PROJECT NO. 0553-17

PROTOCOL

AN ASSESSOR-BLIND, BALANCED, RANDOMIZED, TWO-TREATMENT, TWO-PERIOD, SINGLE-DOSE, TWO-WAY, CROSSOVER, COMPARATIVE, PHARMACOKINETIC AND PHARMACODYNAMIC STUDY OF SUBCUTANEOUS INJECTIONS OF INTP5 OF INTAS PHARMACEUTICALS LTD., INDIA AGAINST NEULASTA OF AMGEN INC., USA 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	:	02 December 2017		[redacted]
Supersedes	:	1.0		
Dated	:	26 August 2017		

Sponsor Address:	**Study centre:**
Intas Pharmaceuticals Ltd,	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[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 the permission of Intas Pharmaceuticals Limited (Biopharma Division).

[redacted]

TABLE OF CONTENTS

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	:	The area under the plasma concentration versus time curve
AUC_%Extrap_obs	:	% of the Area under the Curve that has been derived after Extrapolation or % Residual Area
$AUC_{0-\infty}$	:	Area Under the plasma Concentration versus Time Curve from Time Zero to Infinity
AUC_{0-t}	:	Area Under the plasma Concentration versus Time Curve from Time Zero to the Last Measurable plasma Concentration
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_t	:	Last measurable Plasma concentration
C_{max}	:	Maximum Measured Plasma 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
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]
L/hr	:	Liter/hour
m^2	:	Meter Square
Mg	:	Milligram

mL/ml	:	Milliliter
mm Hg	:	Millimeter of Mercury
M	:	Missing Sample
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
R^2adjusted	:	Goodness of fit statistic for the terminal phase, adjusted for the number of points used in the estimation of λ_z.
RBCs	:	Red Blood Cells
SAE	:	Serious Adverse Event
SAS	:	Statistical Analysis System
SAP	:	Statistical Analysis Plan
SGPT	:	Serum Glutamic Pyruvic Transaminase
SGOT	:	Serum Glutamic Oxaloacetic Transaminase
SOP	:	Standard Operating Procedure
$t_{1/2}$	:	Terminal half-life
T/R	:	Test to Reference ratio.
T_{max}	:	Time of the maximum measured plasma concentration.
USFDA	:	United States Food and Drug Administration
WNL	:	Within Normal Limits

1.0 STUDY SYNOPSIS

1.1	Project number	:	0553-17
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. In this study, the pharmacokinetic and pharmacodynamic effects of Pegfilgrastim from the test formulation will be compared with that from the reference formulation after a single subcutaneous dose administration to healthy, adult, human subjects under fed conditions in a crossover design.
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 pharmacokinetic and pharmacodynamic profiles 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 there are any concerns for PKPD, immunogenicity would be evaluated for that subject.
1.5	Study Design	:	An assessor-blind, balanced, randomized, two-treatment, two-period, single-dose, two-way, crossover, comparative, pharmacokinetic and pharmacodynamic of subcutaneous injection (6 mg/0.6 ml) study in healthy adult human subjects under fed condition.
1.6	Number of subjects	:	144 Subjects*. Efforts will be made to recruit equal number of healthy male and female volunteers. *Study may be conducted in multiple groups.
1.7	Dose	:	Single doses of Pegfilgrastim 6 mg/0.6 ml [either of the Test product or Reference product] will be administered subcutaneously to the outer area of right upper arm in each subject as per the randomization schedule in each period.
1.8	Fasting Criteria	:	➢ Pre-dose: At least 10 hours prior to serving standardized vegetarian breakfast ➢ Post-dose: 4 hours ➢ Further meals will be provided to the subjects at appropriate times during their housing period.
1.9	Water restriction	:	➢ 1 hour pre dose ➢ 1 hour post dose.
1.10	Method of administration	:	➢ In each period, 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 a single 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. ➢ **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	:	➢ The study treatment 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	:	➢ Pre-dose: 11 hours ➢ Post-dose: 72 hours ➢ Subject will have to stay in the clinical facility for 4 consecutive nights in each period. ➢ Subjects will have to report to the clinical facility at and after 96 hours for ambulatory blood sample collection in each period. ➢ 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 check in of period-II. ➢ 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 of each period • Before check-out of each period • Within 3 working days prior to check in of period-II. • End of the study (after last ambulatory sample of period-II) (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) in each period:- ▪ Pre-dose (within 60 minutes prior to dosing) and at 2, 4, 10, 24, 30, 36, 48 and 60 hours post 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 in each period ➢ 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 3

working days prior to check in of period-II and at the end of the study (after last ambulatory sample of period-II).

