PROJECT NO. 0554-17

PROTOCOL

AN ASSESSOR-BLIND, BALANCED, PARALLEL, RANDOMIZED, TWO-TREATMENT, COMPARATIVE IMMUNOGENICITY STUDY OF MULTIPLE DOSES OF INTP5 OF INTAS PHARMACEUTICALS LIMITED, INDIA AGAINST NEULASTA® OF AMGEN INC., USA ADMINISTERED SUBCUTANEOUSLY IN HEALTHY, ADULT, HUMAN SUBJECTS UNDER FED CONDITION

INVESTIGATIONAL MEDICINAL PRODUCTS:		
Test Product-T	**:**	INTP5 (INTP5 is an internal code assigned to Pegfilgrastim manufactured by Intas Pharmaceuticals Limited) Active content: Pegfilgrastim, Strength: 6 mg/0.6 ml Dose for administration: 6 mg/0.6 ml Marketing Authorization Holder: Intas Pharmaceuticals Ltd., India
Reference Product-R	**:**	Neulasta® (Pegfilgrastim) (US Licensed product) Active content: Pegfilgrastim, Strength: 6 mg/0.6 ml Dose for administration: 6 mg/0.6 ml Marketing Authorization Holder: Amgen Inc., Thousand Oaks, California, USA

Version No.	:	2.0		**Principal Investigator:**
Date	:	22 January 2018		[redacted]
Supersedes	:	1.0		
Dated	:	23 August 2017		

Sponsor Address:	**Study centre:**
Intas Pharmaceuticals Ltd, [redacted]	[redacted]

CONFIDENTIAL:

The content of this document as well as the objectives and the results of this study are confidential and must not be made accessible to third parties without permission of Intas Pharmaceuticals Limited (Biopharma Division).

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LIST OF ABBREVIATIONS

Abbreviation	:	Full Name
A/G ratio	:	Albumin/Globulin ratio
°C	:	Degree Celsius
°F	:	Degree Fahrenheit
ADR	:	Adverse Drug Reaction
AE	:	Adverse Event
ALT	:	Alanine Amino Transferase
ANOVA	:	Analysis of Variance
AST	:	Aspartate Amino Transferase
AUC	:	Area under Curve
AUC_{0-t}	:	Area under the serum concentration versus time curve from time zero to the last measurable concentration as calculated by linear trapezoidal method
$AUC_{0-\infty}$	:	Area under the serum concentration versus time curve from time zero to infinity
AUC_% Extrap_obs	:	% of the Area under the Curve that has been derived after Extrapolation or % Residual Area
BA	:	Bioavailability
BE	:	Bioequivalence
ß-hCG	:	Beta-human chorionic gonadotropin
BLQ	:	Below Limit of Quantification
BMI	:	Body Mass Index
CDSCO	:	Central Drugs Standard Control Organization
CL	:	Confidence Limit
C_{max}	:	Maximum Measured Serum Concentration
CNS	:	Central Nervous System
COA	:	Certificate of Analysis
CPMA	:	Clinical Pharmacology and Medical Affairs
CPMP	:	Committee for Proprietary Medicinal Products
CRO	:	Contract Research Organization
C.V.	:	Coefficient of Variation
CVS	:	Cardiovascular System

[redacted]

CS	:	Clinically Significant
DCGI	:	Drug Controller General of India
e-CRF	:	electronic case report forms
e-CTD	:	Electronic Common Technical Document
ECG	:	Electrocardiogram
GCP	:	Good Clinical Practices
GCLP	:	Good clinical laboratory practices
G-CSF	:	Granulocyte -Colony Stimulating Factor
HCV	:	Hepatitis C Virus
HBsAg	:	Hepatitis B surface antigen
HIV	:	Human Immunodeficiency Virus
hr/hrs	:	hour/hours
ICH	:	The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
ICMR	:	Indian Council of Medical Research
ICF	:	Informed consent form
IEC	:	Independent\Institutional Ethics Committee
IMP	:	Investigational Medicinal Product
IMPRAR	:	Investigational Medicinal Product Receipt And Accountability Record
IMPDR	:	Investigational Medicinal Product Dispensing Record
IU	:	International Unit
Kg	:	Kilogram
LAR	:	Legally acceptable representative
ln	:	Logarithmic Value to the Base ‘e’
LC-MS/MS	:	Liquid Chromatography – Tandem Mass spectroscopy
[redacted]	[redacted]	[redacted]
L/hr	:	Liter/hour
m^2	:	Meter Square
Mg	:	Milligram
mL/ml	:	Milliliter

[redacted]

mm Hg	:	Millimeter of Mercury
MSR	:	Medical Screening Record
NTF	:	Note to File
NA	:	Not Applicable
NCS	:	Not Clinically Significant
NR	:	Non Reportable
NSAIDs	:	Non-steroidal Anti-inflammatory Drugs
P/A	:	Postero-anterior
PC	:	Project Coordinator
PI	:	Principal Investigator
QC	:	Quality Control
QA	:	Quality Assurance
RBCs	:	Red Blood Cells
SAE	:	Serious Adverse Event
SAP	:	Statistical Analysis Plan
SAS	:	Statistical Analysis System
SGPT	:	Serum Glutamic Pyruvic Transaminase
SGOT	:	Serum Glutamic Oxaloacetic Transaminase
SOP	:	Standard Operating Procedure
$t_{1/2}$	:	Terminal half-life
T_{max}	:	Time of the maximum measured serum concentration
T/R	:	Test to Reference ratio
USFDA	:	United States Food and Drug Administration
WNL	:	Within Normal Limits

[redacted]

1.0 STUDY SYNOPSIS

1.1	Project number	:	0554-17
1.2	Background	:	Pegfilgrastim is the pegylated form of a colony stimulating factor (Granulocyte colony stimulation factor; G-CSF) that acts on hematopoietic cells by binding to a specific cell surface receptor, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation. The sponsor, Intas Pharmaceuticals Ltd., India, is developing a biosimilar of Pegfilgrastim (INTP5) and is planning to compare the immunogenicity of INTP5 with Neulasta of Amgen Inc, USA. In this study, immunogenicity after multiple doses of INTP5 will be compared with that of Neulasta®.
1.3	Investigational Medicinal Products	:	**Test Product-T** INTP5 (INTP5 is an internal code assigned to Pegfilgrastim manufactured by Intas Pharmaceuticals Limited) Active content: Pegfilgrastim, Strength: 6 mg/0.6 ml Dose for administration: 6 mg/0.6 ml Marketing Authorization Holder: Intas Pharmaceuticals Ltd., India Pharmaceutical form: Pre-filled syringe Route of administration: Subcutaneous Storage: 2ºC - 8ºC **Reference Product-R** Neulasta® (Pegfilgrastim) (US Licensed Product) Active content: Pegfilgrastim, Strength: 6 mg/0.6 ml Dose for administration: 6 mg/0.6 ml Marketing Authorization Holder: Amgen Inc., Thousand Oaks, California, USA Pharmaceutical form: Pre-filled syringe Route of administration: Subcutaneous Storage: 2ºC - 8ºC
1.4	Objective	:	**Primary objectives:** • To assess and compare multiple-dose immunogenicity of INTP5 and Neulasta® in healthy, adult, human subjects.

			Secondary objective: • To assess and compare the safety and tolerability of INTP5 and Neulasta® in healthy, adult, human subjects. • If any subject develops immunogenicity, impact of immunogenicity on pharmacokinetics (PK) and pharmacodynamics (PD) would be evaluated for that subject.
1.5	Study Design	:	An assessor-blind, balanced, parallel, randomized, two-treatment, comparative immunogenicity study of multiple doses of INTP5 of Intas Pharmaceuticals Limited, India against Neulasta® of Amgen Inc., USA (US Licensed Product) administered subcutaneously in healthy, adult, human subjects under fed condition.
1.6	Number of subjects	:	200 Subjects (100 subjects per treatment arm)* Efforts will be made to recruit equal number of healthy male and female volunteers. *Study will be conducted in multiple groups.
1.7	Dose	:	Two doses of Pegfilgrastim 6 mg/0.6 ml [either of the Test product (T) or Reference product (R)] will be administered subcutaneously to the outer area of right upper arm in each subject at an interval of 3 weeks (21 days) between each dose.
1.8	Fasting Criteria	:	**For Each dose:** ➢ Pre-dose: At least 10 hours prior to serving standardized vegetarian breakfast and 1 hour (±10 minutes) after starting of the meal ➢ Post-dose: 4 hours ➢ Further meals will be provided to the subjects at appropriate times during their housing period.
1.9	Water restriction	:	**For Each dose:** ➢ 1 hour pre dose ➢ 1 hour post dose
1.10	Method of administration	:	**For Each dose:** ➢ After an overnight fasting of at least 10 hours, subject will be served standardized vegetarian breakfast which they are required to consume within 30 minutes. Each subject will receive 6 mg/0.6 ml dose of Pegfilgrastim (either test product or reference product) subcutaneously in the outer area of the right upper arm at 1 hour (±10 minutes) after serving standardized vegetarian breakfast in sitting position.

			➢ **Method of drug administration will be as per the procedure defined in a separate drug administration manual.** ➢ Standardized vegetarian breakfast will be provided at 1 hour (±10 minutes) prior to each dose.
1.11	Posture Restriction	:	**For Each dose:** ➢ The study medicine will be administered to subjects while in sitting posture. ➢ Subjects will be in sitting or ambulatory posture for the first 4 hours post dose unless medically necessary due to adverse event or procedurally required or natural exigency; in such cases it would not be considered as protocol deviation. In case of adverse event, appropriate position will be given to the subjects. ➢ Thereafter, the subjects will be allowed to engage only in normal activities while avoiding any strenuous physical activity.
1.12	Housing	:	**For Each dose:** ➢ Pre-dose: 11 hours ➢ Post-dose: 72 hours ➢ Subject will have to stay in the clinical facility for 4 consecutive nights for each dose. ➢ The subjects will have to report to the facility at and after 96 hours post dose for ambulatory blood sample collection for each dose. ➢ Subjects will also have to report to the clinical facility for laboratory estimation (as per details mentioned in Section 13.0) along with complete clinical examination within 3 working days prior to receiving the second dose. ➢ In case of adverse event, subjects may be housed in the clinical facility beyond 72 hours based on the principal investigator's discretion, and necessary action will be taken till the event subsides.
1.13	Clinical Safety Measurements	:	➢ Clinical examination (including recording of vital signs [i.e. sitting blood pressure, radial pulse rate, respiratory rate and oral body temperature]): • Screening • After check-in for each dose • Before checkout of each dose • Within 3 working days prior to dose-2. • End of the study (after last ambulatory sample after dose-2)

(Note: Clinical examination before checkout may be started 120 minutes prior to the schedule time of checkout of each subject).

