	Comparison groups	655 cGy reference item/vehicle vs. 655 cGy sargramostim
		Kaplan-Meier median time to ANC recovery ≥ 500/mL (comparison of time to ANC recovery between groups)	19 days versus 17 days, 655 cGy reference item/vehicle versus sargramostim group, respectively.
		95% Confidence Interval	18, 20 and 16, 18, 655 cGy reference item/vehicle versus sargramostim group, respectively.
		Log-Rank Test p-value	P<0.0001
	Time (days) to ANC recovery ≥ 1 x 10^9/L	Comparison groups	655 cGy reference item/vehicle vs. 655 cGy sargramostim
		Kaplan-Meier median time to ANC recovery ≥ 1000/mL (comparison of time to ANC recovery between groups)	20 days versus 18 days, 655 cGy reference item/vehicle versus sargramostim group, respectively.

		95% Confidence Interval	19, 20 and 17, 18, 655 cGy reference item/vehicle versus sargramostim group, respectively.
		Log-Rank Test p-value	P=0.0001
Notes	The duration of neutropenia (grade 3 and grade 4) was less among animals in the sargramostim treated group. Analysis of time to recovery to ANC ≥ 500/µL and ANC ≥ 1000/mL indicated a significant difference in the two recovery curves in favour of the sargramostim group. Durations do not include data from decedent animals unless recovery occurred prior to death, nor from animals that did not develop neutropenia.		
Analysis description	**Secondary Analysis - Febrile Neutropenia ($LD_{50-60/60}$ dose)** Secondary analysis was pre-specified in the Statistical Analysis Plan (SAP)		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Twice prior to irradiation and days 1 to 30, 35, 40, 45, 50, 55, and 60 after TBI and prior to unscheduled euthanasia, when possible.		
Descriptive statistics and estimate variability	Treatment group	655 cGy Reference item/vehicle	655 cGy sargramostim
	Number of subjects	36	36
	Incidence of Febrile Neutropenia (proportion of animals)	3% (1/36)	22% (8/36)
	Variability statistic	N/A	N/A
	Duration (days) of Febrile Neutropenia (mean)	1	1
	± SE (range)	None	0 (1-1)
Effect estimate per comparison	Incidence of febrile neutropenia	Comparison groups	Not reported
		Test statistic	Not reported
		Variability statistic	Not reported
		P-value	Not reported
Notes	The incidence of febrile neutropenia was higher in the sargramostim group.		
Analysis description	**Secondary Analysis - Platelet Related Parameters ($LD_{50-60/60}$ dose)** Secondary analysis was pre-specified in the Statistical Analysis Plan (SAP)		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Twice prior to irradiation and days 1 to 30, 35, 40, 45, 50, 55, and 60 after TBI and prior to unscheduled euthanasia, when possible.		
Descriptive statistics and estimate variability	Treatment group	655 cGy Reference item/vehicle	655 cGy sargramostim
	Number of subjects	36	36
	Platelet nadir (cells/µL) (mean)	6,944.4	11,805.6
	± SE	1,326.7	1,821.7
	Duration (days) of severe thrombocytopenia	6.0	4.8
	± SE (range)	0.62 (2-12)	0.46 (2-11)
	Time (days) to thrombocytopenia recovery (median)	18	16

	95% Confidence Interval	18, NE	NE, NE
Effect estimate per comparison	Time (days) to thrombocytopenia recovery	Comparison groups	655 cGy reference item/vehicle vs. 655 cGy sargramostim
		Kaplan-Meier median time to thrombocytopenia recovery	18 days vs. 16 days for the 655 cGy reference item/vehicle vs. 655 cGy-sargramostim groups, respectively
		95% confidence interval	18, NE and NE, NE for the 655 cGy reference item/vehicle vs. 655 cGy sargramostim groups, respectively.
		Log-Rank Test p-value	P=0.0008
Notes	The duration of thrombocytopenia was less among animals in the sargramostim treated group. Analysis of time to thrombocytopenia recovery indicated a significant difference in the two recovery curves in favour of the sargramostim group. **Note that durations do not include data from decedent animals unless recovery occurred prior to death, nor from animals that did not develop thrombocytopenia.		
Analysis Description	**Secondary Analysis - Incidence of Infection ($LD_{50-60/60}$ dose)** Secondary analysis was pre-specified in the Statistical Analysis Plan (SAP)		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Time of scheduled or unscheduled euthanasia; Blood culture when febrile neutropenia was identified.		
Descriptive statistics and estimate variability	Treatment group	655 cGy Reference item/vehicle	655 cGy sargramostim
	Number of subjects	36	36
	Incidence of infection-Presence of bacteria (percent of positive samples) (samples positive for bacteria/bacteriology samples taken)	63% (197/312)	32% (89/277)
	95% Confidence Interval	58-69%	27-38%
	Incidence of infection-blood haemoculture (Incidence of positive results)	45% (15/33)	18% (6/33)
Effect estimate per comparison	Incidence of Infection	Comparison groups	655 cGy reference item/vehicle vs. 655 cGy sargramostim
		Difference between groups	31% (63% reference item/vehicle - 32% sargramostim = 31%)
		Variability statistic	Not reported
		P-value	Not reported
Notes	Bacteriology results indicated that incidence of infection was less among sargramostim treated animals. Blood haemoculture results indicated a higher percentage of positive findings in the reference item/vehicle animals.		

Exploratory endpoint

Analysis Description	**Exploratory in animals exposed to a $LD_{70-80/60}$**

Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Day 60 post-TBI		
Descriptive statistics and estimate variability	Treatment group	713 cGy Reference Item/Vehicle	713 cGy sargramostim
	Number of subjects	18	18
	Mortality Rate at Day 60 - $LD_{70-80/60}$ (proportion of animals that died prior to Day 60 scheduled euthanasia)	83% (15/18)	39% (7/18)
	Variability statistic	Not reported	Not reported
	Overall Survival - $LD_{70-80/60}$ (Day 15)	0.56	0.83
	95% Confidence Interval	0.31, 0.75	0.57, 0.94
	Overall Survival - $LD_{70-80/60}$ (Day 30)	0.17	0.67
	95% Confidence Interval	0.04, 0.37	0.40, 0.83
	Overall Survival - $LD_{70-80/60}$ (Day 45)	0.17	0.61
	95% Confidence Interval	0.04, 0.37	0.35, 0.79
Effect estimate per comparison	Mortality Rate at Day 60 - $LD_{70-80/60}$	Comparison groups	713 cGy reference item/vehicle vs. 713 cGy sargramostim
		Differences between groups in Mortality Rate at Day 60	44%
		95% Confidence Interval	16, 73%
		Fisher's exact test one-sided p-value	P=0.0076
	Overall Survival - $LD_{70-80/60}$	Comparison groups	713 cGy reference item/vehicle vs. 713 cGy sargramostim
		Kaplan-Meier median	16 days vs. NE for the 713 cGy reference item/vehicle group versus the 713 cGy sargramostim group, respectively)
		95% Confidence Interval	Not reported
		Log-rank test	P=0.0036
Notes	The analysis of the primary endpoint showed a significantly lower mortality rate at day 60 for the sargramostim treated group compared to the reference item/vehicle group (39% versus 83%). Survival analysis indicated a significant difference in the two survival curves in favour of the sargramostim group. The sargramostim treated group had a hazard ratio of 0.29 (95% CI: 0.12, 0.73).		

Clinical observation (regardless of radiation dose)

At a higher dose of radiation, no substantial difference in the overall incidence of ARS related clinical signs was observed during the period of highest mortality rate (days 15- 20).

The descriptive analysis of incidence of signs/symptoms of interest showed at a radiation dose od DL_{50-}

$_{60/60}$ a higher incidence > 10% in the control group for weakness, skin turgor, tremors, petechiae and changes in stool consistency, while the opposite was noted for the incidence of skin wounds. At higher radiation dose, the differences became less apparent, with a difference in incidence higher that 10% in the control group for weakness, petechiae, while in the sargramostim group could be noted for skin wounds and turgor, buccal ulceration, alteration in stool consistency.

Effect on the other haematological parameters

No statistically relevant effects were noted on RBC/ retyculocytes and lymphocytes.

Immunogenicity

No drug-induced antibodies were detected in this study.

Study 1017-3493 (Time-to-treat AWC efficacy study)

This was a GLP-compliant randomised, blinded, placebo-controlled time-to-treat study to assess the efficacy of sargramostim versus the reference item/vehicle at different timepoints of 48 hours, 72 hours, 96 hours, or 120 hours post-total body irradiation (TBI) at $LD_{70\text{-}80/60}$.

Methods

Study participants

A total of 308 healthy animals were enrolled in this study; 220 were included in the main cohort, while 88 in the exploratory arm with azithromycin. In the main cohort, 6 subgroups of 44 animals (22 males and 22 females) were contemplated: 1 subgroup were treated with vehicle, while in the other 5 subgroups animals were differentiated basing on the time of administration of sargramostim.

Given the nature of the study, no specific inclusion or exclusion criteria were provided, except for healthy, adult rhesus monkeys with an adequate body size (4.0 to 6.0 kg) to receive TBI and tolerate the sequential, invasive monitoring techniques used to assess hematopoietic and immune reconstitution.

Treatments

Sargramostim and reference item/vehicle were administered daily by subcutaneous injection between the scapulas using a needle attached to a syringe. The first treatment injection was be performed 48h, 72h, 96h or 120h ± 1 hour post-end of irradiation, basing on the subgroups; the same time as the first treatment injection (± 3 hours).

The test item and reference item/vehicle were administered subcutaneously daily until the preliminary absolute neutrophil count (ANC) returned to ≥ 1 x 10^9/L post-nadir for 3 consecutive days. Treatment was stopped at any time if the preliminary ANC is ≥ 10^9/L; terminal necropsies were on Day 60.

For groups with treatments starting later than 48 hours after irradiation (Groups 3, 4 and 5), the reference item/vehicle was administered on days before the start of test item administration to have the same level of handling procedures in all groups.

The dose volume was 0.2 mL/kg for all animals. The actual volume administered to each animal was calculated and adjusted based on the most recent body weight of each Animal.

The azithromycin was administered from Day 8 to Day 21 (inclusive) by oral gavage using a gavage tube attached to a syringe.

Herein is reported the preparation of dosing formulations:

- Reference Item/Vehicle: Sterile water for injection, USP (SWFI) was used as provided by the manufacturer.

- Test Item/sargramostim: sargramostim (250 µg/vial) was reconstituted fresh on each dosing occasion with SWFI to obtain a concentration of 250 µg/mL. The solution was gently swirled at room temperature until complete dissolution of the test item was verified and was then placed on wet ice pending use.

sargramostim dosing formulations were prepared daily prior to each dosing occasion and were used for Groups 2, 3, 4, 5 and 7. On the day of injection, dilutions of the stock solution were prepared. The sargramostim dosing formulation was mixed gently by pipetting up and down at least 5 times using a sterile pipette tip with filter. The final volume of these solutions was based on daily requirement and were kept on wet ice pending dosing.

A supportive care with analgesics, parenteral fluids, anti-emetics, wound disinfection and nutritional support was also permitted, based on clinical judgment of the veterinarian.

Objectives

The main objective of trial 1017-3493 was to assess the efficacy of sargramostim versus the reference item/vehicle at different timepoints of 48 hours, 72 hours, 96 hours, or 120 hours post-total body irradiation (TBI). Efficacy was assessed by mortality rate at Day 60 in irradiated rhesus monkeys at an $LD_{70\text{-}80/60}$ dose (713 cGy) with minimal supportive care. The hypothesis was set on a superiority assumption.

Secondary objectives were to evaluate the efficacy of sargramostim on overall survival, haematology parameters and incidence of infection.

An exploratory objective was to assess the effect of administering azithromycin prophylactically in addition to the minimal supportive care regimen as assessed by mortality rate at Day 60 in irradiated rhesus monkeys at an $LD_{70\text{-}80/60}$ dose with minimal supportive care.

Outcomes/endpoints

The primary endpoint was to evaluate the superiority of of sargramostim versus reference item/vehicle when administered 48h, 72h, 96h, 120h post-irradiation as assessed by mortality rate at Day 60 in irradiated rhesus monkeys at an LD70-80/60 dose with minimal supportive care. The statistical hypothesis was set on a superiority assumption.

The secondary endpoints of this trial are reported below:

- Overall survival time of decedents

- Neutrophil-related parameters: ANC nadir [lowest post-TBI ANC value], duration of severe neutropenia [time from first post-TBI ANC $<0.5 \times 10^9$/L and $<0.1 \times 10^9$/L to first post-nadir ANC $\geq 0.5 \times 10^9$/L and $\geq 0.1 \times 10^9$/L, respectively], Time to ANC recovery [Time from TBI to first post-nadir ANC $\geq 0.5 \times 10^9$/L and $\geq 1 \times 10^9$/L, respectively], incidence and duration of Febrile Neutropenia [ANC $<0.5 \times 10^9$/L, core body temperature ≥103°F concurrently].

- Platelet-related parameters: Platelet nadir [lowest post-TBI platelet value], duration of severe

thrombocytopenia [time from first post-TBI platelet count <20 x 10^9/L to first post-nadir platelet count ≥ 20 x 10^9/L], time to thrombocytopenia recovery [Time from TBI to first post-nadir platelet count ≥20 x 10^9/L].

- Incidence of infection: Number and percent of animals with post-TBI infection (i.e., positive blood cultures or tissue or evidence of sepsis at necropsy).
- Incidence of ARS related clinical signs (change in body weights, diarrhoea, change in activity, hunched back posture, stool consistency, emesis, hemorrhage, respiration, alopecia)
- Presence of antibodies to sargramostim
- Haemoculture
- Gross necropsy observations
- Microbiological analysis of select organs (i.e., organ site, severity, organism)
- Histopathological examination of select organs
- Other hematologic parameters (white blood cell, red blood cell, haemoglobin, haematocrit, mean corpuscular haemoglobin, mean corpuscular volume, haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, haemoglobin distribution width, platelet, mean platelet volume, platelet distribution width, plateletcrit/thrombocrit, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells and reticulocytes)

Exploratory objective

An exploratory objective was proposed with the aim of assess the effect of administering azithromycin prophylactically in addition to the minimal supportive care regimen as assessed by mortality rate at Day 60 in irradiated rhesus monkeys at an $LD_{70\text{-}80/60}$ dose with minimal supportive care.

Sample size

Using 44 rhesus animals per group, the trial will provide 80% power at a 2-sided alpha level of 5% to demonstrate a mortality rate at Day 60 of 40% in the sargramostim arm and 70% in the reference item/vehicle arm using a Log-Rank test, and will provide 80% power at a 1-sided alpha level of 2.5% to demonstrate a mortality rate at Day 60 of 50% in the sargramostim arm and 80% in the reference item/vehicle arm using a Fisher Exact test. The number of animals used on the study was approved by IACUC.

Randomisation and blinding (masking)

Animal assignment occurred during the acclimation period of cohorts 1 to 4 and 5 to 6, separately. Male and female animals have been separately assigned to dose groups by a randomized stratification system based on body weights and the animals' providers (Kunming Biomed International LTD and Yunnan Yinmore Bio-tech Co China), as applicable.

The Blinding Procedure was conducted in accordance to Citoxlab North America internal procedures. Personnel conducting the study will be blinded to experimental groups with the exception of staff involved with test item preparation/analysis of the dosing formulations, the study team leader and the Unblinded Supporting Scientist. After randomization, the Unblinded Scientist will verify that the proportion of animals from each of the two suppliers is comparable in each group. Randomization may have been repeated if the proportion of animals from each of the two suppliers is considered different between groups.

Herein is reported the classification of the staff basing on their level of blindness:

- Unblinded: Team Leaders (will prepare the dosing and blood collections worksheets), Unblinded Supporting Scientist (will review all documents which will include unblinded information (e.g. dosing, blood collections and dosing formulations worksheets) and Pharmacy Staff (will prepare the dosing formulations)
- Partially blinded: Analytical and Immunology Staff
- Blinded: All other personnel

Statistical methods

The statistical comparisons will be performed in two stages: the first stage will include only groups 1 to 5 to assess the effect of the first dosing time after irradiation; the second stage will include only groups 1, 2, 6 and 7 to assess the effect of adding the azithromycin.

The sample size calculation of each group was done according the statistical assumption that, using 44 rhesus animals per group, the trial will provide 80% power at a 2-sided alpha level of 5% to demonstrate a mortality rate at Day 60 of 40% in the sargramostim arm and 70% in the reference item/vehicle arm using a Log-Rank test, and will provide 80% power at a 1-sided alpha level of 2.5% to demonstrate a mortality rate at Day 60 of 50% in the sargramostim arm and 80% in the reference item/vehicle arm using a Fisher Exact test.

Stage 1 (including groups 1 to 5)

The group comparisons of each incidence parameter will be done using a one factor generalized linear mixed model with a binomial distribution and a logit link function (logistic regression model). If the overall group effect is significant, each test item treated group will be compared to the reference item/vehicle Group 1 using t-test on least-squares means.

The group comparison of each time related parameter will be done using a log-rank test (survival analysis). Animals without event at Day 60 will be censored in the analysis. If the overall group effect is significant, each test item treated group will be compared to the reference item/vehicle Group 1 using independent log-rank tests. The group comparison of each numerical parameter will be done by occasion using the following one-way analysis method and will not include groups with less than three observations or with zero variance.

For each data set with more than two groups to compare, a one-way analysis of variance (ANOVA) will be performed and the residuals will be tested for normality using a Shapiro- Wilk test.

When the Shapiro-Wilk test is not significant ($p > 0.05$) a Levene test will be performed on the residuals to assess the homogeneity of the group variances. If differences between group variances are not found to be significant ($p > 0.05$) then the results from the related ANOVA will be retained. When significant differences among the group means are indicated by the ANOVA overall F-test ($p \leq 0.05$), a Dunnett test will be used to perform the group mean comparisons between the reference item/vehicle treated Group 1 and each test item treated group. When the Shapiro-Wilk test on the residuals of the second ANOVA is significant or when heterogeneous group variances ($p \leq 0.05$) are revealed by the Levene test, then the ANOVA results will be discarded and the groups will be compared using a non-parametric Kruskal- Wallis test. When the Kruskal-Wallis test is significant ($p \leq 0.05$), a Dunn test will be used to perform the pairwise group comparisons between the reference item/vehicle treated Group 1 and each test item treated group.