- 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 period
- 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 cm2 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 cm2 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 cm2 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 cm2 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 cm2 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 cm2 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 of each period.
- Breath test for alcohol consumption:
  - Screening and before check-in of each period.

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 of period-II).

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 period:** 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 check in of period-II. • **End of the study (after last ambulatory sample of period-II):** Hematology (except sickling test), biochemistry 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 post 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** 6 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 29 blood samples will be collected for PK evaluation and 20 blood samples for PD evaluation. **For PK evaluation:** The venous blood samples will be withdrawn at Pre-dose (collected within 60 minutes prior to dosing) and at 2.000, 4.000, 6.000, 8.000, 10.000, 12.000, 14.000, 16.000, 18.000, 20.000, 22.000, 24.000 (Day 2), 26.000, 30.000, 36.000, 42.000, 48.000 (Day 3), 60.000, 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 168.000 (Day 8), 192.000 (Day 9), 240.000 (Day 11), 288.000 (Day 13), 336.000 (Day 15) and 504.000 (Day 22) hours following drug administration in each period. Blood samples will be collected in a prelabelled serum separator vacutainer. **For PD evaluation:** The venous blood samples will be withdrawn at pre-dose (collected within 60 minutes prior to dosing) and at 6.000, 12.000, 24.000 (Day 2), 36.000, 48.000 (Day 3), 60.000, 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 168.000 (Day 8), 192.000 (Day 9), 216.000 (Day 10), 240.000 (Day 11), 288.000 (Day 13), 312.000 (Day 14), 336.000 (Day 15), 360.000 (Day 16) and 504.000 (Day 22) hours following drug administration in each period. Blood samples will be collected in a prelabelled sample collection tube containing K_2EDTA as anticoagulant. **<u>For Immunogenicity evaluation:</u>** Venous blood samples (06 ml each) will be withdrawn at screening, pre-dose of period-I (Day 1), at 2 weeks (day 15) and at 4 weeks (day 29) after the first dose, at pre-dose of period-II (Day 43) and at 2 weeks (Day 57) and 4 weeks (Day 71) after second 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 immunogenicity evaluation at 336.000 (Day 15) 672.000 (Day 29), 1344.000 (Day 57), 1680.000 (Day 71) hours following drug administration in each period will be collected on an ambulatory basis.

<table>
<tr><td>1.17</td><td>Total Blood Loss</td><td>:</td><td>Not exceeding 391 mL for male subjects and 395 mL for female subjects as follows:
<table>
<tr><td></td><td></td><td></td><td colspan="2">Subjects</td></tr>
<tr><td></td><td></td><td></td><td>Male</td><td>Female</td></tr>
<tr><td>+</td><td>Blood volume for PK sample for two periods (58 samples of 4 mL each).</td><td>:</td><td>232 mL</td><td>232 mL</td></tr>
<tr><td>+</td><td>Blood volume for PD samples for two periods (40 samples of 2 mL each).</td><td>:</td><td>80 mL</td><td>80 mL</td></tr>
<tr><td>+</td><td>Immunogenicity samples – 6 mL per sample</td><td>:</td><td>42 mL</td><td>42 mL</td></tr>
<tr><td>+</td><td>Discarded normal saline solution containing blood for two periods (26 × 0.5 mL)</td><td></td><td>13 mL</td><td>13 mL</td></tr>
<tr><td>+</td><td>Blood withdrawn for screening including serum pregnancy test (for female subjects) prior to study.</td><td>:</td><td>10 mL</td><td>10 mL</td></tr>
<tr><td>+</td><td>Blood withdrawn for post-study safety assessment including serum pregnancy test (for female subjects)</td><td>:</td><td>08 mL</td><td>08 mL</td></tr>
<tr><td>+</td><td>Estimation of hematology, liver function tests, renal function tests parameters (within 3 working days prior to check in of period-II)</td><td>:</td><td>6 mL</td><td>6 mL</td></tr>
<tr><td>+</td><td>Serum pregnancy test prior to check-in of each dose (for female subjects).</td><td>:</td><td>--</td><td>4 mL</td></tr>
<tr><td colspan="2">Total blood loss for each subject</td><td>:</td><td>391 mL</td><td>395 mL</td></tr>
</table></td></tr>
<tr><td>1.18</td><td>Washout Period</td><td>:</td><td>At least 42 days between the dosing days of two consecutive periods.</td></tr>
<tr><td>1.19</td><td>Sample handling and processing</td><td></td><td>Separate laboratory manual will be prepared for PK, PD and Immunogenicity sample handling, separation process and analytical procedure.</td></tr>
<tr><td>1.21</td><td>Analytical Procedure</td><td>:</td><td><u>For PK evaluation:</u>
Serum samples will be analyzed for Pegfilgrastim using a validated method.
<u>For PD evaluation:</u>
Pharmacodynamic marker ANC will be analyzed on an automated cell counter using a validated method.
<u>For immunogenicity evaluation:</u>
The anti-PegG-CSF antibodies will be detected using a validated screening</td></tr>
</table>