- Subject will be instructed not to participate in other clinical trial or donate blood anywhere else during the study.
- Vitals (Sitting blood pressure and radial pulse):
  - Pre-dose (within 60 minutes prior to each dose) and at 2, 4, 10, 24, 30, 36, 48 and 60 hrs after each dose.

(Note: All the post-dose vitals will be performed within ± 40 minutes of the scheduled time)

- Well-being: At the time of clinical examinations, during ambulatory samples and at the time of recording of vital signs for each dose.
- Chest X-ray (P/A view; within the last 6 months) at the time of screening.
- 12-lead ECG recording will be performed at the time of screening, within three working days prior to receiving dose-2 and at the end of the study (after last ambulatory sample after dose-2).
- Abdominal ultrasonography will be done at the time of screening.
- Injection site assessment will be performed after 30 minutes, and at 2, 6 and 12 hours of injection after each dose.
- **Injection site scoring (Scoring to be done separately for Pain, Erythema and Induration/Swelling):**

None	0	No reaction
Mild	1	**Pain:** Pain or tenderness causing no or minimal limitation of use of limb **Erythema:** 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities **Induration/Swelling**: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities
Moderate	2	**Pain:** Pain or tenderness causing greater than minimal limitation of use of limb

		Erythema: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities **Induration/Swelling**: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities
Severe	3	**Pain:** Pain or tenderness causing inability to perform usual social & functional activities **Erythema:** ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities **Induration/Swelling**: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities
Potentially life-threatening	4	**Pain:** Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated **Erythema:** Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue) **Induration/Swelling**: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)

- If there is a lump, swelling or bruising at the injection site, subjects will be instructed to consult the investigator.
- Paracetamol or other NSAIDS can be given in case of bone pain or pain in extremities, which is the most common side effect of Pegfilgrastim.

			➢ The needle cover on the single-use prefilled syringe contains dry natural rubber (latex); subjects with latex allergies should not be administered with this product. ➢ Urine Scan for drug of abuse: ▪ Screening and before check-in for each dose ➢ Breath test for alcohol consumption: ▪ Screening and before check-in for each dose ➢ For female subjects, serum pregnancy test will be performed at the time of screening, prior to check-in of each dose and at the end of the study (after last ambulatory sample after dose-2).
1.14	Laboratory Assessments	:	• **Screening:** Hematology, biochemistry, urine analysis, sickling test immunological tests and serum pregnancy test (for female subjects). • **Prior to check-in of each dose:** Serum pregnancy test for female subjects. • **Pre-check-in:** Estimation of hematology (except sickling test), biochemistry and urine analysis will be done within 3 working days prior to receiving dose-2. • **End of the study (after last ambulatory sample after dose-2):** Hematology (except sickling test), biochemistry (except sodium, potassium, calcium, and chloride), and urine analysis and serum pregnancy test (for females).
1.15	Sample Collection	:	Blood samples will be collected through an indwelling intravenous cannula (Venflon) placed in the forearm vein of the subjects. If required, it may also be collected through a fresh vein puncture. Cannula will be removed after collection of blood sample at 24 hrs after each dose. Samples after 24.000 hrs post dose will be collected through fresh vein puncture. 4 ml of blood per sample for PK evaluation, 2 ml of blood per sample for PD evaluation and 8 mL of blood per sample for immunogenicity assessment will be withdrawn using syringe/adaptor at respective time points
1.16	Sampling Schedule	:	A total of 23 blood samples will be collected for PK and 23 blood samples will be collected for PD evaluations. (Note: The last sample of the previous dose and the pre-dose sample of the following dose will be the same). A total of 8 blood samples will be collected for immunogenicity analysis, including sample at screening. **<u>For Pharmacokinetic evaluation:</u>** **1st and 2nd doses:** Venous blood samples (04 ml each) will be withdrawn at pre-dose (0.000) and at 8.000, 16.000, 24.000 (Day 2), 48.000 (Day 3), 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 240.000 (Day 11), 336.000 (Day 15) and 504.000 (Day 22) hours following 1st and 2nd dose

			administration. Blood samples will be collected in a pre-labeled serum separator vacutainer. (Note: Post-dose sample of 1st dose at 504.000 (Day 22) hours will be considered as pre-dose sample of 2nd dose, which should be collected within 5 minutes prior to 2nd dose). **For Pharmacodynamic evaluation:** **1st and 2nd dose:** Venous blood samples (02 ml each) will be withdrawn at pre-dose (0.000) and at 8.000, 16.000, 24.000 (Day 2), 48.000 (Day 3), 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 240.000 (Day 11), 336.000 (Day 15) and 504.000 (Day 22) hours following 1st and 2nd dose administration. Blood samples will be collected in pre-labeled sample collection tubes containing K_2EDTA as anticoagulant. (Note: Post-dose sample of 1st dose at 504.000 (Day 22) hours will be considered as pre-dose sample of 2nd dose, which should be collected within 5 minutes prior to 2nd dose). **For Immunogenicity evaluation:** Venous blood samples (08 ml each) will be withdrawn at screening, pre-dose, and at 336.000 (Day 15, week 2), 504.000 (Day 22, week 3, **within 60 minutes before 2nd dose**), 840.000 (Day 36, week 5), 1176.000 (Day 50, week 7), 1680.000 (Day 71, week 10) and 2016.000 (Day 85, week 12) hours after the first dose. Blood samples will be collected in a pre-labeled serum separator vacutainer. Note: Pre-dose sample will be collected within 60 minutes before the scheduled time. Post- dose in-house blood samples will be collected within ± 02 minutes and ambulatory blood samples will be collected within ± 01 hours from the scheduled time. Samples for PK and PD at and after 96.000 hours after each dose will be collected on an ambulatory basis. Samples for immunogenicity evaluation at 336.000 (Day 15, week 2), 504.000 (Day 22, week 3, **within 60 minutes before 2nd dose**), 840.000 (Day 36, week 5), 1176.000 (Day 50, week 7), 1680.000 (Day 71, week 10) and 2016.000 (Day 85, week 12) hours after the first dose will be collected on an ambulatory basis.
1.17	Total Blood Loss	:	Not exceeding **232 mL** for male subjects and **236 mL** for female subjects, as follows:

			Subjects	
			Male	**Female**
+	Blood volume for PK evaluation (23 samples of 4 mL each).	:	92 mL	92 mL
+	Blood volume for PD evaluation (23 samples of 2 mL each).	:	46 mL	46 mL

			+	Blood volume for the immunogenicity assessment (8 samples of 8 mL each).	:	64 mL	64 mL
			+	Discarded normal saline containing blood (8 × 0.5 mL)	:	4 mL	4 mL
			+	Blood withdrawn for screening prior to study.	:	10 mL	10 mL
			+	Blood withdrawn for post-study safety assessment (including serum pregnancy test in case of female subjects)	:	8 mL	8 mL
			+	Estimation of hematology and biochemistry (within three working days prior to receiving dose-2)	:	8 mL	8 mL
			+	Serum pregnancy test prior to check in each dose (for female subjects).	:	--	4 mL
				Total Blood Loss for each Subject	**:**	**232 mL**	**236 mL**
1.18	Sample handling and processing			Separate laboratory manual will be prepared for pharmacokinetic, Pharmacodynamic and Immunogenicity sample handling, separation process and analytical procedure.			
1.19	Analytical Procedure	:		**<u>For immunogenicity evaluation:</u>** The anti-PegG-CSF antibodies will be detected using a validated screening assay. All samples deemed positive by the screening assay shall be re-assessed using a validated confirmatory assay, and confirmed positive samples will be submitted for neutralizing antibody (NAb) assay. The presence of NAbs to PegG-CSF shall be assessed using a validated NAb assay **<u>For PK evaluation:</u>** Serum samples will be analyzed for Pegfilgrastim using a validated method. Samples from subjects who are positive for immunogenicity will be analyzed. **<u>For PD evaluation:</u>** Pharmacodynamic marker ANC will be analyzed on automated cell counter using a validated method. All subject's samples will be analyzed.			
1.20	Immunogenicity data analysis			Immunogenicity (Anti-drug antibody; ADA) data will be presented for all subjects' samples collected. Descriptive analysis will be provided for immunogenicity (ADA) data. Percentage incidence within <u>+</u> 10% of the expected ADA positivity incidence of Test (6% ADA in Test is anticipated from literature) would not be considered clinically significant.			

			In case any subject develops positive immunogenicity, impact of immunogenicity on PK and PD would be evaluated for that subject. Pooled PD data along with variability will be presented. PK and PD data of individual subjects having positive immunogenicity will be presented separately
1.21	Pharmacokinetic and Pharmacodynamic Parameters	:	If decided (decision based on Immunogenicity results) to analyze PK data by analytical laboratory, PK parameters would be calculated. PD parameters will be estimated for all subjects. Employing the measured concentration vs. time profiles of Pegfilgrastim, the following PK parameters will be calculated for the samples collected after the first dose and second dose, separately: PK parameters: C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, T_{max}, AUC_% Extrap_Obs, R^2adjusted, λ_z and $t_{½}$ Employing the estimated concentration vs. time profiles of absolute neutrophil counts (ANC), the following PD parameters will be calculated based on baseline non-adjusted and baseline-adjusted data for samples collected after the first and second doses, separately: **For ANC (Baseline Non-Adjusted):** E_{max} and $AUEC_{0-t}$ **For ANC (Baseline-Adjusted):** E_{max}, $AUEC_{0-t}$, T_{max}, λ_z and $t_{½}$ The pre-dose levels will be used for the baseline adjustment of the post-dose levels of ANC. Baseline adjustment will be done for both the dosing-related values separately, by subtracting the baseline value (i.e. pre-dose value) from all the pre and post-dose values. If any negative concentrations result will be obtained after adjustment, it would be set to zero.
1.22	Ethical Issues	:	The study will commence only after a written approval is obtained from the Independent\Institutional Ethics Committee. The study will be conducted as per Schedule Y (with subsequent amendments) of CDSCO (Central Drugs Standard Control Organization), Ministry of health and family welfare, Government of India; Ethical guidelines for biomedical research on human participants, Indian Council of Medical Research (2017); ICH (The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) E6 (R2) Guideline for Good Clinical Practice, (2016); Good Clinical Laboratory practices (GCLP); Declaration of Helsinki (Brazil, October 2013).