For datasets with only two groups to compare (including the reference item/vehicle Group 1), a

Shapiro- Wilk test and a Levene test will be performed as described above but a two sample t-test will replace the one-way ANOVA F-test, and a Wilcoxon rank-sum test will replace the Kruskal-Wallis test, while Dunnett and Dunn tests will not be performed.

Stage 2 (including groups 1, 2, 6 and 7)

The group comparisons of each incidence parameter will be done using a two-way generalized linear mixed model based on a binomial distribution and a logit link function (logistic regression model). The fixed effects in the model were the dose level (0 and 7 µg/kg/day), the azithromycin (presence and absence) and their interaction.

If the interaction dose*azithromycin is not significant ($p > 0.05$), it will indicate that the addition of does not significantly interact with the dose level effect and vice versa. Therefore, the dose level effect will be assessed pooled across presence/absence of azithromycin and the azithromycin effect will be assessed pooled across dose levels using t-tests on least-squares means. If the interaction dose*azithromycin is significant ($p \leq 0.05$), it will indicate that the dose level effect is significantly influenced by the addition of azithromycin and vice versa. Therefore, the dose level effect will be assessed for each presence-absence of azithromycin and the azithromycin effect will be assessed for each dose level using the t-tests on least-squares means.

The group comparison of each time related parameter will be done using a Cox proportional hazards model including the dose level (0 and 7 µg/kg/day), the azithromycin (presence and absence) and their interaction as fixed effect. If the interaction dose*azithromycin is not significant ($p > 0.05$), the dose level effect will be assessed pooled across presence/absence of azithromycin and the azithromycin effect will be assessed pooled across dose levels using Wald Chi-square test for main effects. If the interaction dose*azithromycin is significant ($p \leq 0.05$), the dose level effect will be assessed for each presence-absence of azithromycin and the azithromycin effect will be assessed for each dose level using Wald Chi-square test with contrasts.

The group comparison of each numerical parameter will be done by occasion using two-way analysis of variance (ANOVA) model based on a normal distribution. The fixed effects in the model will be the dose level (0 and 7 µg/kg/day), the azithromycin (presence and absence) and their interaction. If the interaction dose*azithromycin is not significant ($p > 0.05$), the dose level effect will be assessed pooled across presence/absence of azithromycin and the azithromycin effect will be assessed pooled across dose levels using t-tests on least-squares means. If the interaction dose*azithromycin is significant ($p \leq 0.05$), the dose level effect will be assessed for each presence-absence of azithromycin and the azithromycin effect will be assessed for each dose level using the t-tests on least-squares means.

No subgroup analysis nor interim analyses were planned.

No interim or pre-specified subgroups analyses were planned. Methods for multiplicity control are not reported in the trial.

Results

Participant flow

A total of 308 healthy animals were enrolled in this study; 220 were included in the main cohort, while 88 in the exploratory arm with azithromycin. In the main cohort, 6 subgroups of 44 animals (22 males and 22 females) were contemplated: 1 subgroup were treated with vehicle, while in the other 5 subgroups animals were differentiated basing on the time of administration of sargramostim

Prior to or following irradiation, 12 animals were replaced (by spare animals from the same suppliers, maintained under the same environmental conditions) or reassigned between replicates. Only the data of the replacement animals were reported.

Of them, 10/12 were replaced before irradiation, while in two cases animals were replaced after irradiation (1 animals found dead during irradiation and 1 for a misleading dose at day 9 after irradiation).

All survivor animals reached the day 60 and then were euthanized, including the two animals that were replaced during the study.

Recruitment

The study initiation date (date of signature of the study plan) and the experimental start date (date of the first animal transfer) was February 19, 2018. Animals were randomly assigned to the study on March 1 and 2, 2018 (for replicates A to D) and March 28, 2018 (for replicates E and F). Irradiation (Day -1) was performed on March 12 to 14, March 20 to 22, March 28 to 30, April 5 to 7, April 13 to 15 and April 21 to 23, 2018 (for replicates A to F). Dosing (Day 2) was initiated on March 14 to 16, March 22 to 24, March 30 to April 1, April 7 to 9, April 15 to 17 and April 23 to 25, 2018 (for replicates A to F). The last necropsy was performed on June 22, 2018. The experimental completion date was August 24, 2018. Given the nature of the protocol, no follow-up was contemplated after the end of the observation phase (day 60).

Conduct of the study

Seven amendments were approved during the 1017-3493 protocol; no changes in the numerosity or in the primary endpoint were introduced, but the Amendment n7 introduce a clarification about the comparison with group 1 (vehicle group) and stated that, for survival analysis, each test item treated group will be compared to the reference item/vehicle Group 1 using independent log-rank tests regardless of the significance of the overall group effect. This amendment does not elucidate the absence of multiplicity control.

None of them are considered to affect the integrity of the SAP.

Baseline data

Three hundred and eight (308) Rhesus monkeys (154 males, 154 females), including 15 spare animals/sex, were transferred on the study on February 19 and March 19, 2018.

At the onset of dosing, the age of the animals ranged from 2.7 to 5.8 years. The body weights ranged from 3.1 to 7.0 kg and from 2.9 to 6.6 kg for males and females, respectively. Although the weight and/or age of some animals were slightly outside the range indicated in the study plan, all animals were judged suitable for the study as the magnitude of the deviation was small. All animals underwent medical screening for exclude the presence of concomitant diseases that could have an impact on the survival rates. For guarantee the chain of identity of animals, they were individually identified by means of a tattoo.

Numbers analysed

All animals were eligible for the evaluation. Twelve animals were replaced before irradiation according to a specific procedure reported in the protocol: 10/12 were replaced with spare animals before irradiation, while in two cases animals were replaced after irradiation (1 animals found dead during irradiation and 1 for a misleading dose at day 9 after irradiation).

For stage 1 analysis (comparison between groups 1-5), 250 animals were suitable for the planned

analysis, while 176 were included in stage 2 (comparison 1-2 and 6-7).

Outcomes and estimation

Primary endpoint

Stage 1: groups 1 to 5

Herein MR60 were reported for each group:

Group 1 (reference item/vehicle): 86%

Group 2 (sargramostim 48h): 68%, difference (in respect to Group 1) of -18%

Group 3 (sargramostim 72h): 75%, difference (in respect to Group 1) of -11%

Group 4 (sargramostim 96h): 68%, difference (in respect to Group 1) of -18%

Group 5 (sargramostim 120h): 84%, difference (in respect to Group 1) of –2%.

A trend in a better survival was showed when sargramostim was administered from 48 to 96 hours post radiation exposure; at 120h, the MR60 was similar among the two groups.

Table 20: Summary of statistical Comparisons using log-rank test

SURVIVAL
STAGE 1
Time to Mortality

Sex	Last Study Day	Considered Groups	Raw *p*-Value
Males	60	1,2,3,4,5	0.4275
		1,2	0.1562
		1,3	0.9377
		1,4	0.3135
		1,5	0.9592
Females	60	1,2,3,4,5	0.6389
		1,2	0.8120
		1,3	0.2699
		1,4	0.2656
		1,5	0.9138
Combined	60	1,2,3,4,5	0.4568
		1,2	0.2353
		1,3	0.4217
		1,4	0.1426
		1,5	0.9526

For Groups 1 to 5, the log-rank test for comparing the survival curves and the logistic regression model for comparing the mortality frequencies at Day 60 did not reach statistical significance.

Stage 2: groups 1,2,6 and 7

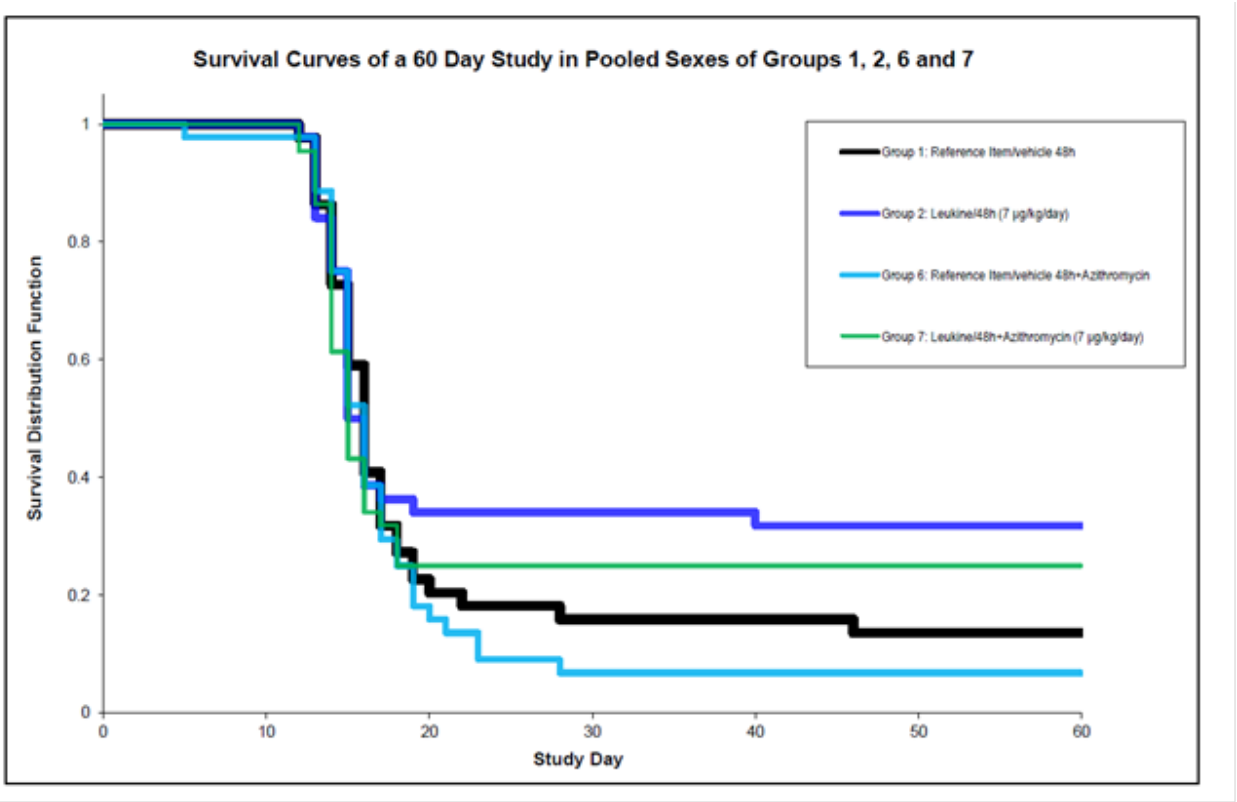
Mortality rate at day 60:

- Group 6 (reference item/vehicle + azithromycin): 93%

- Group 7 (sargramostim 48h + azithromycin): 75%, difference (in respect to Group 6) of -18%.

The comparison resulted statistically relevant in favour of sargramostim when groups 1 and 6 were compared to groups 2 and 7 for male subjects (logistic regression model p: 0.0029) and in the overall population (logistic regression model p: 0.0032).

Figure 7: Kaplan Meier curve for stage 2 analysis



As reported in the statistical analysis plan of the study, Log-Rank test was not applied to the stage 2 of the analysis; in addition for this endpoint and all following ones no statistical test result can be used since the primary endpoint has not shown statistical significance. A visual interpretation of the Kaplan-Meier plot indicate that survival is numerically higher in the sargramostim groups (2 and 7) than in the reference groups (1 and 6). Time to haematological recovery for ANC and platelets were reported in mean values.

Secondary endpoints

Analysis description	**Secondary Analysis - Neutrophil-related parameters**					
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Twice prior to irradiation and days 1-30 (inclusive), 35, 40, 45, 50, 55, and 60 after TBI and prior to unscheduled euthanasia, when possible.					
Descriptive statistics and estimate variability	Treatment group	Reference item/vehicle - 48 hours	sargramostim			
			48 hr	72 hr	96 hr	120 hr
	Number of subjects	44	44	44	44	44

	Duration (days) ANC < 0.5 x 10^9/L (mean)	13.3	10.1	11.1	10.8	11.4
	± SD	1.3	1.8	1.3	1.6	1.4
	Time (days) to ANC recovery ≥ 0.5 x 10^9/L (mean)	19.7	18.0	18.1	18.0	18.3
	± SD	1.0	1.1	0.8	0.9	1.1
	Time (days) to ANC recovery ≥ 1 x 10^9/L (mean)	21.2	18.8	18.7	18.8	19.0
	± DS	1.3	1.1	1.0	1.1	1.0

Effect estimate per comparison	Time (days) to ANC recovery ≥ 0.5 x 10^9/L (mean)	Comparison groups	Reference item/vehicle - 48 hr vs. sargramostim 48 hr vs. sargramostim 72 hr vs. sargramostim 96 hr vs. sargramostim 120 hr
		Kaplan-Meier mean time (days) to ANC recovery ≥ 500/mL	19.7 days for the reference item/vehicle group and 18.0, 18.1, 18.0 and 18.3 days for sargramostim groups at 48 hr, 72 hr, 96 hr and 120 hr respectively.
		Standard Error	± 1.0 for the reference item/vehicle group and ± 1.1, ± 0.8, ± 0.9, ± 1.1 days for sargramostim groups at 48 hr, 72 hr, 96 hr and 120 hr respectively.
		Log-Rank Test p-value	P≤0.01
	Time (days) to ANC recovery ≥ 1 x 10^9/L (mean)	Comparison groups	Reference item/vehicle - 48 hr vs. sargramostim 48 hr vs. sargramostim 72 hr vs. sargramostim 96 hr vs. sargramostim 120 hr
		Kaplan-Meier mean time (days) to ANC recovery ≥ 500/mL	21.2 days for the reference item/vehicle group and 18.8, 18.7, 18.8 and 19.0 days for sargramostim groups at 48 hr, 72 hr, 96 hr and 120 hr
		Standard Error	± 1.3 days for the reference item/vehicle group and ± 1.1, ± 1.0, ± 1.1, and ± 1.0 days for sargramostim groups at 48 hr, 72 hr, 96 hr and 120 hr
		Log-Rank Test p-value	P≤ 0.001
Notes	The Log-Rank Test for comparing the recovery curves suggested an earlier neutrophil recovery (i.e., return to ANC ≥ 0.5x109/L, ANC ≥ 1x109/µL) for all sargramostim groups.		
Analysis description	**Secondary Analysis - Platelet-related parameters**		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: Twice prior to irradiation and Days 1-30 (inclusive), 35, 40, 45, 50, 55, and 60 after TBI and prior to unscheduled euthanasia, when possible.		
	Treatment group		sargramostim

Descriptive statistics and estimate variability		Reference item/vehicle	48 hr	72 hr	96 hr	120 hr
	Number of subjects	44	44	44	44	44
	Time (days) to thrombocytopenia recovery (mean)	17.4	16.6	17.5	17.6	17.4
	± DS	2.3	1.1	1.2	1.7	0.9

Effect estimate per comparison	Time (days) to thrombocytopenia recovery (mean)	Comparison groups	Reference item/vehicle - 48 hr vs. sargramostim 48 hr vs. sargramostim 72 hr vs. sargramostim 96 hr vs. sargramostim 120 hr
		Kaplan-Meier mean time (days) to thrombocytopenia recovery	17.4 days for the reference item/vehicle group and 16.6, 17.5, 17.6 and 17.4 days for sargramostim groups at 48 hr, 72 hr, 96 hr and 120 hr, respectively.
		Standard Error	± 2.3 days for the reference item/vehicle group and ± 1.1, ± 1.2, ± 1.7, and ± 0.9 days for sargramostim groups at 48 hr, 72 hr, 96 hr and 120 hr, respectively.
		Log-Rank Test p-value	P≤0.05 (sargramostim 48 hr. vs. reference item/vehicle group)
Notes	The Log-Rank Test for comparing the recovery curves suggested an earlier platelet recovery (i.e., return to platelet count ≥ 20 x 109/L) for the sargramostim 48 hr group.		

Incidence and duration of Febrile Neutropenia

The global incidence of FN resulted slightly higher number of animals treated with Imreplys, especially at 48h (group 2: 61%, group 7: 45%) in respect to the reference/item vehicle groups (group 1: 39%, group 6: 32%). No substantial differences in terms of duration of the episodes can be noted.

Table 21: Incidence and number of days of fever (rectal temperature ≥ 39.4°C) and febrile neutropenia (fever and ANC <500/µl)

Treatment Group	Incidence of fever	Number of days of fever (range)	Incidence of febrile neutropenia	Duration of febrile neutropenia (days)
1. Reference Item/vehicle 48h	19/44	1-5	17/44 (39%)	2.6 ± 1.5
2. Leukine/48h	29/44	1-7	27/44 (61%)	3.3 ± 2.0
3. Leukine/72h	16/44	1-3	16/44 (36%)	4.3 ± 2.4
4. Leukine/96h	18/44	1-6	17/44 (39%)	2.9 ± 2.1
5. Leukine/120h	19/44	1-4	18/44 (41%)	4.2 ± 2.3
6. Reference Item/vehicle 48h +Azithromycin	16/44	1-4	14/44 (32%)	3.2 ± 1.6
7. Leukine/48h +Azithromycin	22/44	1-5	20/44 (45%)	3.7 ± 2.3

Of note, the definition of FN applied to this protocol was different from the official definition in human of febrile neutropenia (defined as a temperature of ≥38.0ºC (≥100.4ºF) sustained over 1 hour, with an absolute neutrophil count (ANC) of <0.5 x 10^9/L, or an ANC that is expected to decrease to <0.5 x 10^9/L over the next 48 hours). This different definition is based on the general laboratory veterinary practice guidelines (Footman et al, 2002) where 103.1ºF is considered to be indicative of fever in rhesus monkeys. The temperature threshold reflects the naturally higher body temperature of rhesus monkeys compared to humans (98.6 to 103.1ºF vs. 97.7-99.5ºF, respectively).