			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. Note: Decision to analyze the samples of a subject for immunogenicity will be based on the PK/PD results for that subject.
1.22	Pharmacokinetic Parameters	:	Employing the measured concentration vs. time profiles of Pegfilgrastim, the following PK parameters will be calculated: Primary PK parameters: C_{max} and $AUC_{0-\infty}$ Secondary PK parameters: T_{max}, AUC_{0-t}, 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 ANC [baseline non-adjusted data]: Primary PD Parameters: E_{max} and $AUEC_{0-t}$ For ANC [baseline-adjusted data]: Secondary PD parameters: 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 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. Pharmacodynamic assessment will be based on baseline non-adjusted data of ANC only. Baseline-adjusted data of ANC will be presented for supportive information only.
1.23	Statistical Analysis	:	Descriptive statistics will be computed and reported for the PK parameters of Pegfilgrastim and for the PD parameters of ANC. ANOVA, power and ratio analysis will be computed and reported for ln-transformed PK parameters C_{max} and $AUC_{0-\infty}$ for Pegfilgrastim and for ln-transformed PD parameters E_{max} and $AUEC_{0-t}$ of baseline non-adjusted data for ANC. 90% confidence interval using two one-sided tests for bioequivalence for ln-transformed PK parameters C_{max} and $AUC_{0-\infty}$ of Pegfilgrastim, and ln-

			transformed PD parameters E_{max} and $AUEC_{0-t}$ for baseline non-adjusted data of ANC will be computed and reported. Descriptive statistics will be presented for the immunogenicity results.
1.24	Pharmacokinetic Bioequivalence criteria	:	Bioequivalence of the test product with that of the reference product will be concluded if 90% confidence interval for the test to reference ratio of the geometric least squares means for ln-transformed PK parameters C_{max} and $AUC_{0-\infty}$ of Pegfilgrastim falls within the acceptance range of 80.00% to 125.00%.
1.25	Pharmacodynamic Bioequivalence criteria	:	Bioequivalence of the test product with that of the reference product will be concluded if 90% confidence interval for the test to reference ratio of the geometric least squares means for ln-transformed PD parameters E_{max} and $AUEC_{0-t}$ of baseline non-adjusted ANC falls within the acceptance range of 80.00% to 125.00%.
1.26	Immunogenicity Data Analysis	:	Immunogenicity (Anti-Drug Antibody-ADA) data will be presented and evaluated, if required.
1.27	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 (2006); 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).; Declaration of Helsinki (Brazil, October 2013) and USFDA guidelines for bioequivalence studies.

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 (2006); 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); and USFDA guidelines for bioequivalence studies.

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]

04-Dec-17

Date

Principal Investigator

[redacted]

[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 (2006); 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).and USFDA guidelines for bioequivalence studies.

[redacted]

2 Dec 2017

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, pharmacodynamics, biostatistics and programming, clinical data management, quality assurance and clinical safety laboratory services:

Address	:	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

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]

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: Single dose 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 42 days between the dosing days of two consecutive periods.

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 42 days between the dosing days of two consecutive periods. However, study-related health assessments are provided at no cost.

6.4 Rationale

The objective of this study is to assess and compare the PK and PD profiles of INTP5 and Neulasta® after single subcutaneous 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 INTP5 of Intas Pharmaceuticals Ltd., India or Neulasta of Amgen Inc, USA for the PK and PD 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 pharmacokinetic and pharmacodynamic profiles 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 there are any concerns for PKPD, immunogenicity would be evaluated for that subject.