2.0 INVESTIGATOR'S DECLARATION

I, the undersigned, have read and understood this protocol and hereby agree to conduct the study in accordance with this protocol and to comply with all requirements regarding the obligations of investigators and all other pertinent requirements of Schedule Y (with subsequent amendments) of CDSCO (Central Drugs Standard Control Organization), Ministry of health and family welfare, Government of India; Ethical guidelines for biomedical research on human participants, Indian Council of Medical Research (2017); ICH (The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) E6 (R2) Guideline for Good Clinical Practice, (2016); Good Clinical Laboratory practices (GCLP); Declaration of Helsinki (Brazil, October 2013).

I agree to comply with all relevant SOPs required for the conduct of this study. I further agree to ensure that all associates assisting in the conduct of study are informed regarding their obligations.

[redacted]

23/JAN/18

Date

Principal Investigator

[redacted]

[redacted]

3.0 SPONSOR'S APPROVAL

I, on behalf of **Intas Pharmaceuticals Ltd, India,** have read, understood and approved this Protocol. I agree to comply with all requirements regarding the obligations of Sponsor and all other pertinent requirements of Schedule Y (with subsequent amendments) of CDSCO (Central Drugs Standard Control Organization), Ministry of health and family welfare, Government of India; Ethical guidelines for biomedical research on human participants, Indian Council of Medical Research (2017); ICH (The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) E6 (R2) Guideline for Good Clinical Practice, (2016); Good Clinical Laboratory practices (GCLP); Declaration of Helsinki (Brazil, October 2013).

[redacted]

22/01/18

Authorized signatory

Date

Name : [redacted]

Address :

Tel. No. :

Fax No. :

E-mail :

[redacted]

4.0 MEDICAL EXPERT

4.1 Sponsor's Medical Expert:

Name	:	[redacted]
Address	:	
Tel. No.	:	
Fax No.	:	

4.2 Sponsor's Safety Expert:

Name	:	[redacted]
Address	:	
Tel. No.	:	
Fax No.	:	

5.0 FACILITIES

5.1 Clinical facility, Pharmacodynamics, Biostatistics and programming, Clinical Data management, Quality Assurance and clinical safety laboratory Services:

Address	:	[redacted]
Tel. No.	:	
Fax No.	:	

Note: In case of emergency or adverse event management, back-up contractual lab can be used for lab investigations, if required. Monitoring, pharmacokinetic analysis and immunogenicity may also be done at [redacted]

[redacted]

6.0 BACKGROUND INFORMATION

6.1 Investigational Medicinal Products

6.1.1 Test Product-T

INTP5 (INTP5 is an internal code assigned to Pegfilgrastim manufactured by Intas Pharmaceuticals Limited)

Active content: Pegfilgrastim, Strength: 6 mg/0.6 ml

Dose for administration: 6 mg/0.6 ml

Marketing Authorization Holder: Intas Pharmaceuticals Ltd., India

Pharmaceutical form: Pre-filled syringe

Route of administration: Subcutaneous

Storage: 2ºC - 8ºC

6.1.2 Reference Product–R

Neulasta® (Pegfilgrastim) (US Licensed Product)

Active content: Pegfilgrastim, Strength: 6 mg/0.6 ml

Dose for administration: 6 mg/0.6 ml

Marketing Authorization Holder: Amgen Inc., Thousand Oaks, California, USA

Pharmaceutical form: Pre-filled syringe

Route of administration: Subcutaneous

Storage: 2ºC - 8ºC

6.2 Investigational Medicinal Product Summary [1, 2]

6.2.1 General pharmacology

Pegfilgrastim is the pegylated form of G-CSF that acts on hematopoietic cells by binding to a specific cell surface receptor, thereby stimulating proliferation, differentiation, commitment, and end cell functional activation. Human G-CSF is a glycoprotein, which regulates the production and release of neutrophils from the bone marrow. Pegfilgrastim is a covalent conjugate of recombinant human G-CSF (r-met HuG-CSF) with a single 20 kD polyethylene glycol (PEG) molecule. Pegfilgrastim is a sustained duration form of filgrastim due to decreased renal clearance.

6.2.2 Absorption, Distribution, Metabolism, Excretion and Food Effects

After a single subcutaneous dose of Pegfilgrastim, the peak serum concentration of Pegfilgrastim occurs at 16 to 120 hours after dosing and serum concentrations of Pegfilgrastim are maintained during the period of neutropenia after myelosuppressive chemotherapy. The elimination of Pegfilgrastim is non-linear with respect to dose; serum clearance of Pegfilgrastim decreases with increasing dose. Pegfilgrastim appears to be mainly eliminated by neutrophil-mediated clearance, which becomes saturated at higher doses. Consistent with a self-regulating clearance mechanism, the

serum concentration of Pegfilgrastim declines rapidly at the onset of neutrophil recovery. Due to the neutrophil-mediated clearance mechanism, the pharmacokinetics of Pegfilgrastim is not expected to be affected by renal or hepatic impairment. The half-life of Pegfilgrastim ranges from 15 to 80 hours after subcutaneous injection.

6.2.3 Adverse Effects

Blood and lymphatic system disorders: Common: Thrombocytopenia, leukocytosis

Uncommon: Sickle cell crisis, leukocytosis, splenomegaly, splenic rupture

Immune system disorders: Uncommon: Hypersensitivity reactions (including skin rash, urticaria, angioedema, dyspnoea, flushing and hypotension), serious allergic reactions including anaphylaxis, splenomegaly and splenic rupture

Metabolism and nutrition disorders: Uncommon: Elevations in uric acid

Nervous system disorders: Very common: Headache

Vascular disorders: Uncommon: Capillary leak syndrome

Respiratory, thoracic and mediastinal disorders: Uncommon: Adult respiratory distress syndrome, pulmonary adverse reactions (interstitial pneumonia, pulmonary edema, pulmonary infiltrates and pulmonary fibrosis)

Gastrointestinal disorders: Very common: Nausea

Skin and subcutaneous tissue disorders: Uncommon: Sweet's syndrome (acute febrile dermatosis), cutaneous vasculitis

Musculoskeletal and connective tissue disorders: Very common: Bone pain

Common: Musculoskeletal pain (myalgia, arthralgia, pain in extremity, back pain, neck pain)

General disorders and administrative site conditions: Common: Injection site pain, non-cardiac chest pain

Uncommon: Injection site reactions

Investigations: Uncommon: Elevations in lactate dehydrogenase and alkaline phosphatase, transient elevations in LFT's for ALT or AST.

Renal and urinary disorders: Uncommon: Glomerulonephritis

6.2.4 Indications

Pegfilgrastim is approved by FDA for the following indications:

(1) To decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia; and

(2) To increase survival in patients acutely exposed to myelosuppressive doses of radiation (Hematopoietic Subsyndrome of Acute Radiation Syndrome).

6.2.5 Contraindications

Patients with a known hypersensitivity to *E coli*-derived proteins, Pegfilgrastim, filgrastim or any other component of the product.

6.2.6 Dosage

Recommended dose: One 6 mg dose (a single prefilled syringe) of Pegfilgrastim is recommended for each chemotherapy cycle, given at least 24 hours after cytotoxic chemotherapy.

Proposed dose for the study: Two doses of Pegfilgrastim 6 mg/0.6 ml [either of Test product or Reference product] will be administered subcutaneously to the outer area of the upper right arm to each subject at an interval of 3 weeks (21 days) between each dose.

The needle cover on the single-use prefilled syringe contains dry natural rubber (latex); persons with latex allergies should not be administered this product.

The recommended dosage of Pegfilgrastim is a single subcutaneous injection of 6 mg administered once per chemotherapy cycle.

6.3 Risks and Benefits

Participation in this study yields no direct benefit to the subjects. The risks as described above are reduced considering the fact that only two doses are to be administered at an interval of not less than 3 weeks (21 days) between each dose. However, study-related health assessments are provided at no cost.

6.4 Rationale

The objective of this study is to assess and compare the immunogenicity profile of INTP5 of Intas Pharmaceuticals Ltd., India and Neulasta® of Amgen Inc, USA after multiple dose administration of Pegfilgrastim in healthy, adult, human subjects.

Through this study, we will prospectively collect the data on healthy subjects that are randomly assigned to receive either INTP5 or Neulasta® for the immunogenicity profiling. No additional tests apart from those already specified in this protocol, will be performed. The study will, therefore, not place any additional risk/burden on the subjects.

Choice of the Pegfilgrastim dose:

Each pre-filled syringe contains 6 mg of Pegfilgrastim in 0.6 ml solution for injection. The protein concentration is 10 mg/ml. One 6 mg dose (a single pre-filled syringe) of Pegfilgrastim is recommended for each chemotherapy cycle, administered as a subcutaneous injection approximately 24 hours following cytotoxic chemotherapy.

In this study, 6 mg dose will be evaluated to determine safety.

Choice of the injection site:

Outer area of the upper arms, abdomen, except for the two-inch area around the navel, front of the middle thighs and upper outer areas of the buttocks are easy accessible areas for subcutaneous injection. According to the Neulasta prescribing information, above mentioned site can be used for injection. With the aim to standardize the injection procedure through the clinical study, the area

around the outer area of the right upper arm will be used for all subjects for the Pegfilgrastim injection.

7.0 STUDY OBJECTIVES

7.1 Primary objectives

- To assess and compare multiple-dose immunogenicity of INTP5 and Neulasta® in healthy, adult, human subjects.

7.2 Secondary objectives

- To assess and compare the safety and tolerability of INTP5 and Neulasta® in healthy, adult, human subjects.
- If any subject develops immunogenicity, impact of immunogenicity on PK/PD would be evaluated for that subject.