Bacteriology (Blood/Organ Cultures)

The vast majority (93% (225/242) of preterminally fated (found dead or euthanized) animals presented at least 2 positive results for the same bacterial strain at organ and blood culture, suggesting an active infection or sepsis. **The treatment with sargramostim reduced the total number of infections when administered at 48h, 72h and 96h, reaching statistical significance at 96h (p≤0.01) and at 48h when comparing pooled Groups 2 and 7 (sargramostim 48h with and without azithromycin) with pooled Groups 1 and 6 (reference item/vehicle with and without azithromycin) (p≤0.01).** This effect could be triggered by the faster recovery and/or the higher count at nadir for ANC in sargramostim-treated animals compared to reference item/vehicle-treated animals.

The number of animals with signs of sepsis was significantly lower in the group treated with sargramostim at 48h and azithromycin compared to the reference item/vehicle group with azithromycin; however, it was similar to the sargramostim group without azithromycin indicating that administration of azithromycin had no significant beneficial effects on the rate of sepsis.

Exploratory endpoints

Analysis description	**Exploratory analysis**		
Analysis population and time point description	All analysis was performed using the intent-to-treat (ITT) population. The ITT population included all animals randomised with TBI. Time point: 60-Days		
Descriptive statistics and estimate variability	Treatment group	Reference item/vehicle + azithromycin	sargramostim + azithromycin
	Number of subjects	44	44
	Mortality Rate at Day 60 (proportion of animals that died prior to Day 60 scheduled euthanasia [no. decedents/no. in group])	93% (41/44)	75% (33/44)
	Variability statistic	Not reported	Not reported
Effect estimate per comparison	Mortality Rate at Day 60	Comparison groups	Reference item/vehicle + azithromycin vs. sargramostim + azithromycin
		Differences between groups in Mortality Rate at Day 60	18% (93% reference item/vehicle + azithromycin - 75% sargramostim + azithromycin).
		Variability statistic	Not reported
		Logistic regression model p-value	Not significant
	Mortality Rate at Day 60	Comparison groups	Pooled reference item/vehicle -48 hr and reference item/vehicle + azithromycin vs. pooled sargramostim 48 hr + sargramostim + azithromycin
		Differences between groups in Mortality Rate at Day 60	18% (90% in the pooled reference item/vehicle groups vs. 72% in the pooled sargramostim 48 hr + /- azithromycin groups.
		Variability statistic	Not reported
		Logistic regression model p-value	P=0.0032
Notes	When comparing reference item/vehicle and sargramostim groups with and without azithromycin in this exploratory endpoint, sargramostim administration initiated 48 hours after TBI seemed to decrease the Mortality Rate at Day 60 (logistic regression, overall dose level effect on pooled groups with and without azithromycin, t-test p=0.0029 and p=0.0032 for males and pooled sexes, respectively).		

The addition of azithromycin had no statistically significant effect and there was no interaction between sargramostim and azithromycin.

Study FY14-045

The objective of this study was to determine treatment efficacy, specifically any survival benefit at 60 days resulting from early (beginning at day 1 or day 2 post-irradiation) administration of sargramostim following lethal total body irradiation at the $LD_{50/60}$ dose in rhesus macaques.

Methods

Study participants

Healthy male subjects weighted more than 2.5 kg and considered able to tolerate irradiation and subsequent monitoring procedures were enrolled in this trial.

Treatments

Animals received a single irradiation with the linear accelerator (LINAC) at an absorbed dose of 6.8 Gy (the institutional lethality profile LD50/60). Animals were randomized to receive:

water for injection (Water Control, Group 1)

sargramostim at 7 µg/kg/day (experimental arm, Group 2) at 24 h

sargramostim at 7 µg/kg/day (experimental arm, Group 2) at 48 h

Bacteriostatic water for injection and sargramostim were administered to animals once daily by subcutaneous injection beginning on Day 1 or Day 2 post-irradiation and continuing through Day 18 or until the most recent blood neutrophil counts increase to above 1 x 109/L. Dose volumes for water and sargramostim depended on the animal's most recent body weights and ranged from 0.08 to 0.15 mL/day.

Duration of therapy was dependent on the duration of neutropenia and continued until Day 18 or until absolute neutrophil counts (ANC) were above 1 x 109/L, whichever was earlier.

All animals received minimal supportive therapy with antibiotics, fluids and analgesics: in particular, enrofloxacin 5 mg/kg once daily by oral administration was administered from 3 days post irradiation through 30 days post irradiation.

Objectives

The primary objective of this trial was to evaluate the difference in mortality rate between irradiated monkeys treated with sargramostim administered at 24h and 48 h and animals treated with vehicle, evaluated 60 days following lethal total body irradiation at the LD50/60 dose using total body irradiation with minimal supportive care (antibiotics and fluids) in nonhuman primates (NHP, Rhesus macaques).

Outcomes/endpoints

The primary endpoint was the difference in the mortality rate between irradiated monkeys treated with sargramostim administered at 24h and 48 h and animals treated with vehicle.

No key secondary endpoints were listed; the secondary endpoints evaluated the effects of sargramostim on:

- haematology counts,

- clinical findings

- bacteriology

- necroscopic findings

The results of the secondary endpoints were reported as descriptive.

Sample size

The determination of the sample size was based on an expected difference in survival of 25% with an an alpha = 0.05 (power of 0.7); the number for each group was of 35 animals. The expected mortality rate for control was set on the expected 50% of the LD50-60/60, while the expected effect on experimental cohorts was based on the reported effects of growth factors in H-ARS.

The sample size determination was not considered for the evaluation of the secondary endpoint, so their results were considered supportive for the primary objective.

Randomisation and blinding (masking)

All animals will be identified by tattoo prior to arrival. Throughout the study, all animals will be identified by tattoos. All animals will be weighed and randomly assigned by wheight to study group by weight using a computerized data acquisition system (Provantis 8.2, Instem LSS Ltd, Staffordshire, UK).

Statistical methods

One hundred and 5 (105) male rhesus macaques of age between 2-4 years were ordered and screened for the study; animals were randomized by body weight into 3 groups, with 35 males per group. Group sizes were determined based on a power function estimating that 35 animals/group are needed to see a 25% difference in survival with an alpha = 0.05 (power of 0.7).

Data were analyzed using appropriate techniques for the type of data collected using Provantis, SAS®, or GraphPad PrismTM. Kaplan-Meier curves were used to estimate the survival function for each dose group following irradiation. Log-rank tests were used to compare the survival distributions of groups in a pairwise pattern. Additionally, the Fisher exact test was used to determine whether the post-irradiation treatment influenced survival outcomes.

In absence of pre-planned key secondary endpoints, interim and subgroup analyses, multiplicity adjustment was not required.

Results

Participant flow

A total of 105 healthy male rhesus macaques were enrolled in this study, 35 for each group; randomization was based on body weights.

No replacement of animals was reported in the study protocol.

Survivor animals at day 60 were euthanized according to the study protocol.

Recruitment

The study initiation date was April 25, 2014; the original final report date of issue: 30 March 2016, while the revision final report date of issue: 17 May 2017.

Conduct of the study

This study was conducted in accordance with U.S. FDA 21 CFR Part 58 (Good Laboratory Practices for Non-Clinical Laboratory Studies) except for the procedures listed below: analysis conducted at Zoologix and IDEXX, these procedures were conducted per their respective SOPs.

A total of 35 inspections/audits were recorded, none of them considered able to affect the quality of the protocol; three amendments were approved during the study, but none of them were considered

substantial; similarly, 2 protocol deviations were recorded but they were not considered critical for the integrity of the protocol.

Baseline data

One hundred and five (105) male rhesus macaques were ordered and screened for the study: the age of the test subjects was 2-4 years and all animals weighted more than 2.5 kg. All animals came from the same facility.

Numbers analysed

All the 105 animals (35 for each group) were eligible for the evaluation; in 1 case of the control group, an animal in water control group had a severe prolapse that could not be reduced therefore the animal was euthanized due to clinical observations and the GI characteristics associated with the model.

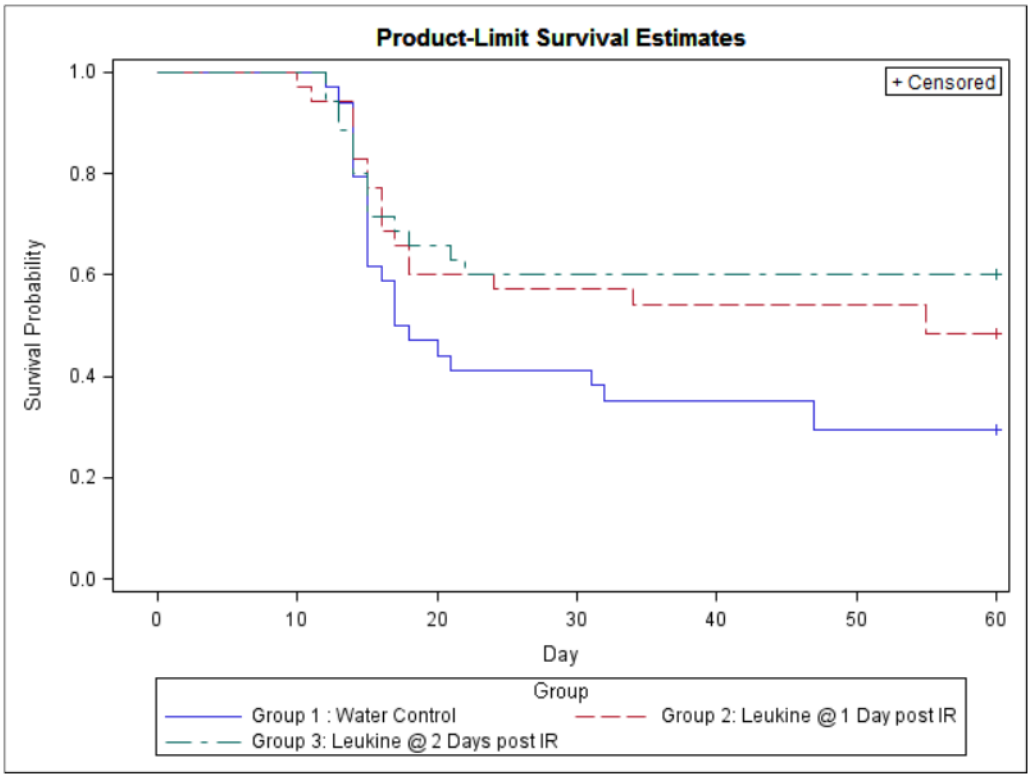
Outcomes and estimation

Primary endpoint

Results and Analysis				
Analysis description	**Primary Analysis**			
Analysis population and time point description	Per protocol: One animal in the Water Control was euthanised for humane purposes due to recurring rectal prolapse not related to TBI Timepoint: Day 60			
Descriptive statistics and estimate variability	Treatment group	Water Control	sargramostim Day 1	sargramostim Day 2
	Number of subjects	34	35	35
	60 Day Survival (proportion of animals surviving to Day 60 [no. of surviving animals/no. in group)	29.4% (10/34)	48.6% (17/35)	60% (21/35)
Effect estimate per comparison	60-Day Survival	Comparison groups	Water control vs. sargramostim Day 1	
		Difference between groups	19.2% (48.6% sargramostim - 29.4% water control = 19.2%).	
		Variability statistic	Not reported	
		Log-rank test p-value	P=0.11	
		Fischer's exact test two-sided p-value	P=0.14	
	60-Day Survival	Comparison groups	Water Control vs. sargramostim Day 2	
		Difference between groups	30.6% (60% sargramostim - 29.4% water control = 30.6%).	
		Variability statistic	Not reported	
		Log-rank test p-value	P=0.03	
		Fischer's exact test two-sided p-value	P=0.02	
	60-Day Survival	Comparison groups	sargramostim Day 1 vs. sargramostim Day 2	
		Difference between groups	11.4% (60% sargramostim day 1 - 48.6% sargramostim day 2)	
		Variability statistic	Not reported	
		Log-rank test p-value	P=0.47	
		Fischer's exact test two-sided p-value	P=0.47	
Notes	Mortality rate (70.6%) for the control group in this study was higher than the targeted rate (50%). The cause of the shift from $LD_{50/60}$ to $LD_{70/60}$ could not be determined. Both sargramostim treatment groups had improved survival compared to water control, but only the group starting treatment on Day 2 was statistically improved compared to water control.			

The Fisher exact test was used to compare outcomes between group 1 (vehicle) with group 2 (sargramostim 24h) and 3 (sargramostim 48h): group 3 was significantly different from group 1 for survival rate at (p: 0.02).

Figure 8: Product limit survival estimates



The log rank tests between Groups 1 and 3 showed a significantly different for survival distribution at a significance level (p:0.03).

Secondary endpoints

Effects of sargramostim on secondary endpoints were reported only as descriptive.

ANC count: ANC counts decreased substantially in all groups from Day 1 through Day 12 post irradiation, reaching severely neutropenic levels in all treatment groups. A difference in ANC increase between both sargramostim dose groups and control group can be noted (day 12 versus day 15) and reached a maximum at baseline levels by Day 24 in all groups, becoming similar at Day 35.

Platelet count: Nadir of platelet count was reached in sargramostim group at day 12, while in the water control it was reached at day 15; in survivor animals, platelet counts in sargramostim dose groups peaked by Day 24 before slightly declining to baseline levels by Day 35, while counts in the water control group reached a maximum and plateaued by Day 30. From Day 35 through scheduled necropsy at Day 60, platelet counts were similar in all treatment groups. These trends were generally similar among survivors and animals that succumbed prior to Day 60.

Lymphocyte count

In survivors, lymphocyte counts fell in all dose groups after Day 1 post irradiation and reached a minimum by Day 12 in both sargramostim treatment groups and by Day 15 in the water control group. Although lymphocyte counts in water controls increased less quickly than sargramostim groups, all

groups reached baseline counts by Day 50 and remained through the duration of the study.

Red Blood Cells count

Haemoglobin and haematocrit values also demonstrated radiation-induced changes, though there was no clear difference between sargramostim treatment groups for either parameter. In all treatment groups, both haemoglobin and haematocrit slightly increased from Day 1 to Day 3 before falling sharply until Day 15 or 18; the decrease was slightly more dramatic for sargramostim treated groups than water controls. Both parameters then increased in all treatment groups, until reaching baseline levels by Day 50.

Bacteriology (blood/organ cultures)

Bacterial culture at the time of euthanasia (blood) and necropsy (kidney, liver) for moribund animals was positive in most animals (60- 92% across treatment groups) for a variety of organisms (largely species of staphylococci and streptococci).

Across all treatment groups, animals positive for bacterial culture of terminal blood, liver or kidney on Day 60 (scheduled euthanasia) ranged from 0 to 33%. Histopathology and culture results both indicated that most invasive bacteria were species of Streptococcus and Staphylococcus.

Clinical observations associated with radiation illness

Common observations included skin redness, swelling, and bruising, changes in appetite, liquid, soft, scant, or lack of stool, and hair loss. Other common observations included a decrease in grooming behaviour and the appearance of a rough hair coat. As expected, based on model development, the majority of animals experienced clinical signs associated with the gastrointestinal and integumentary systems. No statistical differences were noted between sargramostim and water/control patients.

2.6.5.3. Clinical studies in special populations

Table 22: Clinical Studies in Special Populations

	Age 0-17 (Paediatric subjects number /total number)	**Age 65-74 (Older subjects number /total number)**	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Studies	***448 / 899***	***35 / 899***	***0 / 899***	***0 / 899***
Study 301	9 / 44	0 / 44	0 / 44	0 / 44
Study 302	3 / 62	0 / 62	0 / 62	0 / 62
Study 305	0 / 117	33 / 117	0 / 117	0 / 117
Study 9002	23 / 109	1 / 109	0 / 109	0 / 109
Study 501	53 / 207	1 / 207	0 / 207	0 / 207
Study 001.0005 (Part B1)	60 / 60	0 / 60	0 / 60	0 / 60
Study 001.0005 (Part B2)	264 / 264	0 / 264	0 / 264	0 / 264
001.0006	36 / 36	0 / 36	0 / 36	0 / 36
Non-Controlled Studies	***98 / 285***	***43 / 285***	***7 / 285***	***0 / 285***
Study 308001	22 / 22	0 / 22	0 / 22	0 / 22
Study 202	9 / 13	0 / 13	0 / 13	0 / 13
Study 502	26 / 26	0 / 26	0 / 26	0 / 26
Study 701	2 / 58	15 / 58	4 / 58	0 / 58
Study 702	0 / 10	3 / 10	0 / 10	0 / 10
Study 703	14 / 27	0 / 27	0 / 27	0 / 27
Study 704	0 / 12	6 / 12	1 / 12	0 / 12
Study 705	0 / 12	2 / 12	0 / 12	0 / 12
Study 706	4 / 29	0 / 29	0 / 29	0 / 29

Study 707	0 / 10	1 / 10	1 / 10	0 / 10
Study 708	0 / 16	4 / 16	0 / 16	0 / 16
Study 709	0 / 23	10 / 23	1 / 23	0 / 23
Study 710	0 / 6	2 / 6	0 / 6	0 / 6
Study 9208	21 / 21	0 / 21	0 / 21	0 / 21

.

Results in paediatric patients

Clinical data that support the efficacy of sargramostim in paediatric populations come from studies in humans of autologous BMT, allogeneic BMT, and BMT failure or engraftment delay; further supportive data came from study 001.0005A, in which sargramostim was used in prophylaxis for nosocomial infections in pre-term, low weighted newborns.

Study 301 (prospective, double-blind, placebo-controlled trial of sargramostim in patients with ALL or NHL undergoing autologous BMT)

Of the 44 patients enrolled in this trial, there were 9 patients under the age of 18 years (3 to 17 years); 5 paediatric patients were randomised to sargramostim and 4 to placebo.

All patients included in the study received a dose of sargramostim of 250 µg/m2 over a 2 hour period for a total of 21 days, regardless of their age.

All 9 paediatric patients reached the endpoint of ANC recovery $\geqslant$ 0.5 x 10^9/L: the median number of days to an ANC $\geqslant$ 0.5 x 10^9/L was 18 days in the sargramostim group versus 24.5 days in the placebo group, for a difference of 6.5 days.