8.0 STUDY DESIGN

8.1 Design

An assessor-blind, balanced, randomized, two-treatment, two-period, single-dose, two-way, crossover, comparative subcutaneous injection (6 mg/0.6 ml) pharmacokinetic and pharmacodynamic study in healthy, adult, human subjects under fed conditions.

8.2 Numbers of subjects

Based on the in-house study data, maximum intra-subject variability observed for primary PK parameter $AUC_{0-\infty}$ of Pegfilgrastim was ~32%. The sample size was determined using SAS software considering the following assumptions:

a. T/R ratio = 90.0-110.0%

b. Intra-Subject CV (%) ~ 32%

c. Significance Level = 5%

d. Power ≥ 90%

e. Bioequivalence Limits - 80.00-125.00%

Based on the above estimates, **122 completers** will be sufficient to establish bioequivalence between formulations with adequate power for two-way crossover design study. Considering ~15% dropouts/withdrawals, **144* subjects** will be enrolled. Efforts will be made to recruit equal number of healthy male and female subjects.

Above sample size will also be sufficient to prove bioequivalence of baseline non-adjusted data of ANC as intra-subject variability for baseline non-adjusted data of ANC has been observed as low compared to PK data.

Note: Two additional subjects if available, may be checked-in on the day of check-in of period-I to compensate for any dropout prior to the dosing of period-I. These subjects will be dosed if there are dropouts prior to dosing in period-I. If there are no dropouts, these subjects will be checked-out without being dosed after completion of dosing in period-I.

* Study may be conducted in multiple groups.

8.3 Randomization

The order of receiving test (T) and reference (R) products for each subject during both the periods 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.

The sequence of administration of treatments, i.e. "TR" or "RT", to the subjects will be determined according to the randomization schedule.
The personnel involved in dispensing of study drugs 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 in each period.

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

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

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 check in of period-II.

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 Washout Period

There will be a washout period of at least 42 days between the administrations of investigational medicinal products of two consecutive periods.

8.7 Duration of Fasting & Distribution of Meals

All subjects will be required to fast overnight for at least 10 hours prior to serving of standardized vegetarian breakfast which they are required to consume within 30 minutes and 4 hours post dose for each period.

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 period 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 the 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 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.

8.8 Restrictions

8.8.1 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 and quantity 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 washout 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, pharmacodynamics and pharmacokinetics of the non-study medication.

b) The likelihood of a drug-drug interaction, thereby affecting the PK and PD 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.

8.8.2 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) for 24 hours prior to IMP administration of each period and throughout their stay in the clinical facility.

Subject will be instructed to abstain from grapefruit or 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.

Smoking for 6 months prior to start of the study till the completion of the study is prohibited and the subjects will be instructed to abstain from the same.

8.8.3 Postural Restrictions

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.

8.9 Dosing Procedures & Compliance Assessment

8.9.1 Dosing

IMP administration as described below will be done under the supervision of the 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 in each period by the trained study personnel as per the randomization.

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

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.

8.9.1.1 Test Product

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.

8.9.1.2 Reference Product

Single dose of Neulasta® of AMGEN Inc; USA (US Licensed product) 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.

8.9.2 Assessment of compliance for dosing

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

8.10 Blood Sampling for Pharmacokinetic Analysis

8.10.1 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 the scheduled time. Samples for PD and PK and immunogenicity at and after 96 hours post 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 (for both PK and PD). 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 prelabelled (mentioning Project number, Subject number, Sample ID No./Bar code ID no and sampling time point) serum separator vacutainer (in case of samples for PK) and/or K2EDTA sample collection tubes (in case of samples 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 hours post dose. Samples after 24 hours post dose will be collected through fresh vein puncture. The ambulatory samples and pre-check-in sample for serum pregnancy test (for female subjects) in each period will be collected by a fresh vein puncture. The sample to be collected for laboratory estimation within 3 working days prior to check-in of P-II will be collected through a fresh vein puncture.