8.0 STUDY DESIGN

8.1 Design

An assessor-blind, balanced, parallel, randomized, two-treatment, comparative immunogenicity study of multiple doses of INTP5 of Intas Pharmaceuticals Limited, India against Neulasta® of Amgen Inc., USA administered subcutaneously in healthy, adult, human subjects under fed condition.

8.2 Numbers of subjects

As per the data from prescribing information, 6% patients develop antibodies to Pegfilgrastim. Hence, we hypothesize that 94% patients will not develop antibodies, and based on this assumption, the sample size has been calculated. For a comparison of two independent binomial proportions using Pearson's Chi-square statistic with a Chi-square approximation with a two-sided significance level of 5%, 89 completers per group achieves a power of at least 80% when the proportions are 94% for both test and reference and the null proportion difference is assumed as 10%.

Considering dropouts/withdrawals, approximately 100 subjects per treatment group (approximately 200 subjects for two treatment groups)***** will be required.

Subsequent dropouts if any after first dosing will not be replaced.

*Study will be conducted in multiple groups.

Note: Two extra subjects if available may be enrolled on the day of check-in for first dose in each group to compensate for any dropout prior to first dosing of each group. If there are no dropouts, these two subjects will be checked out without being dosed after completion of first dosing for each group.

8.3 Randomization

The allocation to test (T) or reference (R) product for each subject during the study will be determined according to a balanced randomization schedule. The randomization schedule will be generated using SAS® Version 9.3 or higher (SAS Institute Inc., USA) by the biostatistician.

Equal allocation of subjects in each treatment arm (i.e. ‘T’ or ‘R’) will be ensured.

The personnel involved in dispensing of study drug and verification of dispensed study drugs will be accountable for ensuring compliance to the randomization schedule.

This is an assessor-blind study so coded treatment blinding is not required.

8.4 Blinding

The study staff taking care of subject's safety and the laboratory personnel doing the sample analysis of pharmacokinetic, pharmacodynamic and immunogenicity data will be blinded.

A list of blinded and un-blinded team members of the trial will be prepared and documented.

8.5 Housing

Subjects will be housed in the clinical facility at least 11 hours before administration of the dose and will continue to remain in the clinical facility for at least 72 hours after each administration of investigational medicinal product.

The subjects will have to stay in the facility for 4 consecutive nights in each dose.

The subjects will have to report to the facility at and after 96 hours post dose for ambulatory blood sample collection in each dose.

Subjects will also have to report to the clinical facility for laboratory estimation (as per details mentioned in Section 13.0) along with complete clinical examination within 3 working days prior to receiving the second dose.

In case of adverse event, subjects may be housed in the clinical facility beyond 72 hours based on the principal investigator's discretion and necessary action will be taken till the event subsides.

8.6 Duration of Fasting and Distribution of Meals

All subjects will be required to fast overnight for at least 10 hours prior to serving of standardized vegetarian breakfast and 4 hours post dose for each dose; drug administration will be at 1 hour (±10 minutes) after starting of the meal.

Standardized meal will be served to the subjects at appropriate times during their stay in the clinical facility. The contents of the meals served during each dose at various time points will be identical. The subjects will receive lunch at least 4 hours after dosing and further meals will be served at appropriate intervals from then on, until check-out. Information on the amount of meal consumed and the time taken for consuming the meal will be recorded in the source data forms. The actual time of meal distributions will also be recorded. If meals and blood sample collection schedules coincide, samples will be collected at the scheduled time only.

Subjects will refrain from drinking water from 1 hour before till 1 hour after each dosing. Prior to and thereafter, water shall be consumed as required.

Non-compliance to above fasting and water restriction will be recorded as protocol deviation.

Note: In case any subject has any adverse event and requires any change in diet, it will be done after consultation with the Principal Investigator. It will not be considered as protocol deviation. This will however be documented.

8.7 Restrictions

8.7.1 Medications

Paracetamol or other NSAIDS can be given in case of bone pain or pain in extremities which is the most common side effect of Pegfilgrastim. Dose administered will be documented. No other prescribed medicines other than paracetamol or NSAIDs for pain and the investigational medicinal product will be allowed within 1 month prior to first dosing until the last sample collection of trial for each group. Also, no over-the-counter (OTC) medicines will be allowed during the study until the last sample collection of trial, except vitamins, minerals and nutritional supplements that may be taken at the discretion of the Investigator. If drug therapy other than that specified in the protocol is required prior to or during the study including wash-out period (i.e. dosing interval), decisions shall be taken by the Principal Investigator to continue or discontinue the subject based on the following:

a) The pharmacology, immunogenicity impact, pharmacodynamics and pharmacokinetics of the non-study medication.

b) The likelihood of a drug–drug interaction, thereby affecting the immunogenicity, pharmacodynamic and pharmacokinetic comparison of Investigational medicinal products.

c) The time and duration of administration of the non-study medicine.

All such instances will be recorded and reported in the final report.

Note: Any concomitant medication administered during the course of the trial will be documented appropriately in the source data forms.

8.7.2 Diet

All subjects will be instructed to abstain from any xanthine-containing food or beverages (like tea, coffee, chocolates or cola drinks), tobacco, tobacco-containing products (like pan, pan masala, gutkha) and smoking (beedi, cigarette) for 24 hours prior to each dose administration and throughout their stay in the clinical facility for each dose.

Subject will be instructed to abstain from grapefruit, grapefruit products, recreational drugs, alcohol and alcoholic products from 72 hours prior to first IMP administration until the end of the study.

Subjects should be instructed that they should not consume, an unusual diet, for whatever reason (e.g. low-sodium), for 4 weeks prior to receiving the first dose of study medicine till the completion of study.

8.7.3 Postural Restrictions

For Each dose:

The study medicine will be administered to subjects while in sitting posture.

Subjects will be in sitting or ambulatory posture for the first 4 hours post dose unless medically necessary due to adverse event or procedurally required or natural exigency; in such cases it would not be considered as protocol deviation. In case of adverse event, appropriate position will be given to the subjects.

Thereafter, the subjects will be allowed to engage only in normal activities while avoiding any strenuous physical activity.

8.8 Dosing Procedures and Compliance Assessment

8.8.1 Dosing

Each dose administration as described below will be done under the supervision of trained study personnel.

After an overnight fasting of at least 10 hours, subject will be served standardized vegetarian breakfast which they are required to consume within 30 minutes. Each subject will receive the dose of Pegfilgrastim 6 mg/0.6 ml [either test product or reference product] subcutaneously in the outer area of the right upper arm at 1 hour (±10 minutes) after serving standardized vegetarian breakfast in sitting position.

The drug delivery device should be stored and should be destroyed only after written permission from the sponsor.

Details related to the method of drug administration is as per the procedure defined in a separate drug administration manual.

Standardized vegetarian breakfast will be provided at 1 hour (±10 minutes) prior to each dose.

8.8.1.1 Test Product-T

Single dose of INTP5 of Intas Pharmaceuticals Ltd., India will be administered subcutaneously in the outer area of the right upper arm to the subjects at a dose of 6 mg/0.6 ml in sitting posture at ambient temperature by the trained study personnel.

8.8.1.2 Reference Product-R

Single dose of Neulasta® of Amgen Inc; USA will be administered subcutaneously in the outer area of the right upper arm to the subjects at a dose of 6 mg/0.6 ml in sitting posture at ambient temperature by the trained study personnel.

8.8.2 Assessment of compliance for dosing

Compliance for dosing will be assessed by supervision of the entire dosing procedure by the trained study personnel.

8.9 Blood Sampling for Pharmacokinetic/ Pharmacodynamics/ Immunogenicity Analysis

8.9.1 Procedure of Blood Sampling and Collection

Blood samples will be collected through an indwelling intravenous cannula (Venflon) placed in the forearm vein of the subjects. If required, it may also be collected through a fresh vein puncture.

Pain and redness may occur at the injection site. If there is a lump, swelling or bruising at the injection site that does not go away, subjects may consult the investigator.

Pre-dose sample will be collected within 60 minutes before the scheduled time. Post-dose in-house blood samples will be collected within ± 2 minutes, and ambulatory blood samples will be collected within ± 1 hours from scheduled time. Samples for pharmacodynamic and pharmacokinetic at and

after 96 hours after each dose will be collected on an ambulatory basis. The actual time of collection of each blood sample will be recorded immediately after blood collection.

The blood samples will be kept at room temperature during sample collection activity. Post-dose samples not collected within this time frame from the scheduled time will be documented as sampling deviations.

Intravenous indwelling cannula will be kept in situ as long as possible by injecting 0.5 mL of normal saline solution to maintain the cannula patent for collection of the all blood sample from pre-dose to post-dose samples up to 24 hours. In such cases, blood samples will be collected after discarding the first 0.5 mL of normal saline containing blood from the tubing. The blood samples will be collected using syringe or adaptor and transferred into appropriate different pre-labelled (mentioning Project number, Subject number, Sample ID No./Bar code ID no and sampling time point) serum separator vacutainer (for PK and immunogenicity evaluation) and/or K_2EDTA sample collection tubes (for PD evaluation).

Alternatively, if the cannula is blocked or there is difficulty in withdrawing blood through the cannula, blood samples may be withdrawn by a fresh vein puncture using a disposable sterile syringe and a needle at each time of collection. Cannula will be removed after collection of blood sample at 24 hrs after each dose. Samples after 24.000 hrs post dose will be collected through fresh vein puncture. The ambulatory samples, pre-check in sample for serum pregnancy test (for female subjects) in each dose and sample to be collected for laboratory estimation within 3 working days prior to receiving dose-2 will be collected by a fresh vein puncture.

Not exceeding **232 mL** for male subjects and **236 mL** for female subjects as follows:

			Subjects	
			Male	**Female**
+	Blood volume for Pharmacokinetic evaluation (23 samples of 4 mL each).	:	92 mL	92 mL
+	Blood volume for Pharmacodynamic evaluation (23 samples of 2 mL each).	:	46 mL	46 mL
+	Blood volume for the Immunogenicity assessment (8 samples of 8 mL each).	:	64 mL	64 mL
+	Discarded normal saline containing blood (8 × 0.5 mL)	:	4 mL	4 mL
+	Blood withdrawn for screening prior to study.	:	10 mL	10 mL
+	Blood withdrawn for post-study safety assessment (including serum pregnancy test (in case of female subjects)	:	8 mL	8 mL
+	Estimation of hematology and biochemistry (within three working days prior to receiving dose-2)	:	8 mL	8 mL
+	Serum pregnancy test prior to check in each dose (for female subjects).	:	--	4 mL
Total Blood Loss for each Subject		**:**	**232 mL**	**236 mL**

8.9.2 Sampling Schedule

The venous blood samples will be withdrawn at the following times, assuming that the dosing of a subject takes place at 0900.