These data suggest that the treatment was consistent with the overall population (i.e., adults and paediatrics) in which the median number of days to an ANC $\geqslant$ 0.5 x 10^9/L was 18 days in the sargramostim group versus 24 days in the placebo group, for a difference of 6.0 days.

Table 23: Autologous BMT: analysis of clinical response in paediatric patients

Median values (days)	ANC ≥500/mm³	ANC ≥1000/mm³	Duration of hospitalization	Duration of infection	Duration of antibacterial therapy
Sargramostim (n=5)	18	24	28	0	28
Placebo (n=4)	24.5	28	38	0	28

ANC: absolute neutrophil count; BMT: bone marrow transplant; Source: Post-hoc analysis of Study 301 CSR appendices

Study 9002 (prospective, Phase 3, multi-centre, randomized, placebo- controlled study of sargramostim was conducted in patients undergoing HLA-identical sibling BMT for a variety of lymphoid and myeloid malignancies).

Of the 109 patients enrolled, there were 23 patients under the age of 18 years (2.2 – 16.8 years of age); 11 paediatric patients were randomised to sargramostim and 12 to placebo.

All patients included in the study received a dose of sargramostim of 250 µg/m2 over a 4 hour period beginning on Day 0 after bone marrow infusion, regardless of their age. Study drug was discontinued if the patient had an absolute neutrophil count (ANC) > 10 x 10^9/L or platelet count > 600 x 10^9/L for 2 days or had a severe adverse event. If the patient had not achieved an ANC of 1 x10^9/L by Day 20

post bone marrow infusion, study drug was continued for an additional 7 days.

For the paediatric subset, the median number of days to ANC ≥ 0.5 x 10^9/L was 13 days in the sargramostim group versus 17.5 days in the placebo group, for a difference of 4.5 days. These data suggest that the treatment effect observed in paediatric patients was consistent with the overall population, where the median number of days to an ANC ≥ 0.5 x 10^9/L was 13 days in the sargramostim group versus 17 days in the placebo group, for a difference of 4.0 days.

Table 24: Allogenic BMT: analysis of data in paediatric patients

Median values (days or number of patients)	ANC ≥500/mm^3	ANC ≥1000/mm^3	Number of Patients with Infections	Number of Patients with Bacteraemia	Days of Hospitalization
Sargramostim (n=11)	13	15	5	0	25
Placebo (n=12)	17.5	21	7	3	30

Source: Post-hoc analysis of Study 9002 CSR appendices (see Section 9.1.2)

Study 5001 (historically controlled study in patients with failure or delay of engraftment after bone marrow or peripheral stem cell transplantation).
Paediatric patients: 105/203

This study was conducted in order to assess efficacy and safety of patient with a failure to engraft or delayed engraftment, defined as failure to achieve ANC >0.1 x 10^9/L by Day 21 after transplant with active infection; failure to achieve ANC >0.1 x 10^9/L by Day 28 with or without infection; or demonstration of engraftment followed by loss of engraftment.

Of the 203 eligible patients, 105 were paediatric patients, ages 1-17 years. Of these, 48 received sargramostim but only 37 patients were evaluable for efficacy (autologous and allogeneic BMT) and 57 were historical controls.

The treatment schedule in this study was substantially different from the other protocols and from the proposed schedule for treatment of H-ARS: it consists in 3-week course, daily 2-hour IV infusions of 60-1,000 µg/m2/day sargramostim, for a maximum of 3 courses: sargramostim was administered for 14 days, followed by a 7-day rest period.

For the paediatric subpopulation, the autologous BMT patients treated with sargramostim had a median survival of 306 days versus 103 days for controls, for a difference of 203 days. Similarly, in the allogeneic paediatric patients the median survival was 189 days in those treated with sargramostim versus 83 days in controls, for a difference of 106 days. These data suggest that the survival treatment effect observed in paediatric patients was consistent with the overall population.

Table 25: BMT failure of engraftment delay: analysis of paediatric data from a historically- controlled clinical trial – Median survival (days)

	Total population[a]	Paediatric population[b]
Autologous BMT	474 (n=68)	306 (n=24)
Leukine	161 (n=17)	103 (n=23)
Historical		
Allogeneic BMT	97 (n=72)	189 (n=21)
Leukine	35 (n=86)	83 (n=34)
Historical		

[a] only includes patients with known MOF score
[b] includes all paediatric patients regardless of whether MOF score is known
MOF: multiple organ failure
Source: Study 501 [Section 9.1.3]

001.0005A (A Multicenter Phase III Trial to Evaluate the Efficacy of rhu GM-CSF for Prophylaxis of Neonatal Nosocomial Infection - Part A Pilot Study)

Part A is a Phase IIII open-label, non-placebo controlled trial of rhu GM-CSF administered via two-hour IV infusion daily for 28 days in pre term neonates weighing 501-1000 grams, with the aim of evaluate the safety of rhu GM-CSF when administered at 10 µg/kg/day via two-hour intravenous infusion daily for 28 days in preterm neonates weighing 501-1000 grams.

A total of 6 preterms neonates were included: during this trial, the dose was reduced to 7.5 µg/kg/day because of leukocytosis with [ANC] > 20 x 10^9/L). Sargramostim at 10 µg/kg/day by 2-hour infusion in preterm neonates of 501- 1000 grams may cause leukocytosis but it was reported that the drug was otherwise tolerated.

Although there are no differences in the mechanism of radiation toxicity between the paediatric and adult population, paediatric patients are more vulnerable to radiation exposure due to a greater minute ventilation, higher body-surface-area-to-mass ratio, and more rapidly dividing cells (Gardner, Disaster Med Public Health Prep, 2019). As there are no expected differences in the MoA of sargramostim between the paediatric and adult population, the rationale to paediatric use of sargramostim is acknowledged. As described in the PIP (EMA/128509/2024), the paediatric use for the H-ARS indication can been determined based upon 1) extrapolation from adult data, 2) a population PK study to determine dose conversion from animals to humans for the H-ARS indication, and 3) clinical experience in the paediatric population. The proposed paediatric weight-tiered dosing for H-ARS is based on scaling the adult population PK model to paediatrics using allometry and selecting paediatric doses that match adult exposures corresponding to the adult sargramostim dose of 7 mcg/kg/day (please refer to the PK section for comments on the paediatric dosing). Similarly to the adult population, the clinical data in children undergoing autologous or allogeneic BMT following myelosuppressive chemotherapy with or without TBI provide support for the use of sargramostim after radiation exposure. This strategy was also accepted in the PIP. The data in patients <2 years in this setting is limited, but it is agreed with the Rapporteur that given the high mortality of radiation exposure, this uncertainty is accepted and can be reflected in the SmPC. Regarding extrapolation, the PDCO decided to not add any extrapolation plan to the PIP. The PDCO concluded that there was no structured extrapolation in this development because all the efficacy studies are non-clinical. The safety data from the paediatric studies in other indications are not part of an extrapolation but were regarded as supportive as weight of evidence for safety. The current clinical supportive data do not

suggest differences in the efficacy and safety of sargramostim between the paediatric and adult population. It is, therefore, agreed to not request further questions on the extrapolation regarding PD, efficacy, and safety, however, there are questions on the dosing in the paediatric population (please refer to the PK section).

Elderly
Limited data are available from supportive studies about elderly population; in study 305, the 28% of the enrolled population with ANLL was aged 50-70 years. A trend in better survival not reaching the statistical significance was noted in patients treated with sargramostim in the age group 65-70 in respect to placebo, although the median number of survival days was reduced in that patients (258 sargramostim vs 135 placebo) in respect to younger patients (439 sargramostim vs 328 placebo).

2.6.5.4. In vitro biomarker test for patient selection for efficacy
Not applicable.

2.6.5.5. Analysis performed across trials (pooled analyses and meta-analysis)
Not applicable.

2.6.5.6. Supportive studies
Supportive clinical efficacy data for the H-ARS indication comes from the pilot study TSK0143 and from clinical studies of sargramostim in the setting of autologous bone marrow transplant (BMT), allogeneic BMT, and acute myelogenous leukaemia (AML), which led to the other approved indications of sargramostim by FDA. In these clinical settings, patients are exposed to myelosuppressive chemotherapy with or without radiation treatment: the subsequent treatment-related pancytopenia was considered sufficiently in line for mimic the clinical setting of H-ARS.

All studies were conducted before 1994, in the United States of America and were managed by a sponsor different from the current MAH; therefore, it was not possible to verify the conduction of those trials. This limitation in the robustness of data is acknowledged and contributes to the downgrading of the evidence to a supportive level.

Evidence form supportive studies was stratified in three different tiers, according to the level of generalizability of the results in respect to H-ARS:

- in **tier 1** were included the pilot study TSK0143 and other randomized, placebo controlled clinical trials in which patients received TBI as a part of the treatment for the underlying haematological disorders or as a part of the conditioning regimen before transplantation.
- In **tier 2** were included randomized, placebo controlled clinical trials in which patients received chemotherapies able to induce bone marrow aplasia, but not TBI.
- In **tier 3** all other types of clinical trials (single arm, dose-finding trial or trials which used a different dose of sargramostim) and narratives were included.

Table 26: Summary of efficacy from tier 1 studies

	Study TSK 0143	**Study 301**	**Study 9002**
Survival	Trend in favour *(Primary endpoint)*	No effect	-
Effect on ANC	Trend in favour	**Statistical effect *(primary endpoint)***	**Statistical effect *(primary endpoint)***
Effect on PLTS	Trend in favour	**Statistical effect**	No effect
Effect on RBC	Trend in favour	No effect	No effect

Effect on infections	Descriptive	No effects	**Statistical effect**

Table 27: Summary of efficacy from tier 2 studies

	Study 302	**Study 303**	**Study 305**
Survival	No effect	No effect	**Statistical effect on early mortality**
Effect on ANC	Trend in favour but only in AuBMT *(primary endpoint)*	**Statistical effect *(Primary endpoint)***	**Statistical effect *(primary endpoint)***
Effect on PLTS	Trend in favour	No effect	No effect
Effect on RBC	No effect	No effect	No effect
Effect on infections	No effect	No effects	**Statistical effect on infection G4/5**

Table 28: Summary of efficacy from tier 3 study

	Study 5001
Survival	**Statistical effect** ***(Primary endpoint)***
Effect on ANC	No effect *(primary endpoint)*
Effect on PLTS	No effect *(primary endpoint)*
Effect on RBC	No effect *(secondary endpoint)*
Effect on infections	No effect *(secondary endpoint)*

A brief description of study 301 is reported, given its relevance as all patients included were treated with TBI.

Study 301: Safety and effectiveness of rhu GM-CSF (sargramostim) compared with placebo following autologous bone marrow transplantation (AuBMT) in subjects with lymphoid malignancies

This prospective, double-blind, single centre, placebo-controlled study was conducted to assess the efficacy and safety of sargramostim for promoting myeloid engraftment following AuBMT with or without purging in patients with various lymphoid malignancies. All patients received a total dose and duration of TBI whose intensity was depended on the lymphoma classification; ALL received 1.2 Gy 3 x daily for three days, favorable prognosis lymphoma 2 Gy daily for 6 days, unfavorable prognosis lymphoma 2.25 Gy daily for 7 days. After autologous BMT, patients received study drug, 250µg/m2 sargramostim or placebo, as a daily 2-hour intravenous infusion for a maximum of 21 days.

The clinical endpoints of this study were the haematological recovery for ANC at 3 different levels (ANC $\geq 0.1 \times 10^9$/L, $\geq 0.5 \times 10^9$/L , ANC $\geq 1 \times 10^9$/L), WBC $>1 \times 10^9$/L, time to platelet transfusion and red cell transfusions and duration and severity of infections.

Results

WBC and ANC

Table 29: Granulocytes and Leukocytes Response rates

Granulocyte and Leukocyte Response Rates

	Frequency of Positive Response (%)	
	Rhu GM-CSF	Placebo
2 Weeks:		
ANC ≥ 100/cmm	73.9	66.7
ANC ≥ 500/cmm	43.5	14.3
ANC ≥ 1000/cmm	13.0	0.0
WBC ≥ 1000/cmm	65.2	28.6
3 Weeks:		
ANC ≥ 100/cmm	91.3	90.5
ANC ≥ 500/cmm	78.3	52.4
ANC ≥ 1000/cmm	60.9	19.0
WBC ≥ 1000/cmm	87.0	71.4

Granulocyte and Leukocyte Endpoints

	Median Time to Endpoint (days)	
	Rhu GM-CSF	Placebo
ANC ≥ 100/cmm	12.0	13.0
ANC ≥ 500/cmm	15.0	20.5
ANC ≥ 1000/cmm	20.0	28.0
WBC ≥ 1000/cmm	14.0	19.0

The median times to the specified ANC and WBC endpoints were 1-8 days shorter in the sargramostim treatment group than in the placebo group. These differences, favouring sargramostim group, were statistically significant for ANC > 1 x 10^9/L at 3 weeks (20 vs 28 days, Wilcoxon p: 0.027) (Relative Risk estimate: 3.05, 95% CI:1.28-7.2, p: 0.011) and WBC > 1 x 10^9/L at 2 weeks (14 vs 19 days, Wilcoxon test p:0.008) (Relative Risk estimate: 2.203, 95% CI: 1.09-4.45, p: 0.028).

sargramostim showed a statistical effect on median time between AuBMT and last platelet transfusion (21 days vs 30, p: 0.035), while no statistical effects were noted on red cell transfusions or survival.

Infections

Infection incidence was analysed for the 28 day window following AuBMT. Twenty-two of 23 (96%) sargramostim subjects and 19 of 21 (90%) placebo subjects were febrile (temperature> 38°C) for at least one day; the same subjects had fever in association with neutropenia (ANC < 0.5 x 109/L) for at least one day following AuBMT. The median duration of febrile episodes was 8 days in the sargramostim group and 10 days in the placebo group. The median duration of febrile neutropenia was 7 days in the sargramostim group and 9 days in the placebo group. The differences in median number of days febrile and median number of days with febrile neutropenia are not statistically significant.

Narratives

Narratives added further information about the efficacy of GM-CSF in case of accidental exposure to radiation; a comparative analysis of the case series is limited by the large variability in terms of

radiation exposure, timing of start, duration of GM-CSF therapy and administration of other concomitant therapies. It can be noted that in presence of high radiation exposure (> 10 Gy) mortality rate was 100%: for other radiation exposures, it is impossible to define a real trend considering the high variability among the reported cases: mortality rate ranged between 0 to 67%, without a clear trend of association with the received radiation and drug doses.

2.6.6. Discussion on (non) clinical efficacy

The applicant seeks approval of Imreplys under exceptional circumstances for the treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic Sub-syndrome of Acute Radiation Syndrome (H-ARS). Imreplys should be used in accordance with official radiological/nuclear emergency recommendations.). Pivotal data are generated in a NHP H-ARS model.

The dossier lacks comprehensive efficacy and safety data for Imreplys in the claimed indication, but the intrinsic limits and ethical considerations are justified in the context of a marketing authorisation application under exceptional circumstances. The data from studies using the rhesus monkey model is considered adequate for supporting Imreplys in H-ARS due to its similarity to humans in haematopoietic stem cell biology, bone marrow distribution, and radiation effects. Imreplys showed similar biological activity in NHPs and humans, allowing for relevant dose extrapolation. Supportive clinical efficacy data also come from studies in patients undergoing autologous and allogeneic bone marrow transplants or with acute myelogenous leukemia (AML), that led to other approved indications in the US, and from narratives of accidental high-dose radiation exposure.

Design and conduct of (non) clinical studies

A common study design model (blinded, randomized, controlled) was applied to each NHP, with minimal supportive care mimicking conditions of a mass casualty nuclear accident. Differences were based on the study's original purpose regarding radiation doses and timing of sargramostim/vehicle administration. All studies were designed according to ICH M3(R2), FDA Guidance for Industry on Product development under the Animal Rule (October 2015), and ICH S6(R1).

Regarding population selection, it is understood that clinical efficacy studies in humans for the intended indication are unethical and that therefore mainly relied on efficacy data obtained in animals.

However, ethical concerns were raised regarding the conduct of monkey efficacy study 1017-3493. The rationale for using a high TBI dose of 713 cGy in this study on time-to-treatment effects is unclear and the use of even higher TBI doses in confirmatory studies appears unnecessary, as previous studies showed that a lower dose of 655 cGy resulted in interpretable endpoints. Subjecting animals to higher doses, resulted in 86% mortality in control animals, which is extremely high and not justified. The applicant was requested to justify these points as these aspects of the non clinical development were not in line with the "3R principles" (Replacement, Reduction and Refinement) described in Directive 2010/63/EU on the protection of animals used for scientific purposes. The applicant explained that Study 1017-3493 was a Concept of Operations (CONOPS) study run by the United States Government (USG) to inform on use of the product in a radiological or nuclear mass casualty incident.

In each non-clinical study, the proposed dosage of 7 µg/kg/day of sargramostim (84 µg/m²) was selected and the duration of treatment was set according to other indications of sargramostim. Only one dose level of 7 µg/kg was used in efficacy studies. The applicant justified this choice to match and not exceed the expected exposure level (AUC) and pharmacodynamic effects observed in humans. However, PK studies show that a dose of 20 µg/kg/day is within the range of clinical AUC values. This higher dose might have resulted in higher efficacy and less mortality in treated groups, making it a more appropriate choice considering animal welfare.

As reported in the SmPC of the product, in case of high radiation exposure sargramostim should be administered daily until the ANC remains greater than 1 x 10^9/L for three consecutive CBCs, or exceeds 10 x 10^9/L after a radiation-induced nadir. In non-clinical studies, the drug was administered for up to 24 days, aligning with bone marrow reconstitution kinetics after acute damage and applicable to H-ARS management. However, the proposed ANC target is suitable for healthy subjects, with no stopping rules for patients with impaired bone marrow functions (e.g., myelodysplastic syndrome, bone marrow aplasia), where the minimum ANC threshold may not be reached. Treatment duration for these patients may be longer. The applicant argued that sargramostim is for acute situations, potentially overlapping with pre-existing bone marrow disease, and should follow a maximum of 23 consecutive days of administration. However long-term G-CSF plus erythropoietin use in low-risk myelodysplastic syndrome shows an acceptable safety profile, suggesting prolonged sargramostim exposure may have a similar safety profile. Section 4.2 of the SmPC therefore provides the following recommendation: 'Administration of Imreplys should continue until the ANC remains greater than 1 000/mm3 for 3 consecutive CBCs or exceeds 10 000/mm3 after a radiation-induced nadir. If CBCs are not available or in absence of treatment response, Imreplys may be discontinued after 23 consecutive days of dosing.'