Not exceeding **391 mL** for male subjects and **395 mL** for female subjects for two periods as follows:

			Subjects	
			Male	**Female**
+	Blood volume for Pharmacokinetic sample for two periods (58 samples of 4 mL each).	:	232 mL	232 mL
+	Blood volume for Pharmacodynamic samples for two periods (40 samples of 2 mL each).		80 mL	80 mL
	Blood volume for Immunogenicity samples as backup (Samples of 06 mL each).		42 mL	42 mL

+	Discarded normal saline solution containing blood for two periods (26 × 0.5 mL)	:	13 mL	13 mL
+	Blood withdrawn for screening including serum pregnancy test (for female subjects) prior to study.	:	10 mL	10 mL
+	Blood withdrawn for post-study safety assessment including serum pregnancy test (for female subjects)	:	8 mL	8 mL
+	Estimation of hematology, liver function tests, renal function tests parameters (within 3 working days prior to check in of period-II)		6 mL	6 mL
+	Serum pregnancy test prior to check-in of each period (for female subjects).	:	--	4 mL
Total Blood Loss for each Subject		**:**	**391 mL**	**395 mL**

8.10.2 Sampling Schedule

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

Day	Time points (Hours)	(As per 24 hours clock time)	Blood volume (mL)	PK/PD evaluation		
				For PK/PD samples	Only for PK	Only for PD
1	Pre-dose	Within 60 minutes prior to dosing	06	√	-	-
	2.000	1100	04	-	@	-
	4.000	1300	04	-	@	-
	6.000	1500	06	√	-	-
	8.000	1700	04	-	@	-
	10.000	1900	04	-	@	-
	12.000	2100	06	√	-	-
	14.000	2300	04	-	@	-
2	16.000	0100	04	-	@	-
	18.000	0300	04	-	@	-
	20.000	0500	04	-	@	-
	22.000	0700	04	-	@	-
	24.000	0900	06	√	-	-

	26.000	1100	04	-	@	-
	30.000	1500	04	-	@	-
	36.000	2100	06	√	-	-
3	42.000	0300	04	-	@	-
	48.000	0900	06	√	-	-
	60.000	2100	06	√	-	-
4	72.000	0900	06	√	-	-
5	96.000*	0900	06	√	-	-
6	120.000*	0900	06	√	-	-
7	144.000*	0900	06	√	-	-
8	168.000*	0900	06	√	-	-
9	192.000*	0900	06	√	-	-
10	216.000*	0900	02	-	-	X
11	240.000*	0900	06	√	-	-
13	288.000*	0900	06	√	-	-
14	312.000*	0900	02	-	-	X
15	336.000*	0900	06	√		
16	360.000*	0900	02	-	-	X
22	504.000*	0900	06	√	-	-

√: Time points where blood sample will be collected for PK and PD samples
@: Time points where blood sample will be collected for PK samples
X: Time points where blood sample will be collected for PD samples
Day 1: Day of dosing in each period.
Day 2,3,4,5,6,7,8,9,10,11,13,14,15,16,22: Subsequent days after the day of dosing.
*Ambulatory Samples

Sampling Schedule for Immunogenicity samples.

Days	**Time points (hour)**	**Proposed clock time**	**Blood volume**
	Immunogenicity evaluation		
Within 28 days before dosing	Screening	NA	6 ml
1	Pre-dose (0.000)	Within 60 minutes prior to dosing	
15	336.000*	0900	

[redacted]

29	672.000*	0900	
43	Pre-dose (1008.000)	Within 60 minutes prior to dosing of period-II	
57	1344.000*	0900	
71	1680.000*	0900	

*Ambulatory Samples

8.11 Sample Handling and processing

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

Immunogenicity samples will be stored. Immunogenicity sample analysis will be performed if there are any concerns to PKPD. A validated assay will be used to analyze Immunogenicity samples.

8.12 Clinical Safety Measures

A physician will be available within the clinical facility whenever the subjects are housed (from check-in to check-out in each period) and at the end of the study (after last ambulatory sample of P-II). A physician will be available on call during ambulatory sample in each period. 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 in each period, within 3 working days prior to check in of period-II and at the end of the study (after last ambulatory sample of P-II). The clinical examination before check-out in each period 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 check in of period-II and at the end of the study (after last ambulatory sample of period-II)

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 dosing) and at 2, 4, 10, 24, 30, 36, 48 and 60 hours in each period.

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 in each period.

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

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 cm2 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 cm2 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 cm2 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 cm2 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 cm2 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 cm2 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 administer this product.

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

Laboratory assessments will be carried out at the time of screening and prior to each check in (within 3 working days prior to check in of period-II.

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 period.

Laboratory tests for Hematology (except sickling test), biochemistry and urine analysis and serum pregnancy test (for females) will be done at the end of the study (after last ambulatory sample of period-II).