For PK/PD evaluation:

Days	Time points (hour)	Proposed clock time	Blood volume
	PK/PD evaluation		
1	Pre-dose (0.000)	Within 60 minutes prior to dosing	06 ml (04 ml for PK and 02 ml for PD evaluation)
	8.000	1700	
2	16.000	0100	
	24.000	0900	
3	48.000	0900	
4	72.000	0900	
5	96.000*	0900	
6	120.000*	0900	
7	144.000*	0900	
11	240.000*	0900	
15	336.000*	0900	
22	504.000*	0900	

For Immunogenicity evaluation:

Days	Time points (hour)	Proposed clock time	Blood volume
	Immunogenicity evaluation		
Within 28 days before dosing	Screening	NA	8 ml
1	Pre-dose (0.000)	Within 60 minutes prior to dosing	
15	336.000*	0900	
22	504.000	0900	
36	840.000*	0900	
50	1176.000*	0900	

71	1680.000*	0900	
85	2016.000*	0900	

* Ambulatory samples

Day 1: Day of dosing.

Day 2-7, 11, 15, 22, 36, 50, 71, 85: Subsequent days after the day of dosing.

8.10 Sample Handling and processing

Separate laboratory manual will be prepared for pharmacokinetic, Pharmacodynamic and Immunogenicity sample handling, separation process and analytical procedure.

PK samples will be stored till immunogenicity evaluation is complete. PK analysis will be performed for only immunogenicity positive subjects. A validated assay will be used to analyze PK samples.

8.11 Clinical Safety Measures

A physician will be available within the clinical facility whenever the subjects are housed (from check-in to checkout in each dose) and at the end of the study (after last ambulatory sample after dose-2). A physician will be available on call during ambulatory sample after each dosing. A consultant physician will be always available on call during the study period.

Clinical examination of the subjects [including recording of vital signs (i.e. sitting blood pressure, radial pulse rate, respiratory rate and oral body temperature)] will be done at screening, after check-in and before check-out of each dose, within 3 working days prior to dose-2 and at the end of the study (after last ambulatory sample after dose-2). The clinical examination before check-out in each dose may be started 120 minutes prior to the scheduled time of check-out of each subject.

Subjects should meet the criteria for enrolment in the study.

Subject will be instructed not to participate in other clinical trial or donate blood anywhere else during the study.

Chest X-ray (P-A view; within the last 6 months) will be done at the time of screening.

12-lead ECG recording will be performed at the time of screening, within 3 working days prior to receiving dose-2 and at the end of the study (after last ambulatory sample after dose-2).

Abdominal ultrasonography will be done at the time of screening.

Vital signs (Sitting blood pressure and radial pulse) will be measured at pre-dose (within 60 minutes prior to each dose) and at 2, 4, 10, 24, 30, 36, 48 and 60 hours after each dose.

All post-dose vitals will be recorded within ± 40 minutes from the scheduled time.

Subjects will be questioned for well being at the time of clinical examinations, during ambulatory samples and at the time of recording of vital signs for each dose.

Injection site assessment will be performed after 30 minutes and at 2, 6 and 12 hours of injection after each dose.

Injection site scoring (Scoring to be done separately for Pain, Erythema and Induration/Swelling):

None	0	No reaction
Mild	1	**Pain:** Pain or tenderness causing no or minimal limitation of use of limb **Erythema:** 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities **Induration/Swelling**: 2.5 to < 5 cm in diameter OR 6.25 to < 25 cm^2 surface area AND Symptoms causing no or minimal interference with usual social & functional activities
Moderate	2	**Pain:** Pain or tenderness causing greater than minimal limitation of use of limb **Erythema:** ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities **Induration/Swelling**: ≥ 5 to < 10 cm in diameter OR ≥ 25 to < 100 cm^2 surface area OR Symptoms causing greater than minimal interference with usual social & functional activities
Severe	3	**Pain:** Pain or tenderness causing inability to perform usual social & functional activities **Erythema:** ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities **Induration/Swelling**: ≥ 10 cm in diameter OR ≥ 100 cm^2 surface area OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage OR Symptoms causing inability to perform usual social & functional activities
Potentially life-threatening	4	**Pain:** Pain or tenderness causing inability to perform basic self-care function OR Hospitalization indicated

		Erythema: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue) **Induration/Swelling**: Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)

If there is a lump, swelling or bruising at the injection site that does not go away, subjects may consult the investigator.

Paracetamol or other NSAIDS can be given in case of bone pain or pain in extremities, which is the most common side effect of Pegfilgrastim.

The needle cover on the single-use prefilled syringe contains dry natural rubber (latex); subjects with latex allergies should not be administered this product.

Laboratory assessments will be carried out at the time of screening and within 3 working days to receive dose 2.

Serum pregnancy test for the female subjects will be done at the time of screening, prior to check-in of each dose and at the end of the study (after last ambulatory sample after dose-2).

Breath test for alcohol consumption and urine scan for drugs of abuse will be carried out at Screening and prior to check-in of each dose.

Laboratory tests for hematology (except Sickling Test), Biochemistry (except sodium, potassium, calcium, and chloride), urine analysis and Serum pregnancy test (for females) will be done at the end of the study (after last ambulatory sample after dose-2).

8.12 Termination of the Study

[redacted] (for safety reasons) and the sponsor reserve the right to discontinue the study at any time. The sponsor and the IEC will be immediately informed in case the study is terminated by [redacted] Reasons for this termination will be provided to the subjects. The study may be terminated by the IEC (Independent\Institutional Ethics Committee) if there are major violations of ethical considerations or due to any serious adverse event.

9.0 HANDLING, STORAGE, DISPENSING AND ACCOUNTABILITY PROCEDURES FOR INVESTIGATIONAL MEDICINAL PRODUCTS

9.1 Investigational Medicinal Product Receipt and Storage

The Sponsor shall supply adequate units of investigational medicinal product for dose administration and retention purpose. The received investigational medicinal products will be verified for the sealed condition of packs and adequacy of the label, including product name, strength, number of dosage units, lot number or batch number, expiry date/retest date and storage condition mentioned clearly.

The Pharmacy custodian or his/her designated study personnel will receive the investigational medicinal products with certificates of analysis (COA). The investigational medicinal products will be transferred to the pharmacy, after labeling it for project number (as applicable), product type, quantity

and date of receipt, batch number or lot number, manufacturing date, expiry date/retest date, for clinical study use only and storage conditions.

A pre-defined quantity of drug will be randomly identified and stored as retention samples.

Note: Information related to manufactured by/for, marketed/distributed by, marketing authorization holder etc. are based on details provided by the sponsor and/or available literature at the time of protocol development. Exact details will be captured in the investigational medicinal product (IMP) receipt and accountability form by the pharmacy while receiving the IMPs. The same details can be used in the clinical study report for accuracy and compliance purpose.

9.2 Dispensing

The Pharmacy Custodian and Project Coordinator or his/her designated study personnel will dispense the required units of investigational medicinal products before dosing. Six additional units of IMPs (three test and three reference product) will be dispensed in addition to the required number and labeled accordingly. These will be used in any situation such as dropping the IMP etc., if unused they will be handled as described in Section 9.3 (Unused Investigational Medicinal Products). The remaining units of investigational medicinal products will be kept in their original containers as retention samples.

Investigational medicinal product (test and reference products) will be transferred from pharmacy to the clinical facility prior to dose administration along with data logger as per in-house SOP. Dispensing will be performed in the clinical facility.

- The strengths of the solution will be 6 mg/0.6 ml
- Pegfilgrastim should be protected from light, so keep it in its carton until ready to use it.
- Do not leave Pegfilgrastim in direct sunlight.

Note: Pegfilgrastim should be stored in the refrigerator at 2°C to 8°C, but not in the freezer.

The doses intended for administration to the subjects will be transferred to the drug-dispensing containers as per the randomization schedule, and properly labeled for the drug name with strength, dose, product type, project number, subject number, for clinical trial use only and [redacted] Ahmedabad.

The personnel involved in dispensing and verification of dispensed IMPs will be accountable for ensuring compliance to randomization schedule. The Pharmacy Custodian/designate will maintain complete accountability of the investigational medicinal products for the study.

9.3 Unused Investigational Medicinal Products

Units of investigational medicinal products that have not been dispensed will be retained in their original containers. Any product that had been dispensed but not used (e.g. due to the subject being unwell or drop out from the trial etc.) will be labeled as ‘Not For Use’ and returned to the pharmacy and will be retained along with the other investigational medicinal products of its type.

9.4 Retention Samples

Sufficient quantity of retention samples as communicated by the sponsor will be stored. Each reserved sample should be retained and stored under conditions consistent with the product labeling for a period

of at least 5 years following the date on which the study is approved by USFDA or, if the study is not approved, at least 5 years following the date of completion of the study in which the investigational medicinal products were used.

The sponsor will provide [redacted] with a supply of study drug and reference standard sufficient to complete the study and retain the appropriate number of dosage units as reserve samples. [redacted] will randomly select the retention samples from the supply sent by the sponsor.

10.0 SELECTION & WITHDRAWAL CRITERIA

All subjects will undergo physical and clinical screening procedure within 28 days prior to the first dose of IMP administration. The subjects will be selected based on the following inclusion and exclusion criteria.

10.1 Inclusion Criteria

1. Normal, healthy adult human volunteers between 18 to 45 years of age (both inclusive) living in and around Ahmedabad city or western part of India.
2. Having body weight ≥50 kg and body mass index (BMI) between 18.5 and 29.9 (both inclusive), calculated as weight in kg/height in meter2.
3. Not having any significant disease in medical history or clinically significant abnormal findings during screening, abdominal ultrasonography, medical history, clinical examination, laboratory evaluations, 12-lead ECG and X-ray chest (P/A view; within the last 6 months) recordings.
4. Able to understand and comply with the study procedures, in the opinion of the investigator.
5. Able to give voluntary written informed consent for participation in the trial.
6. In case of female subjects:
    a. Surgically sterilized at least 6 months prior to study participation;

       Or

       If a woman of child bearing potential is willing to use a suitable and effective double barrier contraceptive method or intra uterine device during the study.

    b. Serum pregnancy test (for female subjects) must be negative.