Minimal supportive care with antibiotics, fluids, and antiemetics was permitted in each study. Comparing sargramostim to placebo with minimal supportive care is agreed upon, as it mimics limited environmental support in a radiation emergency.

Mortality by day 60 was considered important for trial interpretation; estimands for the primary endpoint were defined accordingly in all NHP studies. Assessing mortality rate (MR) at day 60 from TBI is acceptable for evaluating sargramostim's effects in an acute H-ARS setting.

The secondary endpoints were selected based on sargramostim's mechanism of action as GM-CSF, focusing on haematological recovery, infection rates, microbiological contaminations, and clinical signs and symptoms of H-ARS. Expected effects include myeloid-derived precursor cells such as granulocytes and macrophages/monocytes. Haematological secondary endpoints include white blood cell, neutrophil, and platelet counts. Including monocyte levels as an endpoint is of interest, as it differs from G-CSF products. Data from studies FY14-045 and 1017-3493 show similar effects to neutrophil and platelet counts, with higher nadirs and shorter recovery times post-treatment. For study TSK0144, only individual animal data are provided.

Two types of intercurrent events (deaths from causes other than H-ARS and variability in radiation exposure) were managed in the primary estimands with sufficient minimisation measures. The analysis of secondary endpoints was supportive in substantiating the overall effects of sargramostim in patients affected by H-ARS.

The number of subjects enrolled was set according to the SAP, based on the primary endpoint of each study. No major issues were noted in the study design, which was deemed adequate for isolating the effect of sargramostim compared to placebo, even with minimal supportive care. However, in study 1017-3493, no strategy to control for multiple comparisons was applied, reducing the statistical strength of evidence.

Given the peculiarity of these studies, a limited number of inclusion criteria were defined to exclude confounding factors affecting the primary endpoint of survival. Healthy animals of both sexes, with adequate body weight to tolerate radiation and monitoring, were included in the NHP studies, except for study FY14-045, which included only males. Body weight was used as a stratification factor for randomization to ensure homogeneity between treatment groups. In case of intercurrent events, protocols allowed for animal replacement with spares selected based on the physical features of the main group.

Efficacy data and additional analyses

Reduction in Mortality Rate at Day 60 (MR60)

Sargramostim consistently reduced MR60 compared to vehicle/placebo across all three main studies.

Study TSK0144: sargramostim reduced MR60 by 36% compared to vehicle (MR60: 22% vs 58%). Survival curves showed a significant difference favouring sargramostim (p=0.0023). The Cox proportional hazards model indicated a 69% reduction in risk of death with sargramostim (HR: 0.31, 95% CI: 0.14-0.70).

Study FY14-045: the difference in terms of MR60 was 30.6% (29,4% sargramostim, 60% vehicle) and resulted statistically significant at 48h (Fischer's exact test two-sided p-value=0.02); the log-rank test comparing the survival curves confirms the result in terms of survival advantage (p=0.03).

Study 1017-3493: Logistic regression showed a nominal advantage in MR60 at Day 60 (p=0.0032) when sargramostim was administered at 48 hours. The MR60 difference was 18% in favour of sargramostim. However, the results were not statistically significant overall due to lack of multiplicity control, therefore findings are interpreted with caution.

The gender analysis of NHP results shows some uncertainties regarding sargramostim's effect on female animals:

- **Study TSK0144**: Higher premature death rates in females than males across all groups.
- **Study 1017-3493**: Significant MR60 advantage for combined sexes and males, but only a trend for females.
- **Study FY14-045**: No female animals included, reducing evidence strength for females.

The absence of females in FY14-045 is due to the 2014 standard US practice of excluding females to avoid data interpretation issues from oestrous cycling. Radiation effects might be more intense in females, potentially explaining the suboptimal response in study 1017-3493, but firm conclusions can't be drawn. The mechanism behind the sex difference in mortality among lethally irradiated animals remains unclear. A 2022 NIAID/NIH workshop (Taliaferro LP *et al,* 2024) highlighted the limited data on irradiated female rhesus macaques, as studies predominantly used male animals until the late 2010s. A 2021 study found higher mortality in female rhesus macaques at identical TBI doses, with lower haematological cell nadirs and slower weight recovery compared to males (Beach T *et al,* 2021). This suggests intrinsic biological differences. At a 7 mcg/kg dose, the difference in AUClast between male and female NHPs was less than two-fold, unlikely impacting efficacy. However, higher susceptibility to radiation in females cannot be excluded and would require further data for confirmation.

Reduction of MR60 at different radiation dose levels

Study TSK0144 and FY14-045: The primary endpoint analysis showed a reduction in MR60 at LD_{50-60}. In the exploratory cohort at LD_{70-80}, sargramostim demonstrated a 44% advantage in MR60 compared to vehicle (83% vs 39%, p=0.0076). Survival curves confirmed the survival advantage for sargramostim (log rank: 0.0036), with a 71% reduction in risk (HR: 0.29, 95% CI: 0.12-0.73). The positive effect at LD_{50-60} was also confirmed by study FY14-045.

Study 1017-3493: All animals were irradiated with LD_{70-80}. A numerical advantage was observed at 48 hours, with MR60 reduction between 11% and 18% when sargramostim was administered between 48 and 96 hours, suggesting clinical benefit even at higher radiation doses. This indicates that

sargramostim may reduce mortality rates across different radiation doses (LD_{50-60} and LD_{70-80}), which is clinically relevant for emergency settings where the exact radiation dose absorbed may be uncertain.

Sargramostim should be administered as soon as possible after suspected or confirmed exposure to radiation doses greater than 2 Gray (Gy). Although NHP studies investigated higher doses (LD_{50-60}: 6.55 Gy, LD_{70-80}: 7.13 Gy), results should be transferable to lower doses. The 2 Gy threshold likely comes from literature data indicating increased H-ARS risk above 1.5/2 Gy (Garau 2011, Jones 2014). Exact radiation quantification during an event isn't always available, and H-ARS can occur at lower doses in high-risk groups (children, elderly, immunocompromised). Alternative methods for evaluating H-ARS risk based on clinical signs of haematological alterations have been proposed [Bolduc *et al.* Radiation Protection Dosimetry (2016)]. Currently, no specific NHP studies evaluate sargramostim's efficacy at lower radiation doses. However, its benefits/risks are likely to translate to humans with suspected or confirmed H-ARS from lower doses. Consequently, the SMPC encourages estimation of the absorbed dose but also indicates that treatment should not be withheld if clinical signs and symptoms are present, even if the absorbed dose is estimated to be less than 2 Gy.

Reduction of MR60 in respect to the timing of administration

Study 1017-3493: sargramostim showed a numerical improvement in survival at 48 hours, with a trend in mortality reduction observed up to 96 hours. No significant difference was seen at 120 hours. This aligns with the kinetics of bone marrow precursor response to damage when stimulated by a growth factor (GF), informing the administration guidance in the SmPC for healthcare professionals.

Study FY14-045: Significant survival improvement was confirmed at 48 hours, with a trend in mortality reduction at 24 hours (MR60 difference: 19%, p=0.14). The study wasn't powered to compare 24-hour and 48-hour administration, so no definitive advantage of earlier or later administration can be concluded.

In NHP studies, no data were available for sargramostim administration within 24 hours post-radiation. For chemotherapy-induced neutropenia, G-CSF or GM-CSF administration within 24 hours is contraindicated due to increased risk of impairing neutrophil recovery (GCSF Guidelines Northern Cancer Alliance January 2018 v1.5). The immediate effect of radiation on bone marrow suggests minimal risk of delayed effects after removing the radioactive source, but no efficacy studies have been conducted for sargramostim administered earlier than 24 hours. The kinetics of radiation-induced bone marrow damage differ from chemotherapy, supporting administration as soon as possible post-irradiation, though specific data are lacking. Administration within 24 hours remains at the discretion of healthcare professionals, as noted in section 4.4 of the SmPC:' Imreplys may be used in conjunction with other supportive care to treat H-ARS. Given that the mechanism of radiation toxicity begins at the time of exposure, treatment with Imreplys should start as soon as possible after radiation exposure. However, efficacy studies for sargramostim when administered earlier than 24 hours after exposure to myelosuppressive doses of radiation, have not been conducted in a large animal model of total body irradiation-induced H-ARS.' The SmPC also indicates that Imreplys should not be delayed if a complete blood count is not readily available or absorbed radiation dose cannot be estimated.

Limited survival data for sargramostim were reported in supportive studies. NHP study TSK0143 showed a trend in MR60 reduction (25% survival benefit, p=0.4136). Study 305 demonstrated a statistical effect on early mortality (p=0.015) but no impact on overall survival. Tier 3 study 501 showed increased median survival in both autologous and allogeneic transplant settings (286 vs 161 and 155 vs 35), though generalisability was considered low.

Haematology parameters

The effect of sargramostim on the different blood line cells was investigated as secondary endpoint in all the NHP studies; given the mechanism of action of sargramostim as GM-CSF, its effect is theoretically expected in the myeloid-derived precursor cells (ANC, monocytes, reticulocytes, megakaryocytes) in absence of clear effects on lymphocytes, given the early separation of the two haemopoietic lines.

ANC recovery:

- **Study TSK0144**: Faster ANC recovery and reduced time to recovery were noted. Significant effects on median time to ANC recovery were demonstrated at both 0.5 and 1 x 10^9/L timepoints.
- **Study 1017-3493**: Mean recovery times for ANC ≥ 0.5 x 10^9/L and ≥ 1 x 10^9/L showed faster recovery in sargramostim groups compared to the vehicle group.
- **Study FY14-045**: A trend towards faster neutrophil recovery and reduced time to recovery from neutropenia was observed.

The effect of sargramostim on ANC recovery was largely confirmed by supportive studies. The generalisability of these results is considered acceptable, as most studies focused on ANC recovery. In tier 1 and 2 studies, the primary endpoint of faster ANC recovery versus placebo was reached in 4 out of 5 studies, with the fifth showing a favourable trend without statistical significance.

Platelet recovery:

- **Study TSK0144**: Significant advantage in time to thrombocytopenia recovery was demonstrated at different radiation doses.
- **Study 1017-3493**: Mean duration of platelet count < 20 x 10^9/L was similar across groups, with faster recovery in sargramostim groups.
- **Study FY14-045**: Peak platelet recovery was reached earlier in sargramostim groups compared to the control group.

In supportive studies, sargramostim's effect on platelet recovery was investigated as a secondary endpoint. Some studies reported a trend towards faster platelet recovery, with statistical significance demonstrated in Tier 2 study (302). Pilot study TSK0143 confirmed the effects reported by pivotal studies.

Red Blood Cells and Reticulocytes: Across the three NHP studies, a trend towards faster reticulocyte recovery was observed, but none reached statistical significance. No substantial effects on red blood cells or reticulocytes were noted in supportive studies.

Lymphocytes: No substantial effects on lymphocyte counts were observed. Lymphocytes decline rapidly post-irradiation and reach their nadir around two weeks. Slightly earlier recovery of lymphocyte counts was noted in some studies, but none achieved statistical significance.

Clinical observations: Across all NHP studies, ARS-related symptoms (e.g., skin redness, swelling, changes in appetite, stool changes, hair loss, decreased grooming, posture changes, weight loss) were monitored. Some improvement in symptoms was noted in sargramostim-treated groups, but no statistical significance was demonstrated. Slightly higher incidence of skin wounds, skin turgor issues, and buccal ulcers occurred in sargramostim-treated animals. The daily subcutaneous administration of the drug might have contributed to increased skin wounds, but its role in other events is unclear.

<u>Bacteriology and blood/culture tissue</u>

Sepsis and infection reduction:

- **Study TSK0144**: sargramostim reduced infection rates by 31%.
- **Study 1017-3493**: Reduction in infection rates was observed at 48h, 72h, and 96h, with statistical significance at 96h ($p \leq 0.01$) and at 48h when comparing pooled groups treated with sargramostim and azithromycin ($p \leq 0.01$).

Febrile neutropenia (FN):

- Higher incidence of FN in sargramostim groups in both studies (TSK0144: 22% vs 3%; 1017-3493: 61% vs 39% and 45% vs 32% in azithromycin group). This is likely due to the drug's known side effect of fever (>30% incidence according to FDA SmPC) and longer median survival in treated animals.

Bacterial strains:

- Most frequently isolated strains were normal components of cutaneous and gastrointestinal flora (*Staphylococcus aureus*, *Escherichia coli*). Higher incidence of sepsis in pre-terminally dead animals aligns with tissue damage and recovery time post-radiation.

Antibody absence:

- No anti-sargramostim antibodies were detected across all NHP studies, confirming data from supportive studies about the product's immunology in humans.

Efficacy in special populations

Elderly population: In **Study 305**: Limited data show a trend towards better survival in 28% of patients aged 65-70 with AML treated with sargramostim compared to placebo. However, median survival days were lower in elderly patients (258 days sargramostim vs 135 days placebo) compared to younger patients (439 days sargramostim vs 328 days placebo).

Immunocompromised patients: No specific data are available on sargramostim's efficacy in immunocompromised patients. Indirect extrapolation from supportive studies in patients with lymphoproliferative diseases suggests a higher risk of complications in immunocompromised patients with H-ARS. However, without specific data, conclusions on efficacy in this group cannot be drawn.

Assessment of clinical efficacy in paediatric patients

Data of efficacy of sargramostim in the paediatric population came from supportive studies in which the drug was studied in patients affected by haematological malignancies and from a trial in pre-term, low weighted newborns in which sargramostim was used as prophylaxis of infection.

- **Studies 301 and 9002**: Approximately 20% of enrolled patients were paediatric. In study 301, all patients received TBI. Efficacy results were consistent with the general population, showing faster ANC recovery (18 days vs 24.5 days), reduced hospitalisation days (25 days vs 30 days), and fewer infections (5 cases vs 7 cases). Compared to historical controls, sargramostim increased survival in paediatric patients with engraftment failure (autologous BMT: 306 days vs 103 days; allogeneic BMT: 189 days vs 83 days).
- **Pre-term newborns**: Data from pre-term newborns have limitations in generalisability, making extrapolation to H-ARS difficult. There is a lack of information on sargramostim's efficacy and safety in patients under 2 years old which is outweighed by the high mortality rate of H-ARS. Sargramostim's use in paediatric patients aged 2 years and older is established in the US for other approved indications, but efficacy data for those younger than 2 years are still lacking.

Additional efficacy data needed in the context of a under exceptional circumstances

The applicant requested a Marketing Authorisation under exceptional circumstances in accordance with Article 14(8) of Regulation (EC) No 726/2004. The request was accepted in light of the following considerations:

- H-ARS is a life-threatening condition that occurs only with high radiation exposure, typically following nuclear accidents or detonations. The unpredictability of this condition and the difficulty in generating high-level evidence are acknowledged. Limited data from nuclear accidents or weapons use are available, but the number of reported cases and the quality of evidence are low. Therefore, it is recognised that the applicant cannot be expected to provide comprehensive data on the efficacy of Imreplys in the H-ARS setting.
- The unpredictability of the clinical condition, combined with the challenges of conducting clinical trials for registration purposes in the event of nuclear exposure (limited patients in accidental exposure, logistical issues in massive nuclear blasts), makes it impossible to generate comprehensive information at the current state of knowledge.
- Imreplys efficacy studies cannot be conducted in humans with H-ARS for ethical reasons, as it would be contrary to accepted medical ethics due to the harmful radiation levels required to induce H-ARS.

As such, H-ARS meets the criteria for exceptional circumstances and the following measures are required to reflect the limitations in this unique setting:

- In order to further characterise the efficacy and safety of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall conduct and submit the results of study PTX-01-001, a retrospective observational study to evaluate the efficacy and safety of sargramostim in individuals exposed to myelosuppressive doses of radiation following an ionising radiation event, according to an agreed protocol. Final protocol submission by 30 June 2025 and final study results within 6 months after the use of the product in an incident.
- In order to ensure adequate monitoring of safety and efficacy of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall provide yearly updates on any new information concerning the safety and efficacy of sargramostim as part of the annual re-assessment

The CHMP requested a single master protocol for Study PTX-01-001 applicable to all countries in case of a nuclear accident. The applicant agreed and implemented a single master protocol, with details to be finalised after sargramostim approval. Additional updates will include procedural guidance and instructions to maximise data collection during a nuclear event, including assessing the impact of the radiation dose a person was subjected to.

2.6.7. Conclusions on the (non) clinical efficacy

It is concluded that, from an efficacy point of view, Imreplys fulfils the criteria for being approved under exceptional circumstances for the treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic Sub-syndrome of Acute Radiation Syndrome (H-ARS). Imreplys should be used in accordance with official radiological/nuclear emergency

recommendations.

The CHMP considers the following measures necessary to address the missing efficacy data in the context of a MA under exceptional circumstances:

- In order to further characterise the efficacy and safety of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall conduct and submit the results of study PTX-01-001, a retrospective observational study to evaluate the efficacy and safety of sargramostim in individuals exposed to myelosuppressive doses of radiation following an ionising radiation event, according to an agreed protocol.
- In order to ensure adequate monitoring of safety and efficacy of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall provide yearly updates on any new information concerning the safety and efficacy of sargramostim.