8.13 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 IMP (three test products and three reference products) 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 and 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 to 29.9 (both inclusive), calculated as weight in kg/height in meter^2.
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. Volunteer who is a Non-smoker
5. Able to understand and comply with the study procedures, in the opinion of the investigator.
6. Able to give voluntary written informed consent for participation in the trial.
7. 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 or 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 vaccines, immunoglobulin preparations, or immunomodulator's within the past 6 months prior to receiving the 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. Smokers, or who have smoked within last six months prior to start of the study.
11. Ingestion or use of any prescribed medication at any time within 1 month prior to receiving first dose in period I.
12. 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).
13. 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 in period-I.
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. Receipt of an investigational medicinal product or participation in a drug research study within a period of 180 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.
16. Positive result for human immunodeficiency virus (HIV I &/or II) and/or hepatitis B and C tests.
17. History or presence of cancer because of which anticipated life span is less than 5 years as per the investigator's assessment.
18. History or presence of psychiatric disorders.
19. Presence of tattoo or scars or any type of skin lesions due to infection, burning, wound or inflammation at the proposed site of injection.
20. An unusual diet, for whatever reason (e.g. low-sodium), for 4 weeks prior to receiving the study medicine in period-I. In any such case, subject selection will be at the discretion of the Principal Investigator.
21. Consumption of grape fruit or grape fruit products within 72 hours prior to receiving study drug in period-I.
22. A history of difficulty in donating blood.

23. Females, pregnant or lactating, or planning to become pregnant during the course of the study or found positive in pregnancy test at screening.
24. Any infections in the last 4 weeks before receiving study medication in period-I.

10.3 Withdrawal Criteria

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

1. 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.
2. 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.
3. 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.
4. Any subject found to hide important medical history which in opinion of Principal Investigator may compromise his/her safety during participation in this study.
5. Any subject found as cross-participated in other drug trial or trial screening.
6. 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 the concomitant medications will be discussed with the sponsor. The final decision will be based on the impact of the concomitant medications on the primary endpoint and safety. The decision should be taken blinded before the unblinding of the data on the key endpoints.
7. If it is felt in Principal Investigator's opinion that it is not in the subject's best interest to continue.
8. Any subject who wishes to withdraw his/her consent for whatever reason.
9. Any other justifiable reason, which would be adequately documented.
10. 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 Pharmacokinetic, Pharmacodynamic and Statistical Analyses

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

11.2 Pharmacokinetic and pharmacodynamic Analysis

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

Criteria for exclusion of PK and PD parameters will be as below:

a. Three consecutive missing (M) / Non-Reportable (NR) samples in the elimination phase may significantly influence AUC_{0-t}/$AUEC_{0-t}$ and elimination phase-dependent parameters ($AUC_{0-\infty}$/$AUEC_{0-\infty}$, $t_{1/2}$, λ_z, AUC_% Extrap_obs/AUEC_% Extrap_obs, R^2 adjusted). Inclusion of such parameters in the statistical analysis may mislead the final outcome. Hence, AUC_{0-t}/$AUEC_{0-t}$ and elimination phase dependent-parameters ($AUC_{0-\infty}$/$AUEC_{0-\infty}$, $t_{1/2}$, λ_z, AUC_%Extrap_obs/AUEC_% Extrap_obs, R^2 adjusted) will be excluded.

b. AUC_%Extrap_obs/AUEC_% Extrap_obs is found to be > 20% with R^2 adjusted < 0.80 for λ_z estimation: In such cases elimination phase- dependent parameters will not be reliably characterized. Hence, elimination phase- dependent parameters ($AUC_{0-\infty}$/$AUEC_{0-\infty}$, $t_{1/2}$, λ_z, AUC_%Extrap_obs/AUEC_% Extrap_obs, R^2 adjusted) will be excluded.

c. Subject having pre-dose value >5% of C_{max} for Pegfilgrastim and for ANC on baseline-corrected data [Note: statistical analysis with including the same will be provided for supportive information].

11.3 Pharmacokinetic parameters

The following pharmacokinetic parameters will be computed for Pegfilgrastim using non-compartmental model of Phoenix® WinNonlin® Version 6.4 or higher (Certara L.P.):

Primary PK Parameters:		
C_{max}	:	Maximum measured serum concentration.
$AUC_{0-\infty}$	:	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.
Secondary PK Parameters:		
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.
T_{max}	:	Time to reach the maximum measured serum concentration.