10.2 Exclusion Criteria

1. Known hypersensitivity to the study drug or its constituents and/or hypersensitivity to *E. coli*-derived proteins, and/or previous exposure to the study drug.
2. History or presence of any disease or condition which might compromise the haemopoietic, renal, hepatic, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal or any other body system.
3. Known case of hereditary fructose intolerance.
4. Subjects with latex allergies will be excluded as the needle cover on the single-use prefilled syringe contains dry natural rubber (latex).

5. Any clinically significant laboratory finding including ANC, platelet, RBC count, and hemoglobin level at the time of screening.
6. Prior exposure to any peptide colony stimulating or growth factor, including erythropoietin, filgrastim or Pegfilgrastim; Prior exposure to any vaccines, immunoglobulin preparations or immunomodulators within the past 6 months prior to receiving first dose; evidence of *E coli* diarrhea or diseases within 3 months.
7. Any history or presence of asthma (including aspirin-induced asthma) or nasal polyp or NSAIDs induced urticaria.
8. Subjects with a history of pulmonary infiltrate or pneumonia in the last 6 months.
9. History of any hematologic disease including sickle cell disorders.
10. Ingestion or use of any prescribed medication at any time within 1 month prior to receiving first dose.
11. Receipt of over-the-counter medicines which have not yet cleared from the body (5 half-lives must have passed for the medicine to be considered to have cleared from the body).
12. A recent history of harmful use of alcohol, i.e. alcohol consumption of more than 14 standard drinks per week for men and more than 7 standard drinks per week for women (A standard drink is defined as 360 ml of beer or 150 ml of wine or 45 ml of 40% distilled spirits, such as rum, whisky, brandy etc.) or consumption of alcohol or alcoholic products within 72 hours prior to receiving study medicine.
13. Smokers, who smoke 10 or more than 10 cigarettes/day or inability to abstain from smoking during the study.
14. Use of any recreational drugs or history of drug addiction or testing positive in pre-study drug scans.
15. Donation of blood (1 unit or 350 mL) or equivalent amount of blood substitute.
16. Receipt of an investigational medicinal product or participation in a drug research study within a period of 90 days prior to the first dose of study medication. Elimination half-life of the study drug should be taken into consideration for inclusion of the subject in the study.
17. Positive result for human immunodeficiency virus (HIV I &/or II) and/or hepatitis B and C tests.
18. History or presence of cancer because of which anticipated life span is less than 5 years as per the investigator's assessment.
19. History or presence of psychiatric disorders.
20. Presence of tattoo or scars or any type of skin lesions due to infection, burning, wound or inflammation at the proposed site of injection.
21. An unusual diet, for whatever reason (e.g. low-sodium), for 4 weeks prior to receiving the study medicine. In any such case, subject selection will be at the discretion of the Principal Investigator.
22. Consumption of grape fruit or grape fruit products within 72 hours prior to receiving study drug.
23. A history of difficulty in donating blood.

24. Females, pregnant or lactating, or planning to become pregnant during the time the subject is on study or found positive in pregnancy test at screening.
25. Any infections in the last 4 weeks before receiving study medication.

10.3 Withdrawal Criteria

The investigator may withdraw a subject from the study for any of the following:

a) The subject suffers from significant inter-current illness or undergoes surgery during the course of the study or the subject has any significant symptoms or signs during the course of the study.

b) Adverse event: Subject reports symptoms, which are considered unacceptable by the subject and/or the Investigator and due to safety concern or the nature of adverse event subject is not fit to continue in the trial.

c) Any subject found to have entered the study in violation of this protocol. This would include pre-study directions regarding alcohol and drug use, fasting/fed or if the subject is uncooperative during the study. The individual details of violation of the protocol will be discussed with the sponsor. The final decision will be taken based on the impact of the protocol violation on the primary endpoint and safety. The decision should be taken in blinded manner before the exposure to the data on the key endpoints.

d) Any subject found to hide important medical history which in opinion of Principal Investigator may compromise his/her safety during participation in this study.

e) Any subject found as cross-participated in other drug trial or trial screening.

f) Any subject who requires the use of an unacceptable concomitant medication (prescription medication other than paracetamol or NSAIDS for pain and OTC medicine). The individual details of such concomitant medications will be discussed with the sponsor. The final decision will be taken based on the impact of the concomitant medications on the primary endpoint and safety. The decision should be taken in blinded manner before the exposure to the data on the key endpoints.

g) If it is felt in Principal Investigator's opinion that it is not in the subject's best interest to continue.

h) Any subject who wishes to withdraw his/her consent for whatever reason.

i) Any other justifiable reason, which would be adequately documented.

j) Found positive in serum pregnancy test (for female subjects)

All instances of subject withdrawal, including the date and reason for withdrawal, will be documented and handled as per the in-house procedure. Any untoward effect reported by the subjects who withdraw will be incorporated into the final study report. These subjects will be followed up for their safety as per in-house SOP**.**

11.0 EVALUATION PARAMETERS

11.1 Immunogenicity, Pharmacokinetic and Pharmacodynamic Analyses

A detailed statistical analysis plan (SAP) will be prepared prior to database lock.

Immunogenicity and safety analysis will be performed using SAS® Version 9.3 or higher (SAS Institute Inc., USA).

Pharmacokinetic and pharmacodynamic analysis will be performed on the available concentration data/ANC count of all the subjects.

11.2 Immunogenicity Data Analysis

Immunogenicity (anti-drug antibody; ADA) data will be presented for all subjects' samples collected.

Descriptive analysis will be provided for immunogenicity (ADA) data.

Percentage incidence within + 10% of the expected ADA positivity incidence of Test (6% ADA in Test is anticipated from literature) would not be considered clinically significant.

If any subject develops positive immunogenicity, impact of immunogenicity on PK and PD would be evaluated for that subject. Pooled PD data along with variability will be presented. PK and PD data of individual subjects having positive immunogenicity will be presented separately.

11.3 Pharmacokinetic parameters

If decided (decision based on Immunogenicity results) to analyze PK data by analytical laboratory, the following pharmacokinetic parameters would be computed for Pegfilgrastim for the samples collected after the first dose and second dose, separately using non-compartmental model of Phoenix® WinNonlin® Version 6.4 or higher (Certara L.P.):

PK Parameter:		
C_{max}	:	Maximum measured serum concentration.
AUC_{0-t}	:	Area under the serum concentration versus time curve from time zero to the last measurable concentration as calculated by linear trapezoidal method.
$AUC_{0-\infty}$	:	Area under the serum concentration versus time curve from time zero to infinity. Where $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$, C_t is the last measurable concentration and λ_z is the terminal rate constant.
T_{max}	:	Time to reach the maximum measured serum concentration.
λ_z	:	First order elimination rate constant associated with the terminal (log-linear) portion of the curve. This is estimated via linear regression of time vs. log concentration. This parameter will be calculated by linear least squares regression analysis using at least last 3 or more non-zero serum concentration values.

$t_{1/2}$	:	The terminal half-life will be calculated as $0.693/\lambda_{z.}$
AUC_% Extrap_obs	:	The residual area in percentage will be determined by the formula, $[(AUC_{0-\infty} - AUC_{0-t})/AUC_{0-\infty}] \times 100$.

For all the above computations, actual time points of the sample collection will be used.

All concentration values below the lower limit of quantification will be set to zero for the pharmacokinetic and statistical calculations.

No value of λ_z, $AUC_{0-\infty}$, AUC_% Extrap_obs, R^2adjusted and $t_{1/2}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

11.4 Pharmacodynamic parameters

Using the measured ANC, the following variables will be calculated on baseline non-adjusted and baseline-adjusted data for the samples collected after the first dose and second dose, separately using non-compartmental model of Phoenix® WinNonlin® Version 6.4 or higher (Certara L.P.):

<u>For ANC [Baseline Non-Adjusted]:</u>

$E_{max,}$ and $AUEC_{0-t}$

<u>For ANC [Baseline-Adjusted]:</u>

E_{max}, $AUEC_{0-t}$, $T_{max,}$ λ_z and $t_{½}$

E_{max}	:	Maximum measured ANC.
$AUEC_{0-t}$	:	Area under the ANC versus time curve from time zero to the last measurable concentration as calculated by linear trapezoidal method.
T_{max}	:	Time to reach the maximum measured ANC.
λ_z	:	First order rate constant associated with the terminal (log-linear) portion of the curve. This is estimated via linear regression of time vs. log concentration. This parameter will be calculated by linear least squares regression analysis using at least last three or more non-zero values.
$t_{1/2}$	:	The terminal half-life will be calculated as $0.693/\lambda_{z.}$

For all the above computations, actual time points of the sample collection will be used.

All concentration values below the lower limit of quantification will be set to zero for the pharmacodynamic and statistical calculations.

No value of λ_z or $t_{1/2}$ will be reported for cases that do not exhibit a terminal log-linear phase in the concentration versus time profile.

The pre-dose levels will be used for the baseline adjustment of the post-dose levels for ANC.

Baseline adjustment will be done by subtracting the baseline value (i.e. pre-dose value) from all the pre and post-dose values. If any negative concentrations result will be obtained after adjustment, it would be set to zero.

11.5 Assessment of Eligibility/Safety

11.5.1 Safety data

All subjects who have received at least one dose of the study medicine will be included in the safety evaluation. Results obtained when evaluating safety and tolerability [adverse events, ECG, vital signs, serum pregnancy test (for female subjects) and clinical laboratory tests that are out of the range] will be listed and evaluated descriptively.