2.6.8. Clinical safety

2.6.8.1. Patient exposure

Clinical studies

Clinical safety data of Imreplys provided for safety evaluation for H-ARS indication is based on data from 22 studies and is presented separately for three populations:

1. Haematological patients pertinent to H-ARS based on data from 153 cancer patients after autologous bone marrow transplant (BMT) or autologous peripheral blood progenitor cell (PBPC) transplantation who participated in 3 placebo-controlled clinical studies, which enrolled patients that had total body irradiation (TBI) as inclusion criteria. Data aim at demonstrating the safety of the product in adults and paediatrics that received TBI.
2. Healthy volunteer subjects: Data from 317 healthy volunteer subjects who participated in 7 studies of sargramostim; they support the use of sargramostim in an otherwise healthy general population following a radiation exposure incident.
3. Paediatric patients: Data from a total of 337 paediatric patients who received sargramostim in 15 clinical studies, which enrolled recipients of bone marrow (BM) or peripheral stem cell (PSC) transplant, patients with other oncology or bone marrow indications, patients with Crohn's disease, and premature neonates. The studies enrolling preterm neonates were placebo-controlled trials to evaluate sargramostim in the prevention of nosocomial infection. It is important to note that the legacy data (prior to Partner Therapeutics, Inc. [PTx] ownership), for paediatric patients presented in this document are not complete.

Exposure

Haematological patients: 77 patients received daily infusions of 250 µg/m² IV lyophilised sargramostim for 21 days and 76 patients received a placebo.

Healthy volunteer subjects: 317 subjects received sargramostim in various formulations, doses, and routes of administration.

- **Formulation**: 136 subjects received lyophilised sargramostim and 78 subjects received liquid sargramostim without EDTA. 189 subjects received liquid sargramostim with EDTA.

- **Route and dose:** 304 subjects received SC sargramostim (125 or 250 µg/m2; 2, 6 or 8 µg/kg, or 25, 125, 250 or 500 µg). 44 subjects received IV sargramostim (250 µg/m² or a fixed 500 µg dose). 6 subjects received sargramostim via inhalation (250 µg). 66 subjects received doses greater than or equal to the proposed dose for the H-ARS indication (7 µg/kg).

Paediatric patients: 337 paediatric patients were exposed to sargramostim across 15 studies Approximately 30 patients received doses equal or higher than the proposed H-ARS dose.

- **Oncology and bone marrow indications**: **120 paediatric patients** received sargramostim and 42 paediatric patients received placebo. Most patients received IV, with 6 patients receiving SC sargramostim. Most patients received the lyophilised formulation with the most common dose approximately 250 µg/m²/day.
- **Crohn's disease study (Study 308001)**: 22 children (ages 8-16) received liquid sargramostim with EDTA SC at 4 or 6 µg/kg/day for 8 weeks. There was no placebo group.
- **Preterm neonates**: **190 neonates** received lyophilised sargramostim IV and **161 neonates** received placebo. The **Doses** ranged from 0.05 µg/kg/day to 10 µg/kg twice daily.

Demographic and other characteristics of the study populations

Haematological adult patients were aged up to 62 years old. The majority of subjects were male and white. The paediatric patients in these studies ranged in age from 1 day to 17 years. Of the 337 paediatric patients receiving sargramostim in these studies, 190 patients were 0-1 month of age, 6 patients were >1 month to <2 years of age, 1 patient was <1 year of age (not otherwise specified), 86 patients were 2 to <12 years of age, and 49 patients were 12 to <18 years of age. Most paediatric patients were white, and there were more male than female patients overall.

Post-approval safety data

sargramostim was authorised by the FDA with the tradename Leukine in 1991. Currently 6 indications are approved. The safety of sargramostim has been monitored through more than 33 years of postmarketing pharmacovigilance activities. The estimated number of patients treated with marketed sargramostim from approval in 1991 through the 04 March 2023 is approximately 547,583 patients.

2.6.8.2. Adverse events

Common adverse events

Haematological patients

In patients receiving sargramostim in the haematological studies the most common AEs were Grade 1 or 2 events. In studies 301 (8802), 302 (8803) and 303 (8810) Diarrhoea, Rash, Asthenia and Malaise were the only events observed at a rate ≥5% higher in the sargramostim arm compared to the placebo arm (see Table below).

Table 30: Adverse reactions after autologous bone marrow of PBPC transplantation in at least 10% of patients receiving intravenous sargramostim or ≥5% higher than the placebo arm

Adverse Reaction Events by Body System	Sargramostim (N = 77) %	Placebo (N = 76) %	Adverse Reactions by Body System	Sargramostim (N = 77) %	Placebo (n = 76) %
Body, General			**Metabolic, Nutritional Disorder**		
Fever	95	96	Oedema	34	35
Mucous membrane disorder	75	78	Peripheral Oedema	11	7
Asthenia	66	51	**Respiratory System**		
Malaise	57	51	Dyspnoea	28	31
Sepsis	11	14	Lung disorder	20	23
Digestive System			**Blood and Lymphatic System**		
Nausea	90	96	Blood dyscrasia	25	27
Diarrhoea	89	82	**Cardiovascular Vascular System**		
Vomiting	85	90	Haemorrhage	23	30
Anorexia	54	58	**Urogenital System**		
GI disorder	37	47	Urinary tract disorder	14	13
GI haemorrhage	27	33	**Nervous System**		
Stomatitis	24	29	CNS disorder	11	16
Liver damage	13	14			
Skin and Appendages					
Alopecia	73	74			
Rash	44	38			

Abbreviation: PBPC: peripheral blood progenitor cell
Source: [Post-hoc analysis of Studies 301, 302, 303]

Healthy volunteer subjects

Across the healthy volunteer studies, AEs most commonly occurred in for the following SOCs: General disorders and administration site conditions, Musculoskeletal and connective tissue disorders, and Nervous system disorders. Most events were Grade 1 or Grade 2. There were few Grade 3 and no Grade 4 events. Overall, the most frequent AEs observed after sargramostim administration in healthy volunteers were headache and back pain.

Paediatric patients

In sargramostim-treated paediatric recipients of bone marrow or peripheral stem cell transplants and with other oncology and bone marrow indications, the AEs that were most frequent in most studies included alopecia, anaemia, anorexia, asthenia, back pain, chills, diarrhoea, fever/pyrexia, febrile neutropenia, headache, malaise, mucous membrane disorder/mouth ulceration/stomatitis, nausea, pruritus, rash, sepsis, urticaria and vomiting. Bleeding events such as epistaxis, haematuria, and haemorrhage were common events in a small number of studies. In the controlled studies, the most common AEs and most severe AEs were similar in the sargramostim and control groups. In all of the studies, most AEs were reported as Grade 1 or Grade 2 events.

In paediatric patients with Crohn's disease, the most common AEs were headache, back pain, vomiting, pyrexia, nausea, and abdominal pain.

In premature neonates, the most frequent AEs included leucocytosis, lung disorder, abnormalities of vital signs (e.g., hypertension, hypotension, bradycardia, apnoea), and abnormalities related to laboratory values (e.g., hyponatraemia, hyperglycaemia, respiratory acidosis).

2.6.8.3. Serious adverse event/deaths/other significant events

Serious adverse events

Haematological patients

In adult and paediatric patients receiving sargramostim in the haematological studies, almost all had SAEs, most were comparable with placebo patients and were expected to occur in this population.

Healthy volunteer subjects

One nonfatal serious adverse event (SAE) occurred in the healthy volunteer studies. Subject 017 in Study 001.0004, a 31-year-old Hispanic male, experienced severe back pain, chest pain, hypotension, and shortness of breath with the first dose of IV sargramostim. The infusion was discontinued after 32 minutes, the subject was treated with IV saline and norepinephrine, and he recovered immediately.

Paediatric patients

In paediatric patients receiving bone marrow or peripheral stem cell transplant and patients with other oncology or bone marrow indications, SAE information is available only for Study 302 (8803). All patients receiving sargramostim in this study had SAEs, but specific information on these events is not available. In a single study of paediatric patients with Crohn's disease (Study 308001), SAEs occurred in 23% of patients (5 of 22). In the studies of premature neonates, the percentage of patients with SAEs ranged from 5% (1 of 21 patients; [Study 9208]) to 50% (3 of 6 patients; [Study 001.0005 Part A]).

Deaths

Haematological patients

Of the 77 patients treated with summarised for the SCS, 11 patients had AEs with fatal outcomes. The most common causes of death in patients receiving sargramostim following autologous BMT or PSCT were relapse of primary disease and infection.

Healthy Volunteer Subjects

There were no deaths in the studies of healthy volunteer subjects.

Paediatric patients

Of the 337 paediatric patients treated with sargramostim in the 15 studies summarised for the SCS, 48 patients had AEs with fatal outcomes during study participation or post-treatment follow-up.

These deaths included:

- Recipients of bone marrow or peripheral stem cell transplant: 32 of the 83 paediatric patients (39%) in the sargramostim group (compared with 24 of 54 paediatric patients [44%] in the placebo or historical control group);
- Other oncology or bone marrow indications: 4 of 37 paediatric patients (11%) treated with sargramostim;
- Premature neonates: 12 of 190 neonates (6%) treated with sargramostim; and
- Crohn's disease: None of the 22 paediatric patients treated with sargramostim.

The most common AEs with fatal outcomes in children receiving sargramostim following bone marrow and peripheral stem cell transplant were relapse of primary disease and infection. In controlled studies, the most common causes of death were similar in the sargramostim and control group. In the studies of preterm neonates, the events with fatal outcomes that occurred in patients receiving sargramostim included respiratory events, cardiopulmonary failure, infection, and shock.

2.6.8.4. Laboratory findings

Haematological patients: In the haematological patients, laboratory values were similar in the treatment groups. No laboratory abnormalities potentially related to sargramostim administration were identified.

Healthy volunteer subjects: Across the studies of sargramostim in healthy volunteers, there were very few clinically significant abnormalities in laboratory values. In healthy volunteer subjects with normal haematology values at baseline, increases in white blood cell (WBC) count and eosinophils are expected due to the mechanism of action of sargramostim.

Paediatric patients: The limited data on clinical laboratory evaluations for paediatric patients in the 15 studies of sargramostim reveal no safety signal. In the controlled studies for which data are available, the most common AEs related to abnormalities in laboratory values were similar in the sargramostim and control groups or were elevations in ANC and white blood cell (WBC) parameters consistent with the mechanism of action of sargramostim. In the study of sargramostim in Crohn's disease, the only notable changes from baseline in laboratory parameters were increases in ANC, eosinophils, and white blood cell (WBC) counts that can occur in patients with normal baseline values receiving sargramostim.

2.6.8.5. In vitro biomarker test for patient selection for safety

N/A

2.6.8.6. Safety in special populations

No safety data have been provided for the elderly population.

2.6.8.7. Immunological events

As with all therapeutic proteins, there is the potential for immunogenicity with sargramostim.

Treatment with sargramostim may induce anti-drug antibodies (ADAs) and neutralising antidrug antibodies (nAbs). The nAbs can bind to sargramostim and inhibit its pharmacological function by preventing its binding to target receptors on cells, leading to neutralisation of GM-CSF biological

activity. Clinical relevance of these antibodies remains unknown.

PTx has developed and validated an assay for detection of anti-sargramostim nAbs in human serum. This method complies with the EMA and FDA guidance. The method uses a direct cell-based assay format with commercially available GM-CSF reporter cells. The assay is qualitative, with a sample considered nAb positive or negative, based on its signal relative to the assay cut point.

No ADAs and nAbs have been observed in irradiated myelosuppressed animals treated with sargramostim. In the NHP study (Study DDK0111) animals exposed to a single uniform total body radiation (TBI) dose of 6.46 Gy did not develop ADAs and nAbs when sargramostim was administered daily SC for 14-days at human equivalent doses (HED) of 84 µg/m2 or 250 µg/m2 body surface area.

There is variability in reported incidences of sargramostim ADAs and nAbs in non myelosuppressed individuals. Studies in patients treated with sargramostim who have not been exposed to myelosuppressive doses of radiation are summarised below:

- In a study of Parkinson's disease patients (n=10 sargramostim-treated) who received 6 µg/kg sargramostim SC daily for 56 days (8 weeks), ADAs were detected by week 4 of treatment. Four weeks after sargramostim cessation, ADA levels were diminished (Gendelman, 2017).
- In a study of Crohn's disease patients (n=78) receiving 6 µg/kg sargramostim SC daily for 56 days and no other immunosuppressive drugs, only 1 patient had detectable nAbs on day 57. Thirty days after sargramostim cessation, nAb levels were diminished. No drug-related adverse events were observed in association with ADA development (Korzenik, 2005).

In summary, no clinical safety signals have been associated with anti-sargramostim ADAs and nAbs to date.

2.6.8.8. Safety related to drug-drug interactions and other interactions

Interactions studies between sargramostim and other drugs have not been performed.

2.6.8.9. Discontinuation due to adverse events

Haematological patients

In adult and paediatric patients receiving sargramostim in the haematological studies, 4 patients receiving sargramostim in the 3 studies discontinued treatment due to AEs/intercurrent illness. In Study 301 (8802) 2 patients discontinued due to pulmonary infiltration and 1 subdural haematoma related to thrombocytopenia and in Study 303 (8810) 1 patients discontinued treatment due to fever and chills.

Healthy volunteer subjects

Across the healthy volunteer studies, 7 of the 317 subjects discontinued study treatment because of AEs considered related to sargramostim treatment:

- 1 subject from Study 001.004 discontinued IV treatment following the nonfatal SAEs of back pain, chest pain, hypotension and shortness of breath,
- 1 subject from Study 309901 discontinued SC treatment after developing musculoskeletal chest pain,
- 1 subject from Study 309901 discontinued because of a hematoma AE,
- 1 subject in Study 001.0019 discontinued after developing Grade 3 nausea and vomiting AEs,

- 1 subject in Study 308626 discontinued because of Grade 2 proteinuria and Grade 1 haematuria AEs,
- 1 subject from Study 15367 (PH36647) discontinued because of mild increases in AST and LDH and
- 1 subject from Study PTX-001-005 discontinued due to multiple Grade 1 or 2 AEs. Due to the cumulation of all the treatment emergent adverse events (TEAE), the subject was withdrawn from the study after reporting pain in jaw.

Furthermore, among reported TEAEs, a few (including but not limited to nausea, vomiting, flushing, and coldness) were potentially indicative of hypersensitivity or infusion-related reactions.

Paediatric patients

In 3 of the 6 studies of sargramostim in paediatric recipients of bone marrow or peripheral stem cell transplant, no patient discontinued because of an AE. In the remaining 3 studies the percentage of patients discontinuing because of an AE ranged from 7% (2 of 29 patients; Study 501) to 25% (1 of 4 patients; Study 706 (8705)). Information on the specific AEs that led to discontinuation of study treatment in these studies is limited, but the available data do not suggest a relationship to sargramostim.

In the 3 studies of paediatric patients in other oncology or bone marrow indications, no patient discontinued because of an AE in 2 studies, and no information is available for the remaining study.

One of 22 paediatric patients in the study of Crohn's disease (5%) discontinued because of 2AEs, although the 1 event recorded as leading to discontinuation occurred while sargramostim was being withheld and the other occurred after the last dose of study medication was administered.

In 3 studies of sargramostim in premature neonates, no patient discontinued because of an AE. In the fourth neonatal study (5.3.5.2 [Study 001.0005 Part A]), 2 of 6 premature neonates (33 %) had AEs that led to discontinuation; in both neonates, the AEs that led to discontinuation are known complications of prematurity.

2.6.8.10. Post marketing experience

Sargramostim was originally developed and launched by Immunex; it was acquired by Berlex in 2002, by Bayer in 2006, by Genzyme in 2009, and by Sanofi, through the acquisition of Genzyme, in 2011. PTx acquired global rights from Sanofi in 2018 to develop, manufacture, and commercialise sargramostim.

Sargramostim is only approved in the US. The lyophilised formulation of sargramostim launched in 1991 is the only formulation that is presently manufactured for commercial and Health Security use.

Currently sargramostim is FDA-approved for use in 5 indications at a dose of 250 µg/m2/day, and the H-ARS indication is at a dose of 7, 10 or 12 µg/kg (dependent on age). The approved indications are as follows:

- Following induction chemotherapy in older adult patients with acute myelogenous leukaemia (AML) to shorten time to neutrophil recovery and to reduce the incidence of severe and life-threatening infections and infections resulting in death IV route of administration)
- Mobilisation of haematopoietic progenitor cells into peripheral blood for collection by leukapheresis (IV or SC route of administration)
- Acceleration of myeloid recovery in patients with non-Hodgkin's lymphoma (NHL) acute lymphoblastic leukaemia (ALL) and Hodgkin's disease undergoing autologous bone marrow transplant (BMT; IV route of administration)

- Acceleration of myeloid recovery in patients undergoing allogeneic BMT from human leukocyte antigen (HLA)- matched related donors (IV route of administration)
- Patients who have undergone allogeneic or autologous BMT in whom engraftment is delayed or has failed (IV route of administration)
- To increase survival in adult and paediatric patients from birth to 17 years of age acutely exposed to myelosuppressive dose of radiation H-ARS (SC route of administration).

As sargramostim was not originally developed by PTx, it is not possible to provide cumulative subject exposure to sargramostim in all completed clinical studies as clinical cases in the legacy GPAE database are difficult to identify and some clinical case information remains blinded although the studies were completed. The estimated number of patients treated with marketed sargramostim from approval on 05 March 1991 through 04 March 2023 is approximately 547,583.

A Safety Summary Report was prepared for sargramostim Partner Therapeutics from the Pharmacovigilance Adverse Event Database for the period from the international birth date (IBD) of 05 March 1991, is also presented. Since the March 2017 cutoff of this report, safety evaluations from postmarketing and clinical trials were prepared/reviewed annually as part of the Periodic Adverse Drug Experience Report (PADER), and/or the Development Safety Update Report (DSURs), as applicable. Data from the biomedical literature on the safety of sargramostim in pregnancy, lactation, fertility, its use in paediatric patients, and its immunogenicity are also summarised.

Safety Summary Report from the Global Pharmacovigilance Adverse Event Database for the period from 05 March 1991 to 04 March 2017

As with all data collection in volunteer databases, it is not possible to reliably estimate event frequency due to the potential under-reporting, limited follow-up information, and the imprecision of patient exposure calculations in the real-world setting. In addition, loss of information (for both solicited and unsolicited cases) due to the transfer of the global safety database between multiple Marketing Authorization Holders should be considered when interpreting these data.