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 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, $[(AUC_{0-\infty} - AUC_{0-t})/AUC_{0-\infty}] \times 100$.
R^2adjusted	:	Goodness of fit statistic for the terminal phase, adjusted for the number of points used in the estimation of λ_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 PK 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 based on baseline non-adjusted and baseline-adjusted data using non-compartmental model of Phoenix® WinNonlin® Version 6.4 or higher (Certara L.P.):

For ANC [baseline non-adjusted data]:

Primary PD Parameters: E_{max} and $AUEC_{0-t}$

For ANC [baseline-adjusted data]:

PD parameters: 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, after adjustment, any negative concentration values are obtained, they will be set to zero.

11.1.1 Statistical Analysis

All the statistical and safety analyses will be performed using SAS® Version 9.3 or higher (SAS Institute Inc., USA).

Statistical analysis will be performed on subjects completing both the treatment periods.

Descriptive statistics will be calculated for PK parameters of Pegfilgrastim and for PD parameters of ANC (baseline non-adjusted and baseline-adjusted data).

PD assessment will be based on the baseline non-adjusted data of ANC only. The baseline-adjusted data of ANC will be presented for supportive information only.

i. **Analysis of variance**

The ln-transformed PK parameters C_{max} and $AUC_{0-\infty}$ for Pegfilgrastim and ln-transformed PD parameters E_{max} and $AUEC_{0-t}$ for baseline non-adjusted data of ANC will be subjected to ANOVA.

ANOVA model will include group, sequence, sequence*group, formulation, group*formulation, and period (group) as fixed effects, and subject (sequence*group) as a random effect. Group, sequence and sequence*group effects will be tested using subject (sequence*group) as an error term.

If group*formulation effect is found to be statistically insignificant at 5% level, this interaction term (group*formulation) will be dropped from the model and statistical analyses will be re-performed excluding this interaction term.

Each ANOVA will include calculation of least-squares means, the difference between adjusted formulation means, and the standard error associated with this difference. The above statistical analyses will be done using the appropriate SAS® procedure.

An F-test will be performed to determine the statistical significance of the effects involved in the model at a significance level of 5% (alpha=0.05).

ii. **Two one-sided tests for Bioequivalence**

Using two one-sided tests for bioequivalence, 90% confidence intervals for the test to reference ratio of geometric least squares means will be calculated for ln-transformed C_{max} and $AUC_{0-\infty}$ for Pegfilgrastim and E_{max} and $AUEC_{0-t}$ for baseline non-adjusted ANC.

iii. **Power**

The power of the study will be computed and reported for ln-transformed C_{max} and $AUC_{0-\infty}$ for Pegfilgrastim and ln E_{max} and $AUEC_{0-t}$ for baseline non-adjusted ANC.

iv. **Ratio analysis**

Ratio of geometric least squares means of test and reference formulations will be computed and reported for ln-transformed C_{max} and $AUC_{0-\infty}$ for Pegfilgrastim and E_{max} and $AUEC_{0-t}$ for baseline non-adjusted ANC.

v. **Inter and Intra-subject variability**

Inter and Intra-subject variability will be computed and reported for ln-transformed C_{max} and $AUC_{0-\infty}$ for Pegfilgrastim and E_{max} and $AUEC_{0-t}$ for baseline non-adjusted ANC.

vi. **Missing and Non-reportable values**

Any missing samples (M) or non-reportable (NR) concentration value will be disregarded in PK, PD and statistical analysis.

vii. **Pharmacokinetic Bioequivalence criteria**

Bioequivalence of the test product with that of the reference product will be concluded if 90% confidence interval for the test to reference ratio of the geometric least squares means for ln-transformed PK parameters C_{max} and $AUC_{0-\infty}$ of Pegfilgrastim falls within the acceptance range of 80.00% to 125.00%.

viii. **Pharmacodynamic Bioequivalence criteria**

Bioequivalence of the test product with that of the reference product will be concluded if 90% confidence interval for the test to reference ratio of the geometric least squares means for ln-transformed PD parameters E_{max} and $AUEC_{0-t}$ of baseline non-adjusted ANC falls within the acceptance range of 80.00% to 125.00%.

ix. **Immunogenicity Data Analysis**

Immunogenicity (Anti-Drug Antibody-ADA) data will be presented and evaluated, if required.