11.5.2 Eligibility Assessments

The following assessments will be done before the entry of the subjects into the study:

1.	Demographic Data.
	Age, height, weight and BMI.
2.	Vital Signs.
	Blood pressure, radial pulse, respiratory rate, oral body temperature.
3.	Medical History and Current Status.
	The subject's status as a healthy volunteer will be confirmed.
4.	Medication and Therapy History
	Current medication and use of any concomitant therapy or ingestion of any prescription drugs (i.e medication other than paracetamol or NSAIDS for pain) for previous 1 month of dosing. Also no over-the-counter (OTC) medicines will be allowed during the study until the last sample collection of trial, except vitamins, minerals and nutritional supplements that may be taken at the discretion of the Investigator.
5.	Clinical Examination.
	A standard clinical examination will be conducted, including 12-lead ECG and chest X-ray (P-A view if not done within the last 6 months), abdominal ultrasonography and clinical significant finding will be recorded if any.
6.	Clinical Laboratory Screening.
	Blood and urine will be tested for standard parameters (Section 13: List of laboratory Parameters).

11.5.3 Study Assessments

The following will be recorded during the conduct of the study:

1. Clinical examination and recording of vital signs at regular intervals;
2. Concomitant therapy changes;
3. Adverse event monitoring and reporting.

Definitions:

Adverse Event:

Any untoward medical occurrence in a clinical investigation subject, administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not related to the medicinal (investigational) product.

Serious Adverse Event (SAE):

Any untoward medical occurrence that at any dose:

a) results in death,

b) is life-threatening,

c) requires inpatient hospitalization or prolongation of existing hospitalization

d) Results in persistent or significant disability/incapacity,

e) Results in a congenital anomaly/birth defect. or

f) It is a medically important event or reaction. This would include important medical events that may not be immediately life threatening or results in death or hospitalization but may jeopardize the patient/subject or may require intervention to prevent one of the other outcomes listed in the definition above.

(Note: The term "life threatening" in the definition of "serious adverse event" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event, which hypothetically might have caused death if it was more severe).

Handling of Adverse Events

Subjects will be monitored throughout the study period for adverse events. Subjects will be instructed to bring to the notice of the nurse or the doctor or study personnel (e.g. Custodian), any adverse event that may occur during their stay at the clinical facility.

Subjects will also be specifically asked about any adverse events throughout the study period during the recording of vital signs. A physician will be available 24 hours during the time of subjects stay/housing at the clinical facility. All adverse events will be treated by the attending physician at the clinical facility, or in a nearby reputed hospital. All adverse events will be followed up wherever possible until resolution or until the investigator believes there will be no further change. This may involve additional visits.

All adverse events, including both observed or voluntarily reported problems; complaints, signs or symptoms occurring after the first dose administration shall be recorded on the "Adverse Event/Medical Event Record Form" irrespective of its association with the ongoing study medication. Prior to first dose administration in each subject, the event will be considered as a medical event and the aforementioned form will be completed by encircling "Medical Event" in the title.

Each adverse event shall be evaluated for duration, severity, seriousness and unexpectedness, action taken, date and time of resolution and association with the study treatment. The study may be suspended or terminated depending on the seriousness of the adverse events.

The IEC, regulatory bodies and the Sponsor shall be informed regarding the same as per local regulatory requirements.

Handling of Serious Adverse Event:

In case of Serious Adverse Events, the Sponsor or his representative, licensing authority and IEC will be informed by any available mode of communication within 24 hours of their occurrence or as soon as the initial treatment is provided. In case, the Investigator fails to report any serious adverse event within the stipulated period, he/she shall have to furnish the reason for the delay to the satisfaction of the Licensing Authority along with the report of the serious adverse event. The report of serious adverse event, after due analysis shall be forwarded by principal investigator to sponsor, Licensing Authority, as referred to in clause (b) of rule 21, the Chairman of the Ethics Committee (and the Head of the institution where the trial has been conducted, if applicable) within 14 days of the occurrence of the serious adverse event.

Handling of pregnancy:

Handling and reporting of pregnancy during the clinical study will be done as per in-house SOP.

11.5.3.1 Determining the severity of the adverse event:

Determine severity of the adverse event based on the following:

a) Mild: The adverse event does not limit usual activities; the subject may experience slight discomfort.

b) Moderate: The adverse event results in some limitation of usual activities; the subject may experience significant discomfort.

c) Severe: The adverse event results in an inability to carry out usual activities; the subject may experience intolerable discomfort or pain.

d) Life-threatening consequences

e) Death related to AE

11.5.3.2 Causality Assessment of the Adverse Event to the Investigational Medicinal Product.

Causality assessment of the adverse event will be done based in the following criteria:

Causality term	Assessment criteria
Certain	• Event or laboratory test abnormality, with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically)

	• Event definitive pharmacologically or phenomenologically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon) • Rechallenge satisfactory, if necessary
Probable/Likely	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	• Event or laboratory test abnormality, with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal may be lacking or unclear
Unlikely	• Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations
Conditional/ Unclassified	• Event or laboratory test abnormality • More data for proper assessment needed, or • Additional data under examination
Unassessable/ Unclassifiable	• Report suggesting an adverse reaction • Cannot be judged because information is insufficient or contradictory • Data cannot be supplemented or verified
Unrelated	• The adverse event is clearly NOT related to the investigational medicinal product • A clinical event with an incompatible time relationship and which could be explained by underlying disease or other drugs or chemicals

11.5.4 Post Study Assessments

A clinical examination including recording of vital signs, oral body temperature of the subjects will be conducted at the end of the study (after last ambulatory sample after dose-2). This examination also includes the assessment of blood samples for haematology, biochemistry tests and serum pregnancy test for females (Section 13 List of Laboratory Parameters).

Post study laboratory parameters that are out of specified ranges would be individually assessed and repeated if deemed necessary by the medically qualified reviewer. If any out of range parameter is found clinically significant, an adverse event will be recorded for the same. The subject would be

treated and/or followed up as advised by the physician in charge until resolution/stabilization of the adverse event.

11.5.5 Evaluation Criteria for Laboratory Parameter

Laboratory parameters obtained during the process of screening will be evaluated as follows:

a) Out of range values of hematology, Sickling Test, biochemistry and urine parameters will be individually evaluated and/or repeated for their clinical significance. A subject will be enrolled only if the medically qualified reviewer deems the values clinically insignificant or acceptable.

b) All safety immunology parameters are required to be negative.

c) Serum pregnancy test for female subjects must be negative.

Note: *In case any extra tests are analyzed, it will not have any impact on the study if the parameters and the values are clinically insignificant.*

12.0 ASSESSMENT SCHEDULE:

Phase	**Screening***	**Dose-1**						**Dose-2**									
Procedure																	
Day		**-1**	**1**	**2**	**3**	**4**	**5-7, 11, 15**	**21**	**22**	**23**	**24**	**25**	**26-28**	**32, 36**	**43, 50**	**71**	**85**
Attendance		X						X									
Urine Drug Scan	X	X						X									
Breath test for alcohol consumption	X	X						X									
Informed consent	X$^{\surd}$	X$^{\infty}$															
Serum pregnancy test for females	X	X						X									X$^{\#}$
Compliance Assessment		X					X	X					X	X	X	X	X
Baggage and Body search		X						X									
Sickling test	X																
Clinical Lab Investigation (Hematology/ Immunology/Biochemistry/ Urine analysis) $^{+}$	X							X$^{@}$									
Clinical Lab Investigation at post study (Hematology/Biochemistry/ urine analysis) $^{+}$																	X$^{\#}$
Ultra sound scan of abdomen	X																
12-Lead ECG	X							X									X$^{\#}$
Chest X-ray$^{\$}$	X																
Clinical Examination	X	X				X		X				X					X$^{\#}$
Local injection site examination**			X						X								
Pre-dose vital sign			X						X								
Dosing			X						X								
Blood sampling			X	X	X	X	X		X	X	X	X	X	X	X	X	X
Vital signs^			X	X	X				X	X	X						
Well being		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Standardized vegetarian breakfast			X						X								

* Within 28 days prior to first dosing

√Consent for screening

∞Study specific consent

^Post-dose vitals as mentioned in protocol

+ Clinical lab investigation will be performed as per Section 13 (list of laboratory parameters)

Meals will be provided to the subjects as mentioned in the protocol.

#End study (after last ambulatory sample after dose-2)

@ Estimation of hematology (except Sickling Test), biochemistry and urine analysis will be done within 3 working days prior to receiving dose-2.

$ Chest X-ray is valid up to 6 months.

**At 30 minutes, and at 2, 6 and 12 hrs post dose

Notes:

1:-Check-in days (Day -1 (for dose 1), Day 21 (for dose 2))

2:-Dosing Days (Day 1 (1st Dose), Day 22 (2nd Dose))

3:- Housing days (Day -1 to Day 3 (for dose 1), Day 21 to Day 24 (for dose 2))

4:-. Checkout day (Day 4 (for dose 1), Day 25 (for dose 2)

5:-. Ambulatory sample (days 5-7, 11, 15, 26-28, 29, 43, 57, 85)

6:-. End study (Day 85, after last ambulatory sample after dose-2)

7:-. Sampling time-points

Pharmacokinetic evaluation: 1st and 2nd dose: Venous blood samples (04 ml each) will be withdrawn at pre-dose (0.000) and at 8.000, 16.000, 24.000 (Day 2), 48.000 (Day 3), 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 240.000 (Day 11), 336.000 (Day 15) and 504.000 (Day 22) hours following 1st and 2nd dose administration (Post-dose sample of 1st dose at 504.000 (Day 22) hours will be considered as pre-dose sample of 2nd dose).

For Pharmacodynamic evaluation: 1st and 2nd dose: Venous blood samples (02 ml each) will be withdrawn at pre-dose (0.000) and at 8.000, 16.000, 24.000 (Day 2), 48.000 (Day 3), 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 240.000 (Day 11), 336.000 (Day 15) and 504.000 (Day 22) following 1st and 2nd dose administration (Post-dose sample of 1st dose at 504.000 (Day 22) hours will be considered as pre-dose sample of 2nd dose).

For Immunogenicity evaluation: Venous blood samples (08 ml each) will be withdrawn at screening, pre-dose, and at 336.000 (Day 15, week 2), 504.000 (Day 22, week 3, **within 60 minutes before 2nd dose**), 840.000 (Day 36, week 5), 1176.000 (Day 50, week 7), 1680.000 (Day 71, week 10) and 2016.000 (Day 85, week 12) hrs after first dose.