Overall AEs:

- 6869 AEs were reported (44% from clinical trials).
- 97% of AEs were from US sources (43% of these were from clinical trials).
- Approximately half of AEs occurred after use of sargramostim in oncology indications.
- Pyrexia, Injection site reaction, and Dyspnoea were the most common AEs (3.6%, 3.1%, and 2.9% of all AEs, respectively). Approximately half of Pyrexia AEs and one third of Dyspnoea AEs were serious.
- 3326 events (48% of all AEs) were SAEs. Pyrexia was the most common SAE (2% of AEs overall and from US sources).
- 437 events (6% of all AEs) had fatal outcomes. Most of these events were related to the patient's underlying disease state.
    - Neoplasms benign, malignant and unspecified was the most common MedDRA SOC for events with fatal outcomes. Malignant neoplasm progression, Sepsis, Malignant melanoma, and Disease progression were the most common AEs with fatal outcomes (0.5%, 0.4%, 0.3% and 0.3% of all AEs, respectively).

Paediatric population:

- 293 events (4% of all AEs) from 113 cases occurred in the paediatric population.
- Pyrexia, Abdominal pain and Injection site pain were the most common paediatric AEs (7.8%, 2.7%, and 2.7% of all paediatric AEs, respectively).
- 22 AEs in 14 paediatric cases had fatal outcomes, and these events appear to be related to progression of underlying disease. Neoplasms benign, malignant and unspecified was the most common MedDRA SOC for paediatric AEs with fatal outcomes. However, the patients were medically complex and the available information is incomplete; concomitant medications including sargramostim cannot be ruled out as a contributing factor.
- 150 events in the paediatric population were SAEs. Pyrexia was the most common SAE in the paediatric population (5.1% of paediatric AEs). Most paediatric Pyrexia SAEs occurred in the setting of myelosuppression following treatment of advanced cancer, including bone marrow transplant.

Unsolicited AEs:

- 3384 AEs (49% of all AEs) were unsolicited.
- General disorders and administration site conditions were the most common SOC for unsolicited AEs (33.5% of all unsolicited AEs). Injection site reaction, Dyspnoea, Pyrexia, and Chest pain were the most common unsolicited AEs (5.6%, 4.2%, 3.7%, and 3.4% of all unsolicited AEs, respectively).

Searches that included the biomedical literature:

- 5 cases of overdoses (range: 2-fold to 10-fold) occurring in patients 2 days to 69 years of age were identified. One was associated with a rash; no other AEs were attributed to the overdose.
- One pregnancy occurred in a female patient with Crohn's disease and normal baseline WBC indices who completed an 8-week course of sargramostim (longer than recommended in the USPI). The outcome was a spontaneous abortion approximately 3 weeks after the last dose of sargramostim.

2.6.9. Discussion on clinical safety

It is acknowledged that clinical studies for safety evaluation in H-ARS are not feasible. Sargramostim's safety profile is inferred from other patient populations and US post-marketing data from sargramostim (commercialised as Leukine). The applicant's proposal to evaluate sargramostim's safety using all available data sources (placebo-controlled and open-label clinical studies, post-marketing data) is agreed upon.

To evaluate Imreplys's safety profile for H-ARS treatment, the applicant analysed data from three major groups:

- **Haemato-oncology patients**: 3 placebo-controlled studies (77 sargramostim-treated, 76 placebo).
- **Healthy volunteers**: 7 studies (n=317).
- **Paediatric subjects**: 15 studies (n=337; 5 placebo-controlled, 2 open-label, 1 follow-up study of 190 premature neonates).

Haemato-oncology adults and children treated with TBI are considered the most relevant for mirroring

hematopoietic tissue injury in H-ARS. Data from healthy volunteers and preterm neonates help describe sargramostim's tolerability in subjects without underlying clinical disorders. Safety data from healthy volunteers can also help distinguish the contribution of underlying haematological disorders to adverse events. Data from preterm neonates are relevant for the paediatric indication. Importantly, myelosuppression in H-ARS may vary in severity, and baseline CBC is not mandatory for sargramostim treatment according to the SmPC. Therefore, safety data from healthy volunteers, children with Crohn's disease, and preterm neonates may provide useful information for those with partially preserved bone marrow function despite radiation exposure.

Study Population Characteristics: For haemato-oncology paediatrics and adults patients TBI was administered for autologous BMT or PSCT. Adult patients were up to 62 years old, mostly Caucasian males. Safety data for those over 60 were requested but not provided due to outdated AE coding. No specific safety concerns are however anticipated for the older population.

Dose and Administration: The proposed dose is weight-based, administered once daily until ANC response. Sargramostim's safety database is heterogeneous due to diverse formulations, posology, and exposure times. IV administration in haematological studies limits assessment of SC administration AEs.

Device and Usability: sargramostim SmPC specifies a syringe to be used, obtained separately. For doses >1 mL, an appropriate metered syringe is needed. This could be critical for usability, especially for laypersons. The applicant was asked to consider a human factor study to inform the SmPC/PL. The applicant noted that in mass casualty events, controlling syringe type is impractical. Instead, a comprehensive instructional leaflet has been prepared for proper preparation and administration.

The following contraindications have been included in section 4.3 of the SmPC: 'History of serious hypersensitivity reactions, including anaphylaxis, to human GM-CSF or yeast derived products, or to any of the excipients listed in section 6.1.'

Based on the data available, the following warnings have been included in section 4.4 of the SmPC to reflect the risks of hypersensitivity and anaphylaxis, haemodynamic oedema, effusions and fluid overload, supraventricular arrhythmias, potential effect on malignant cells, immunogenicity, risk of leucocytosis, and limitations of effectiveness.

In the absence of dedicated interaction studies, section 4.5 of the SmPC refers to the fact that 'Limited data are available on drug-drug interactions. Patients receiving both sargramostim and medicinal products that induce leucocytosis (e.g., corticosteroids, other colony-stimulating factors, lithium) may have an increased risk of leucocytosis (see section 4.4).'

With regards to pregnancy and lactation, no contra-indications are proposed but considering that There are no or limited data on the use of sargramostim in pregnant women. Studies in animals have shown reproductive toxicity (see section 5.3). the following recommendations are stated in section 4.6 of the SmPC: for pregnancy: 'Acute exposure to myelosuppressive doses of radiation has per se' a toxic effect on fertility and embryo/foetal development. This should be considered for clinical judgement on the use of Imreplys in pregnant and/or lactating women; for lactation: Breast-feeding may be considered during treatment with Imreplys, keeping in mind that the newborns may also need treatment.

Most common AEs

In haematological placebo-controlled adult studies, diarrhoea, rash, asthenia, and malaise were observed at a rate ≥5% higher in the sargramostim arm compared to placebo. In healthy volunteers, headache (21-23%), back pain (17-24%), and injection site reactions (10-17%) were more frequently

reported after sargramostim administration. Most adverse events (AEs) were mild to moderate in severity. While the safety profile in children is similar to adults, a higher frequency of fever, alopecia, and rash was noted (without cumulative frequencies). Leucocytosis and lung disorder AEs are common in preterm neonates and considering their lack of underlying haematological disorders a strong rise in WBC can be anticipated.

Given the fragmented description of AEs across numerous studies, the applicant was asked to provide side-by-side tables of AE frequencies observed in pooled placebo-controlled studies by patient group and age. However, assessing AEs associated with sargramostim is challenging due to the lack of specific drug-related AE summaries, outdated coding incompatible with current MedDRA terminology, and inconsistencies in laboratory data presentation.

Adverse drug reactions (ADR)

The applicant included all adverse reactions (ADRs) occurring at a higher rate with sargramostim (>5% absolute difference) in placebo-controlled studies, regardless of causality, due to study limitations. These limitations in safety data collection and analysis impact causality interpretation. Acknowledging that section 4.8 of the SmPC should only report adverse reactions with at least a reasonably possible causal relationship to the medicinal product, adverse events were included based on a higher incidence in the active arm compared to placebo. Adverse reactions from post-authorisation safety studies and spontaneous reports, including previously identified important risks (hypersensitivity, anaphylaxis, supraventricular arrhythmia, oedema, capillary leak syndrome, pleural and peritoneal effusion), are reported in Section 4.8. Due to the same data collection limitations, the incidence of these events cannot be established.

ADRs of special interest

The applicant identified Injection Site Reactions (ISRs) as adverse drug reactions (ADRs) of special interest, providing data for adult healthy volunteers from a single study. Since most paediatric subjects and all preterm neonates received sargramostim intravenously, limited data are available for children, and none for infants. The applicant has provided all available information regarding ISRs, noting different incidence rates of ISRs between adult and paediatric populations which is reflected in the SmPC.

Serious Adverse Events

Overall, the type and frequency of serious adverse events (SAEs) in haematological adult and paediatric patients treated with sargramostim are comparable to those in the placebo group. In preterm neonates, 9 SAEs were observed; one was probably related and six were possibly related to sargramostim treatment. Cases of pericardial effusion and pleural effusion were also described.

Deaths

Regarding fatal events in haematological adults and children, Study 301 (8802) showed a higher incidence rate for sargramostim-treated subjects compared to placebo (34.8% vs 23.8%). However, the causes of death were mostly due to relapse of the underlying haematological disease, sepsis, or severe infections, and none were considered related to the study drug by investigators. No deaths were observed in healthy volunteers or paediatric patients with Crohn’s disease.

In preterm neonates, the frequency of fatal events was similar between sargramostim-treated subjects and placebo. However, deaths in neonates receiving sargramostim were often due to respiratory complications or failure, which could be due to critical clinical conditions associated with prematurity but also related to systemic capillary leak syndrome (SCLS), a life-threatening idiopathic angiopathy and an important identified risk of sargramostim. No causality assessment was provided by

investigators. Sargramostim doses in the preterm neonate study ranged from 0.05 µg/kg/day to 10 µg/kg twice daily, with most fatal cases receiving doses below the proposed 8-10 µg/kg/day IV.

The applicant was asked to evaluate AEs suggestive of SCLS in infants and discuss the potential risk based on study findings, post-marketing data, and published literature and propose precautions for if necessary. The applicant however did not believe that sargramostim is associated with increased risk of SCLS in neonates, noting that AEs in Studies 001.0005 B1 and B2 occurred after intravenous administration, and no SCLS reports were found in post-marketing data. Study data were also considered unsuitable for SMQ analysis by the applicant due to their format, and no discussion on study findings and literature data was provided.

However the applicant's position was not endorsed. While information on SCLS in neonates is limited, its occurrence in adults is recognized. Multiple drugs, including GM-CSF and lenograstim, have been linked to SCLS cases. This risk is therefore specified in Section 4.4 of Imreplys's SmPC, noting that oedema, capillary leak syndrome, and pleural and/or pericardial effusion have been reported after Imreplys administration.

Discontinuation due to adverse events

Most adults and children receiving sargramostim completed their treatment, with only a few discontinuing due to adverse events (AEs).

Safety related to drug-drug interactions and other interactions

No analyses of drug interactions were performed. A discussion on the potential for clinically relevant interactions between sargramostim and systemic cancer chemotherapy or radiation therapy was requested. SmPC section 4.4 includes "Patients with pre-existing, or a history of, cancer should begin sargramostim therapy as soon as possible following radiation exposure due to the life-threatening nature of the exposure and consult an oncologist as soon as practical".

Laboratory and other findings

Laboratory abnormalities did not show significant differences between sargramostim-treated adults and children compared to controls. In preterm neonates, a high frequency of leucocytosis and increased bilirubin was noted. However, increased bilirubin is common in neonates and is unlikely to be causally related to sargramostim, so it did not warrant inclusion in the AE table in SmPC Section 4.8. Leucocytosis is expected, as the study population received sargramostim for nosocomial infection prevention and had no underlying bone marrow condition.

For safety evaluation of sargramostim in H-ARS, excessive leucocytosis could be relevant for radiation-exposed subjects with minimal myelosuppressive damage or without neutropenia. The applicant was asked to elaborate on expected post-nadir ANC response kinetics and propose a stopping rule for subjects not meeting the proposed results. Due to inter-individual variability, the applicant could not provide specific estimates for post-nadir ANC response kinetics. Based on animal data, treatment response is estimated to occur 14-28 days after treatment initiation. The applicant's justification for the chosen thresholds (ANC >1,000/mm³ for 3 consecutive CBCs) for treatment interruption is based on what is generally considered to indicate haematopoietic recovery, which is recognized. While the applicability of this endpoint to H-ARS remains to be established, the proposed strategy for treatment interruption is acceptable. The risk of leucocytosis is therefore reflected in Section 4.4 together with a recommendation to discontinue Impreplys if WBC counts exceed ≥ 50 000/mm3.

Post marketing experience

From March 5, 1991, to March 4, 2023, approximately 547,583 patients were treated with marketed sargramostim, and 417 subjects were exposed to sargramostim in clinical trials. According to the GPAE database (March 1991 to March 2017), the most common adverse events (AEs) were pyrexia (3.6%),

injection site reaction (3.1%), and dyspnoea (2.9%). In paediatric subjects, the most common AEs were Pyrexia (7.8%), Abdominal pain (2.7%), and Injection site pain (2.7%).

Additional safety data needed in the context of a MA under exceptional circumstances

Recommending a marketing authorisation under exceptional circumstances is considered acceptable due to the unfeasibility of clinical studies for the H-ARS indication. The safety profile of sargramostim is bridged from clinical studies in haematological cancer patients (adults and children) and 33 years of US post-marketing data. Overall, the safety profile seems to mirror what is known for medicinal products of the same class (i.e. G-CSF) with pyrexia, injection site reactions, rash, asthenia, headache, and dyspnoea being the most common AEs. The majority of adverse reactions are not serious in severity and remain manageable.

The CHMP considers the following measures necessary to address the missing safety data in the context of a MA under exceptional circumstances:

- In order to further characterise the efficacy and safety of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall conduct and submit the results of study PTX-01-001, a retrospective observational study to evaluate the efficacy and safety of sargramostim in individuals exposed to myelosuppressive doses of radiation following an ionising radiation event, according to an agreed protocol.
- In order to ensure adequate monitoring of safety and efficacy of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall provide yearly updates on any new information concerning the safety and efficacy of sargramostim.

The CHMP requested a single master protocol for Study PTX-01-001 applicable to all countries in case of a nuclear accident. The applicant agreed and implemented a single master protocol, with details to be finalised after sargramostim approval.

All adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

2.6.10. Conclusions on the clinical safety

It is concluded that from a safety point of view Imreplys fulfils the criteria for being approved under exceptional circumstances for the treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic Sub-syndrome of Acute Radiation Syndrome (H-ARS). Imreplys should be used in accordance with official radiological/nuclear emergency recommendations.

The CHMP considers the following measures necessary to address the missing efficacy data in the context of a MA under exceptional circumstances:

- In order to further characterise the efficacy and safety of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall conduct and submit the results of study PTX-01-001, a retrospective observational study to evaluate the efficacy and safety of sargramostim in individuals exposed to myelosuppressive doses of radiation following an ionising radiation event, according to an agreed protocol to be submitted by end of June,.
- In order to ensure adequate monitoring of safety and efficacy of sargramostim in the

treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall provide yearly updates on any new information concerning the safety and efficacy of sargramostim as part of the annual reassessment.

2.7. Risk Management Plan

2.7.1. Safety concerns

None.

2.7.2. Pharmacovigilance plan

No additional pharmacovigilance activities.

2.7.3. Risk minimisation measures

None.

2.7.4. Conclusion

The CHMP considers that the risk management plan version 0.4 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 05.03.1991. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Imreplys (sargramostim) is included in the additional monitoring list as it is a biological medicinal product approved under exceptional circumstances [REG Art 14(8), DIR Art (22)].

Therefore, the summary of product characteristics and the package leaflet include a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-risk balance

3.1. Therapeutic context

3.1.1. Disease or condition

The agreed therapeutic indication for Imreplys is:

- Imreplys is indicated for treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic Sub-syndrome of Acute Radiation Syndrome (H-ARS). Imreplys should be used in accordance with official radiological/nuclear emergency recommendations.

HARS, also known as radiation sickness or radiation toxicity, occurs when individuals are acutely exposed to high doses of whole body or significant partial-body irradiation at doses greater than 1 Gy over a relatively short period of time. H-ARS occurs after whole-body or partial-body (>60%) irradiation to doses >0.7 Gy, causing damage to rapidly dividing tissues, including bone marrow. Exposure to doses >2 Gy causes moderate to severe pancytopenia that may lead to infection, sepsis, bleeding, and death. Children are more radiosensitive than adults, which means that a lower lethal dose for 50% of the paediatric population within 60-days of exposure would be expected.

3.1.2. Available therapies and unmet medical need

H-ARS is a life-threatening condition in adults and paediatric patients for which there is no approved treatment in the EU.

The type of a possible radiation exposure event influences the nature of the exposure (i.e., non-ionising versus ionising radiation), types of ionising particles (e.g., alpha particles, beta particles, neutrons) and/or x-ray/ high-energy gamma rays, the amount and duration of radiation exposure, and the consequent biologic effects. It is estimated that 10.000 to 100.000 individuals may be at risk for life-threatening H-ARS in a large-scale radiological and/or nuclear incident. At present, the need is mainly related to **preparedness** activities in case of an acute radiation event at population level (e.g. in the context of nuclear power plant accident, deliberate sabotage of a nuclear facility, transportation incident or following detonation of a radiological dispersal device ("dirty bomb") or nuclear weapons attack), although it could also be beneficial in case of single subject accident.

Besides applying the principles of protection from radiation (maximising the distance from the source, minimising the time of exposure, shielding from exposure), at present, in the EU pharmacological management of hematopoietic toxicity relies on supportive therapies (i.e., transfusion support, fluids, antiemetic, antifungal and antibiotic therapy, anticonvulsants) and is stratified according to the degree of myelosuppression as determined by complete blood count with differential and signs of bleeding

At present none of the available erythropoietin growth factors are recommended by WHO for management of radiation induced anaemia, which could be treated only with red blood cell transfusions.

Eltrombopag is authorised in adult patients with acquired severe aplastic anaemia (SAA) who were either refractory to prior immunosuppressive therapy or heavily pretreated and are unsuitable for haematopoietic stem cell transplantation.