11.2 Assessment of Eligibility/Safety

11.2.1 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, vital signs, serum pregnancy test (for female subjects) and clinical laboratory tests that are out of the range] will be listed and evaluated descriptively.

11.2.2 Eligibility Assessments

The following assessments will be conducted 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).

11.2.3 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 unless,

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 and 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.

11.2.3.1 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

11.2.3.2 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

11.2.4 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 of period-II). This examination also includes the assessment of blood samples for hematology (except sickling test), 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.

11.2.5 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 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***	**Period-I**									**Period-II**									
Procedure																				
Day		**-1**	**1**	**2**	**3**	**4**	**5-11, 13-14**	**15**	**22**	**29 ##**	**-1**	**1**	**2**	**3**	**4**	**5-11, 13-14**	**15**	**22**	**57## #**	**71 ##**
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		
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	X
Immunogenicity								X		X									X	X

evaluation##																			
Standardized vegetarian breakfast		X							X										

* Within 28 days prior to dosing in period-I

√Consent for screening

∞Study specific consent

^Post-dose vitals as mentioned in protocol

+ Clinical lab investigation will be performed as per section 13.0 (list of laboratory parameters).

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

#End study (after last ambulatory sample of period-II).

@ Estimation of hematology (except sickling test), biochemistry and urine analysis will be done within 3 working days prior check in of period-II.

$ Chest X-ray is valid up to 6 months

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

** Blood sampling time points:

Pharmacokinetic Sampling points : Pre-dose (collected within 60 minutes prior to dosing) and at 2.000, 4.000, 6.000, 8.000, 10.000, 12.000, 14.000, 16.000, 18.000, 20.000, 22.000, 24.000 (Day 2), 26.000, 30.000, 36.000, 42.000, 48.000 (Day 3), 60.000, 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 168.000 (Day 8), 192.000 (Day 9), 240.000 (Day 11), 288.000 (Day 13), 336.000 (Day 15) and 504.000 (Day 22) hours following drug administration in each period.

Pharmacodynamic sampling time points: Pre-dose (collected within 60 minutes prior to dosing) and at 6.000, 12.000, 24.000 (Day 2), 36.000, 48.000 (Day 3), 60.000, 72.000 (Day 4), 96.000 (Day 5), 120.000 (Day 6), 144.000 (Day 7), 168.000 (Day 8), 192.000 (Day 9), 216.000 (Day 10), 240.000 (Day 11), 288.000 (Day 13), 312.000 (Day 14), 336.000 (Day 15), 360.000 (Day 16) and 504.000 (Day 22) hours following drug administration in each period.

##For Immunogenicity evaluation: Venous blood samples (08 ml each) will be withdrawn at screening, pre-dose of period I (Day 1) , and at 336.000 (Day 15) 672.000 (Day 29), pre-dose of period II (Day 43) 1344.000 (Day 57), 1680.000 (Day 71) hours post dose..

Notes:

1:-Check-in days (Day -1)

2:-Dosing Days (Day 1)

3:- Housing days (Day 1 to Day 3)

4:-. Checkout day (Day 4)

5:-. Ambulatory sample (days 5-11, 13-16, 22,29,57,71)

6:-. End study (Day 71, after last ambulatory sample of period-II)

13.0 LIST OF LABORATORY PARAMETERS

HAEMATOLOGY	BIOCHEMISTRY		
CBC 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	Triglycerides
	Total cholesterol		

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

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 PERIOD			
(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 period: 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 check in of period-II.

End of the study (after last ambulatory sample of period-II): Hematology (except sickling test), biochemistry and 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.

Bio-analytical laboratory:

Study director as specified in the bioanalytical study plan will be responsible for the bioanalytical component of 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 the 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 (2006); 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) and USFDA guidelines for bioequivalence studies.

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. 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 for period-I 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 legally accepted representative (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 performance. 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.

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 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 at least 20% of the raw data generated during the course of the study including the final reports will be conducted by the 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 the 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 the 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 PK and PD, immunogenicity calculations, the statistical analysis of the data and a clinical report along with the raw data.

22.0 ARCHIVING

A representative sample of the drug supplies used in the study will be retained at [redacted] in accordance with the contract obligations. All data generated in connection with this study, together with the copy of this protocol, 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 the 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: 04/2016
2. Summary of Product Characteristics (NEULASTA® 6 mg solution for injection). last updated on the eMC: Updated 24-Jun-2015.