13.0 LIST OF LABORATORY PARAMETERS

HAEMATOLOGY	**BIOCHEMISTRY**		
CBC (including RBC count, platelet count) along with ANC (absolute neutrophil count) and Sickling Test	Total Protein	Blood Urea	Sodium
	Albumin	SGOT (AST)	Potassium
	Serum Globulin	SGPT (ALT)	Chloride
	A/G ratio	Creatinine	Calcium
	Bilirubin Total	Random glucose	
	GGT	Alkaline phosphatase	

IMMUNOLOGICAL TESTS	
Anti HIV AB (I & II)	Anti HCV
HBsAg	

URINE PARAMETER
Specific Gravity, pH, Glucose, Protein, Bilirubin, ketones, Urobilinogen, Erythrocytes, Leucocytes, Nitrite and, if necessary, microscopic examination

HORMONAL ASSAY:	β-HCG*

AT SCREENING AND PRIOR TO CHECK-IN OF EACH DOSE			
(A)	**URINE SCAN FOR DRUG OF ABUSE**		
	Morphine (MOR)	Cannabinoids (THC)	Amphetamines (AMP)
	Cocaine (COC)	Barbiturates (BAR)	Benzodiazepines (BZD)
(B)	**BREATH TEST FOR ALCOHOL CONSUMPTION**		

Details:

Screening: Hematology, biochemistry, urine analysis, sickling test, immunological tests and serum pregnancy test*

Prior to check-in of each dose: Serum pregnancy test*

Pre-check-in: Estimation of hematology (except Sickling Test), biochemistry and urine analysis will be done within 3 working days prior to receiving dose-2

End of the study (after last ambulatory sample after dose-2): Hematology (except sickling test), biochemistry (except sodium, potassium, calcium and chloride), urine analysis and Serum pregnancy test (for females)*

Note: All the laboratory tests will be done as per clinical/contractual laboratory SOPs

* For female subjects only

14.0 RESPONSIBILITIES OF PERSONNEL INVOLVED IN THE STUDY

Principal Investigator:

[redacted] will be responsible for overall conduct of the study.

Protocol Author:

[redacted] will be responsible for the preparation and finalization of the protocol and ICF.

Clinical Research Physician:

[redacted] will be responsible for the selection of subjects, and Duty doctors will be responsible for the medical care of subjects during the study

Clinical Laboratory:

[redacted] will be responsible for the Clinical Laboratory component of the study.

Pharmacokinetic and Pharmacodynamic Data analysis:

[redacted] will be responsible for the pharmacokinetic and pharmacodynamic data analysis.

Biostatistics and Programming:

[redacted] will be responsible for statistical data analysis.

Quality Assurance:

[redacted] will be responsible for Quality Assurance.

Quality Control:

Project Coordinator along with the designated QC team will be responsible for quality control of the study.

Sponsor's responsible person(s):

[redacted] will be responsible for overall conduct of the study in compliance to the protocol and applicable regulatory requirement on behalf of the sponsor.

[redacted] will be responsible for providing IMP for the study on behalf of the sponsor.

15.0 ETHICAL CONSIDERATIONS

15.1 Basic Principles

The study will be conducted according to Schedule Y (with subsequent amendments) of CDSCO (Central Drugs Standard Control Organization), Ministry of health and family welfare, Government of India; Ethical guidelines for biomedical research on human participants, Indian Council of Medical Research (2017); ICH (The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) E6 (R2) Guideline for Good Clinical Practice, (2016); Good Clinical Laboratory practices (GCLP); Declaration of Helsinki (Brazil, October 2013).

15.2 Independent\Institutional Ethics Committee (IEC)

This Protocol and the corresponding Informed Consent Form (ICF) to obtain consent of study subjects will be reviewed by the Independent\Institutional Ethics Committee and the study will commence only after a written approval is obtained from the Independent\Institutional Ethics Committee.

15.3 Written Informed Consent

The Principal Investigator or his/her designate will explain the conduct of the study and information regarding the investigational medicinal product to the subjects before check-in of the study. This will be done through an oral presentation regarding the purpose, procedures to be carried out, potential hazards and rights of the subjects, in their local language. Subjects will be encouraged to ask questions and clarify their doubts regarding any aspect of the study. Subjects will be required to sign/put a thumb impression on the informed consent form summarizing the discussion prior to check-in for the study. Signature of the LAR/Impartial witness (if applicable) will be taken on the informed consent form in case the subject is not able to sign on the informed consent form. Subjects who fail to understand the informed consent procedure and/or are unable to communicate with the study personnel will not be enrolled.

The subjects will give their consent for participation in the study by signing/putting a thumb impression on Informed Consent Form (ICF), which will also be signed by the LAR/Impartial witness (if applicable) and [redacted] person conducting the ICF presentation, principal investigator or designate. A photocopy of signed informed consent form with the signature or thumb impression will be given to the subject for reference after the procedure is over, while the original will be retained at [redacted]

15.4 Subject Compensation

The subjects will be paid an adequate compensation on account of their contribution towards the conduct of this study. In case of dropout/withdrawal of a subject before completion of the study, the recommendation of the Independent\Institutional Ethics Committee on compensation of the withdrawn subjects will be final and binding on both [redacted] and the study subjects.

16.0 INSURANCE POLICY

[redacted] will take insurance and will ensure its effect throughout the study. The insurance will cover [redacted] liabilities for professional indemnity in the current study. The sponsor will arrange for adequate insurance to cover treatment of (Serious) Adverse Events and provide compensation for clinical study related injury or death.

[redacted]

In the case of an injury occurring to the subject during the study, free medical management will be provided to the subjects as long as required or till such time it is established that the injury is not related to the clinical trial, whichever is earlier.

17.0 AMENDMENT TO THE PROTOCOL

Any significant change in the study procedure or study design will only be effected upon mutual agreement with the Sponsor and [redacted] and after obtaining a favorable opinion from the Ethics Committee. All such changes will be documented in the amended version of the protocol and a list of changes with reference to the previous version will be generated and submitted to the IEC as soon as possible. In cases where there is an immediate safety hazard to the subjects, the amended protocol will be effective immediately and approval of the IEC will be obtained as soon as possible. If required based on the amendment, local regulatory approval will be obtained

18.0 SOURCE DATA ACCESSIBILITY

The Independent\Institutional Ethics Committee, Sponsor, Quality Assurance and Regulatory Agencies such as USFDA, DCGI, etc will have the access to the raw data during inspection and audits.

19.0 QUALITY ASSURANCE AUDITS

In-process audit of various clinical and bioanalytical activities and retrospective audits of 100% of the raw data generated during the course of the study including the final reports will be conducted by Quality Assurance Department of [redacted] These audits will be performed to ensure conformance to this protocol, GCP, GLP and the governing SOPs of [redacted] In addition, each department will implement internal Quality Control measures.

The study may be monitored at the discretion of the sponsor by any of its representatives.

20.0 DATA HANDLING & RECORD KEEPING

All clinical raw data generated during conduct of the study will be directly entered in the respective electronic source forms. Electronic software (Biznet) will be used for capturing the data generated during conduct of the study as per in-house SOPs. A dynamic list of paper source/forms (if required to be used due to temporary non-functioning of software or no provision to document in software) will be maintained and filed with project specific Trial Master File. All source data and transcribed data forms will be compiled by the study personnel assisting in the study and will be checked wherever applicable for completeness.

All bioanalytical raw data of sample processing will be directly entered in the respective source data forms. The data acquisition system software will be used for the quantitative determination and applicable software shall be used to review the chromatographic data as per in-house SOPs. All data related to the project will be in the custody of the designated study personnel until transferred to archives.

21.0 REPORT

All appropriate data from the study will be reported in the final report in eCTD format. The report will contain data regarding the analytical methodology, the pharmacokinetic, pharmacodynamic, immunogenicity calculations, the statistical analysis of the data and a clinical report along with raw data.

22.0 ARCHIVING

A representative sample of the drug supplies used in the study will be retained at the [redacted] in accordance with contract obligations. All data generated in connection with this study, together with the copy of this protocol, Informed Consent Form (ICF) and the final report will be archived after issuance of the audited reports.

All such material will be retained at [redacted] as mentioned in Research Service Agreement. Beyond this period, the sponsor will arrange for the maintenance of these materials.

23.0 CONFIDENTIALITY OF DATA

The data identifying each study subject by name will be kept confidential and will be accessible only to the study personnel (involved in check-in, quality control and dosing procedure), Quality Assurance Auditor during audits, if necessary, to the IEC and various regulatory authorities such as USFDA, DCGI etc. The sponsor, while monitoring or auditing the study, will also have access to data without violating the confidentiality of the subjects, to the extent permitted by applicable laws and regulations. All data related to the project will be in the custody of the designated study personnel until transferred to archives.

24.0 PUBLICATION POLICY

The results of the study including all obtained data will be the property of the sponsor. However, the investigator may seek permission to publish results of the study from the sponsor. Unpublished data cannot be disclosed to any third party by the investigators without written approval of the sponsor and as per the sponsor publication policy.

25.0 STANDARDIZED VEGETARIAN BREAKFAST MENU

Food Items	*Servings*	*Ingredients*	*Qty. Of Ingredients*	*Calorie*	*CHO*	*Fats*	*Proteins*
Aloo Paratha	2 pcs (200gms)	Wheat flour	100 gms	341.00	69.40	1.70	12.10
		Potato	100 gms	97.00	22.6	0.10	1.60
		Ghee	1gm	9.00	0.00	1.00	0.00
		Oil	15 ml	135.00	0.00	15.00	0.00
Milk	1 Glass	Skim milk	150 ml	43.50	6.90	0.15	3.75
Total				**625.50**	**98.90**	**17.95**	**17.45**
Nutrient Calories					**395.60**	**161.55**	**69.80**
Nutrient Calories As % Total					**63.24%**	**25.82%**	**11.16%**

Lemon, Green Chili (Fried & Salted), Rock Salt, Jeera Powder, Onion slices, etc. (whichever is available) will be served as meal enhancers

26.0 REFERENCES

1. Prescribing information (Neulasta® (Pegfilgrastim) injection, for subcutaneous use. Revised: 12/2017
2. Summary of Product Characteristics (NEULASTA® 6 mg solution for injection). last updated on the eMC: Updated 24-Jun-2015.