Medicines authorised for myelodysplastic syndrome (MDS) by EMA include: epoetin, filgrastim, pegfilgrastim, efbemalenograstim, azacitidine, luspatercept, lenalidomide, imetelstat. Iron overload may develop in MDS as a result of repeated RBC transfusions, which are a major part of the supportive care for anaemic MDS patients.

Medicines authorised in the treatment of immune thrombocytopenia (ITP) include: romiplostim, Immunoglobulins, avatrombopag and fostamatinib.

Treatment options also include allogeneic stem cell transplantation (allo-SCT). GM-CSF has been used over many years following HSCT and chemotherapy to help white blood cell levels recover.

Growth factors like filgrastim, peg-filgrastim, sargramostim (from 2018), and romiplostim are FDA-approved in the US for treatment of H-ARS, while in the EU their utilisation is an unapproved off-label use. On April 2023, Ukraine issued an Emergency State Registration for sargramostim given the ongoing threat of a radiation incident.

Overall, the **unmet medical need** for treatment of H-ARS is acknowledged.

3.1.3. Main clinical studies

Efficacy studies of sargramostim for the H-ARS indication could not be conducted in humans because the conduct of such studies is contrary to generally accepted principals of medical ethics and field studies after accidental or deliberate exposure to life-threatening doses of ionising radiation are not feasible. Therefore, 3 adequate and well-controlled studies (i.e., randomised, blinded, placebo-controlled) were conducted in a well-characterised Rhesus monkey model of total body irradiation (TBI)-induced H-ARS.

These studies provided minimal supportive care, mimicking the limited resource environment following a radiological and/or nuclear mass casualty incident. No whole blood, blood products, or individualised antibiotics were provided.

Study 1017-3493 (Time-to-treat AWC efficacy study) (study 1): GLP compliant randomised, blinded, placebo-controlled time-to-treat study conducted in Rhesus monkey to assess the efficacy of sargramostim versus the reference item/vehicle at different timepoints of 48 hours, 72 hours, 96 hours, or 120 hours post-total body irradiation (TBI) at LD70-80/60. 108 Non-Human Primates (NHP, 54 male, 54 female). Animals exposed to 6.55 Gy (36 male:36 female) or 7.13 Gy (18 male:18 female). NHPs randomised to receive sargramostim (7 mcg/kg/day) or placebo (water for injections). Treatment began 48 ± 1 hours post-TBI and continued daily until ANC ≥ 1 000 cells/µL for 3 consecutive days or ANC ≥ 10 000 cells/µL.

Study FY14-045 (study 2): the objective of this study was to determine treatment efficacy (delayed response (expectant haematopoietic recovery response)), specifically any survival benefit at 60 days resulting from early (beginning at day 1 or day 2 post-irradiation) administration of sargramostim following lethal total body irradiation at the LD50/60 dose in rhesus macaques. at the $LD_{50/60}$ dose with minimal supportive care (antibiotics and fluids) in Rhesus monkey. 105 male NHPs randomised into 3 groups (n=35 per group). Treatment Daily for 18 days or until ANC count ≥1 000 cells/µL. Radiation dose 6.80 Gy TBI. Healthy male subjects weighted more than 2.5 kg and considered able to tolerate irradiation and subsequent monitoring procedures were enrolled in this trial. For study FY14-045, the primary endpoint was 60-day survival post-radiation, with secondary endpoints including hematology recovery, infection rates, and clinical signs of H-ARS. The number of animals was based on the primary endpoint.

Study TSK 0144 (Confirmatory AWC efficacy study) (study 3): confirmatory, blinded randomized study in which sargramostim or vehicle were administered daily starting 48h post-TBI at LD50-60/60; the survival benefit and efficacy of sargramostim on hematological parameters were also explored in separate cohorts of irradiated rhesus monkeys at a LD70-80/60 dose (713 cGy).. 308 NHPs (154 male: 154 female). Treatment daily starting 48-, 72-, 96-, or 120-hours post-irradiation until ANC returned to ≥ 1000/µL for 3 consecutive days. Treatment was stopped if the ANC was ≥ 10 000/µL. Radiation dose 7.13 Gy TBI.

Study designs were adequate to isolate Imreplys' effects, though there was no multiplicity control, limiting statistical conclusions. Inclusion criteria aimed to exclude confounding factors, and procedures minimized intercurrent events. There was also some supportive efficacy data from clinical studies in patients with haematological cancers treated with sargramostim. The safety data for Imreplys in treating H-ARS is based on 22 clinical studies. It includes data from haematological patients, healthy volunteers, and paediatric patients. Haematological data comes from 153 cancer patients who had total body irradiation. Healthy volunteer data includes 317 subjects from seven studies, supporting its use after radiation exposure. Paediatric data involves 337 children from 15 studies, including those with various conditions and preterm neonates.

3.2. Favourable effects

Sargramostim has been shown to reduce the mortality rate after the exposure to myelosuppressive doses of radiation: the reduction of mortality rate (estimated reduction in between 18-36%) was statistically consistent ($p < 0.05$ at 48 h) across all NHP studies except for one study, was present at different radiation doses and confirmed in a wide interval time from radiation (until 96h).

A benefit in terms of faster ANC and platelet recovery was also shown across all pivotal studies: the reduction in the time to recovery of ANC was estimated in 1,4-2 days and for time to thrombocytopenia recovery was between 0.8-4 days; also in this case, the effect was statistically consistent across NHP studies and was confirmed at different radiation levels and during a wide interval time from radiation exposure.

Sargramostim determined a reduction in terms of infection rate and signs of sepsis, with a decrease in infections rate estimated between 14- 47% across all NHP studies.

Data from the supportive studies in humans with haematological cancer confirm the favourable effects reported in the NHP models; in particular, the effects in terms of ANC recovery were reported in the majority of patients.

3.3. Uncertainties and limitations about favourable effects

The main source of uncertainties lies in the impossibility of obtaining data in the human species, due to the nature of the intended indication also justifying the request of approval under exceptional circumstances. Limitations in obtaining more comprehensive data under normal conditions of use, inherent to the therapeutic indication, and the intrinsic limits in the generalisability of data obtained in the NHP model to humans is the main source of uncertainties which are considered within the scope of approval under exceptional circumstances.

There are less data available in females; the absence of females in FY14-045 is due to the 2014 standard US practice of excluding females to avoid data interpretation issues from oestrous cycling. Radiation effects might be more intense in females, potentially explaining the suboptimal response in study 1017-3493, but firm conclusions can't be drawn. The mechanism behind the sex difference in mortality among lethally irradiated animals remains unclear. A 2022 NIAID/NIH workshop (Taliaferro

LP *et al,* 2024) highlighted the limited data on irradiated female rhesus macaques, as studies predominantly used male animals until the late 2010s. A 2021 study found higher mortality in female rhesus macaques at identical TBI doses, with lower haematological cell nadirs and slower weight recovery compared to males (Beach T *et al,* 2021). This suggests intrinsic biological differences. At a 7 mcg/kg dose, the difference in AUC last between male and female NHPs was less than two-fold, unlikely impacting efficacy. However, higher susceptibility to radiation in females cannot be excluded.

Few data are available about the efficacy in immunocompromised patients; only an indirect extrapolation of results could be postulated from supportive studies in patients affected by lymphoproliferative diseases, considering that most of them are associated with an immunocompromised status. These uncertainties are reflected in section 4.4 of the SmPC.

In study TSK 0144 (study 3) the 60-day survival results have not been statistically controlled for multiplicity. As a result, the strength of the evidence is limited, and findings should be interpreted with caution as mentioned in section 5.1 of the SmPC.

Sargramostim should be administered as soon as possible after suspected or confirmed exposure to radiations doses greater than 2 gray (Gy) although in NHP studies no data were available when drug is administered within 24 hours after radiation. Therefore, there is an uncertainty of efficacy in this time frame. Moreover, although literature data indicate that 2 Gy exposure is considered as the lower threshold for occurrence of H-ARS, in some patients clinical signs could occur also at a lower radiation level and need treatments.

3.4. Unfavourable effects

In the haematological placebo-controlled adult studies, diarrhoea, rash, asthenia and malaise were the events observed at a rate ≥5% higher in the sargramostim arm compared to the placebo arm. In HV headache (21-23%), back pain (17-24%), and Injection site reactions (10-17%) were the events more frequently reported after sargramostim's administration. AEs were mostly mild/moderate in severity. Although the safety profile in children seems similar to that observed in adults, a higher frequency of fever, alopecia, and rash is to be noted (no cumulative frequencies are provided). Leucocytosis and lung disorder AEs are commonly reported in preterm neonates. Injection site reactions were identified as ADR of special interest and data are provided for adult HVs by single study. Laboratory abnormalities did not show relevant differences between sargramostim-treated adults and children when compared to controls. However, in preterm neonates a high frequency of leucocytosis and bilirubin increased is noted.

According to the post marketing experience, the following AE are reported: pyrexia, injection site reaction, and dyspnoea were the most common AEs (3.6%, 3.1%, and 2.9% of all AEs, respectively). In paediatric subjects, pyrexia, abdominal pain and injection site pain were the most common AEs (7.8%, 2.7%, and 2.7% of all paediatric AEs, respectively).

3.5. Uncertainties and limitations about unfavourable effects

Clinical studies for safety evaluation of sargramostim in the claimed indication of H-ARS are not feasible. Thus, safety profile was inferred from other patient populations as well as US post-marketing information when sargramostim was used for different indications.

The applicant did not submit information specifically related to ADRs in terms of assessment of causality, severity and frequency. Nevertheless, the limitations described by the applicant associated with safety data collection and analysis in sargramostim studies and the impact on causality

interpretation were acknowledged. It was agreed that adverse events are reported based on a higher comparative incidence in the active arm clinical trials in respect to placebo.

No safety data were provided for the elderly population.

3.6. Effects table

Table 31: Effects table for Imreplys in the treatment of H-ARS (based on efficacy data from NHP studies and safety data from CTs and post-marketing data for other indications).

Effect	Short Description	Unit	Imreplys	Control	Uncertainties/ Strength of evidence	Ref
Favourable effects						
MR60 at LD 50-60	Mortality rate at day 60 at Lethal Dose of 50-60	%	22	58	P: 0.0018	TSK0144
MR60 at LD 70-80	Mortality rate at day 60 at Lethal Dose of 70-80	%	39	83	P: 0-0076	TSK0144
MR 60 at LD 70-80	Mortality rate at day 60 at LD 50-60	%	68	86	Statistical significance was not reached with Fisher exact test.	1017-3493
MR60 at LD50-60	Mortality rate at day 60 at Lethal Dose of 50-60	%	71	40	P: 0.02 Only male animals were enrolled in this study; no data are available for female subjects	FY14-045
Unfavourable effects -						
Post marketing (Summary Report from the Global Pharmacovigilance AE Database for the period from 05 March 1991 to 04 March 2017)						
Incidence	**SAE** Pyrexia (paediatrics)	%	48 2 (5.1)	N/A	Unc: Frequencies refer to the GPAE database-based Report for March 1991 to March 2017.	
	Fatal events	%	6			
	Overall Pyrexia Injection site reaction Dyspnoea	%	 3.6 3.1 2.9			
	Paediatrics Pyrexia, Abdominal pain Injection site pain	%	 7.8 2.7 2.7			
Clinical studies						
Incidence	**Haematological pts** Diarrhoea Rash Asthenia Malaise		"Most common AEs"		Unc: SC administration only in HV studies Different doses in some studies Frequencies reported only for individual studies	
Incidence	**HV** Headache Back pain Injection site reactions		 21-23 17-24 10-17		No causality assessment In haematological studies effects of sargramostim cannot be isolated from those related to underlying condition and concomitant treatments	
Incidence	**Paediatrics** Fever Alopecia Rash		"Higher than in adults"		Unc: No pooled frequencies reported No causality assessment	

Effect	Short Description	Unit	Imreplys	Control	Uncertainties/ Strength of evidence	Ref
	Preterm neonates Leukocytosis Lung disorder		"Commonly reported"		In haematological studies effects of sargramostim cannot be isolated from those related to underlying condition and concomitant treatments (not applicable to preterm neonates).	

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The reduction of mortality rate (estimated reduction in between 14-41%) was statistically consistent across all but one NHP studies and at different radiation dose and present in a wide interval time from radiation exposure (until 96h); limited evidence came also from a minority of supportive studies in humans which investigated survival as secondary endpoint. Given that H-ARS is a life-threatening condition which is associated with a high mortality rate in absence of treatment, the effects of sargramostim on survival is considered important, considering also the lack of other approved therapies in EU for H-ARS.

The reduction in the time to recovery of ANC (estimated reduction of 1,4-2 days) and platelets (0.8-4 days) recovery was also statistically consistent across all but one NHP studies and was confirmed at different radiation levels and during a wide interval time from radiation exposure. The analysis of the efficacy results showed also a trend in favour of sargramostim treated animals in all the NHP studies. Both effects were confirmed also by the analysis of supportive. The effects on haematological parameters and infections are considered of importance because the main causes of death during aplasia are represented by infections and severe bleedings: therefore, it could be assumed that the demonstrated faster recovery of ANC and platelets could have contributed to the reported reduction in mortality.

The safety of sargramostim was described in patients with haematological conditions treated with TBI relevant to H-ARS and was fully aligned with the well-known safety profile of other approved medicinal products of the same drug class. Sargramostim has a manageable safety profile and is considered able to address the unmet medical needs of H-ARS by reducing mortality after exposure to myelosuppressive dose of radiation.

3.7.2. Balance of benefits and risks

In terms of clinical benefit, the most important effect of sargramostim was the reduction in the mortality rate after exposure to myelosuppressive dose of radiation: this effect is considered clinically relevant and was observed across all the NHP studies. Beneficial effects were also reported in terms of reduction of infections, time to ANC and platelet recovery; all of them would concur in improving the survival of patients affected by H-ARS. Moreover, it should be outlined also that the favourable effects reported in the NHP models were indirectly demonstrated also in supportive studies in humans. Sargramostim's safety profile seems aligned to what is known for the drug class and is therefore considered manageable.

The features of the provided body of evidence for support the MA request of sargramostim in H-ARS reflected the limitations which led to the request of the under exceptional circumstances; although the demonstration of a consistent effects in terms of reduction of mortality, reduction of infection and

faster ANC and platelets recovery, the intrinsic limits related to the extrapolation of data from a NHP model maintain a grade of uncertainty that cannot be overcome by the evidence of supportive studies in humans and therefore the clinical data are not considered comprehensive. However, these intrinsic limitations could be tolerated, in consideration that more comprehensive data in humans cannot be obtained and in the absence of approved therapies in EU for treatment of H-ARS.

Therefore, the benefit risk balance for sargramostim in the treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with H-ARS **is considered positive in the context of a marketing authorisation under exceptional circumstances.**

3.7.3. Additional considerations on the benefit-risk balance

The CHMP considers the following measures necessary to address the missing safety data in the context of a MA under exceptional circumstances:

- In order to further characterise the efficacy and safety of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall conduct and submit the results of study PTX-01-001, a retrospective observational study to evaluate the efficacy and safety of sargramostim in individuals exposed to myelosuppressive doses of radiation following an ionising radiation event, according to an agreed protocol.
- In order to ensure adequate monitoring of safety and efficacy of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall provide yearly updates on any new information concerning the safety and efficacy of sargramostim.

The CHMP requested a single master protocol for Study PTX-01-001 applicable to all countries in case of a nuclear accident. The applicant agreed and implemented a single master protocol, with details to be finalised after sargramostim approval.

Marketing authorisation under exceptional circumstances

As comprehensive data on the product are not available, a marketing authorisation under exceptional circumstances was requested by the applicant in the initial submission.

The CHMP considers that the applicant has sufficiently demonstrated that it is not possible to provide comprehensive data on the efficacy and safety under normal conditions of use, because the applied indication is encountered so rarely that the applicant cannot reasonably be expected to provide comprehensive evidence and it would be contrary to generally accepted principles of medical ethics to collect such information.

Specifically, the request is considered sufficiently justified based on the unpredictability of the occurrence of the sought indication and on the fact that collection of efficacy data would be contrary to generally accepted principles of medical ethics. Thus, recommending a marketing authorisation under exceptional circumstances is considered appropriate in the interest of public health preparedness for a nuclear/radiological incident.

The agreed post-approval conditions are also considered coherent with the afore-mentioned limitations described before; in particular, the retrospective nature of both studies is accepted given the difficulty in conducting clinical trial in presence of a massive emergency setting.

3.8. Conclusions

The overall benefit/risk balance of Imreplys is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of Imreplys is favourable in the following indication:

> Imreplys is indicated for treatment of patients of all ages acutely exposed to myelosuppressive doses of radiation with Haematopoietic Sub-syndrome of Acute Radiation Syndrome [H-ARS]. Imreplys should be used in accordance with official radiological/nuclear emergency recommendations.

The CHMP therefore recommends the granting of the marketing authorisation under exceptional circumstances subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other conditions and requirements of the marketing authorisation

- **Periodic Safety Update Reports**

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Obligation to conduct post-authorisation measures**

The MAH shall complete, within the stated timeframe, the below measures:

Specific obligation to complete post-authorisation measures for the marketing authorisation under exceptional circumstances

This being an approval under exceptional circumstances and pursuant to Article 14(8) of Regulation (EC) No 726/2004, the MAH shall conduct, within the stated timeframe, the following measures:

Description	Due date
In order to further characterise the efficacy and safety of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall conduct and submit the results of study PTX-01-001, a retrospective observational study to evaluate the efficacy and safety of sargramostim in individuals exposed to myelosuppressive doses of radiation following an ionising radiation event, according to an agreed protocol.	Protocol submission: 30 June 2025 Final study results within 6 months after the use of the product in an incident
In order to ensure adequate monitoring of safety and efficacy of sargramostim in the treatment of acute exposure to myelosuppressive doses of radiation with Haematopoietic Syndrome of Acute Radiation Syndrome (H-ARS), the MAH shall provide yearly updates on any new information concerning the safety and efficacy of sargramostim.	To be submitted as part of the annual re-assessment

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.