CENTER FOR DRUG EVALUATION AND RESEARCH AND CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

APPLICATION NUMBER:

125031/0

PHARMACOLOGY REVIEW(S)

TOXICOLOGIST'S REVIEW

STN: 125031/0
SPONSOR: Amgen, Inc.
PRODUCT: recombinant human pegylated granulocyte-colony stimulating factor; PEG-CSF; PEG-G-CSF; Filgrastim-SD/01; Pegfilgrastim
FORMULATION/CHEMISTRY: Active ingredient - r-metHuG-CSF, produced in a bacterial (*E. coli*) expression system in a — form composed of 175 amino acids with a MW of — kDa. The protein is a single chain polypeptide with 2 disulfide bonds & containing methionine as the N-terminal amino acid. This protein is covalently attached [at the N-terminal residue] to a 20 kDa PEG group. Formulated as a sterile, clear, aqueous solution with — sodium acetate, pH — with —sorbitol & — Tween 20, at concentrations of — — . There is no preservative, thus vials are for single use only.
PROPOSED INDICATION: —

ABBREVIATIONS: recombinant human pegylated granulocyte-colony stimulating factor = PEG-G-CSF; absolute neutrophil count = ANC

received 4/4/01; completed 12/17/01

CROSS-REFERENCES: IND # —

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INTRODUCTION:

The protein G-CSF is involved in the proliferation, differentiation, and/or survival of cells of the neutrophil lineage. This protein also stimulates responses in mature neutrophils, including phagocytosis & superoxide production. Filgrastim SD/01 [PEG-G-CSF] is produced by covalent attachment of a 20 kD PEG molecule to the amino terminal methionine residue of the Filgrastim [G-CSF, Neupogen®] polypeptide chain. This modified protein binds to, and activates, the G-CSF receptor on the surface of cells of the neutrophil lineage, resulting in stimulation of neutrophil production. In vitro studies have indicated that the bindings affinities of PEG-G-CSF

and G-CSF to the G-CSF receptor are similar. Product characterization evaluation revealed that Filgrastim SD/01 displays similar biological effects as Filgrastim by acting through the G-CSF receptor & via the same signaling pathway. In addition, the primary structure of PEG-G-CSF is identical to G-CSF, with the addition of a PEG molecule to the N-terminus.

The sponsor estimates that Filgrastim, initially approved in 1991, has been used in [redacted] patients to treat neutropenia, including acute myeloid leukemia, chemo-induced neutropenia, BMT, & severe chronic neutropenia. The pegylation of G-CSF results in a prolonged serum half-life & a prolonged pharmacodynamic effect compared to G-CSF, although displaying similar receptor binding affinities & an identical effector mechanism of action [binding the G-CSF receptors] compared to Filgrastim.

The proposed clinical indication [per the package insert] for PEG-G-CSF is to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with [redacted]

The PI-recommended dose of PEG-G-CSF for the specified patient population is a single SC injection of 6 mg/human/dose [about 100 μg/kg], administered once per chemotherapy cycle. This injection should be given approximately 24 hrs after the last dose of cytotoxic chemotherapy. The "Precautions" section of the PI states that [redacted] In addition, the safety & efficacy of PEG-G-CSF has not been assessed in patients exposed to antimetabolites [i.e., 5-FU] or in patients undergoing radiation therapy.

Formulation – PEG-G-CSF will be distributed as a preservative-free solution containing 6 mg protein (10 mg/mL, 0.6 mL) in prefilled syringes (PFS). The solution in the syringe contains 0.35 mg acetate, 30 mg sorbitol, 0.02 mg polysorbate 20, [redacted] mg sodium, and [redacted] water for injection, USP.

Preclinical Pharmacology Studies

In vitro

1.Investigation of the Role of Receptor Mediated Clearance of SD/01; study #Ang00-147; performed at Amgen; lot #unknown; date unknown

2.Competitive Displacement Binding Studies of SD/01 & G-CSF on Isolated Human Neutrophils; study #Ang00-237; performed at Amgen; lot #unknown; 1/01

In vivo

1. Timing of SD/01 Relative to Chemotherapy; study #Ang00-146; performed at Amgen; lot #unknown; date unknown

2. Dose Response to SC Injection of SD/01 or Neupogen; study #Ang00-149; performed at Amgen; lot #unknown; date unknown

3. Continuous Infusion of SD/01; study #Ang00-150; performed at Amgen; lot #unknown; date unknown

4. IV vs. SC SD/01 in Normal Mice & Rats; study #Ang00-151; performed at Amgen; lot #unknown; date unknown

5. Diurnal Fluctuations in Neutrophils During Daily Neupogen or a Single Injection of SD/01; study #Ang00-152; performed at Amgen; lot #unknown; date unknown

6. Mobilization & Transplant of PBPC by SD/01 or Neupogen; study #Ang00-153; performed at Amgen; lot #0201077, APD32; date unknown

7. Immunological Response to rhuG-CSF or Filgrastim SD/01 in Dogs; study #Ang00-154; performed at Amgen; lot #unknown; 2/01

8. The Efficacy & Immunogenicity of Filgrastim SD/01 Relative to Filgrastim in Chimps; study #00-8748; performed at Amgen; lot #unknown; 2/01

Pharmacology Studies

In vitro

1. Investigation of the Role of Receptor Mediated Clearance of SD/01
Human neutrophils obtained from healthy subjects were cultured with 500 ng of PEG-G-CSF or G-CSF +/- 1 mM Pefabloc SC [a sulfonyl fluoride which acts as a serine protease inhibitor] + 1 μM staurosporine [which acts as a protein kinase C inhibitor]. The inhibitors were included in an attempt to delineate the contribution made by protease digestion of the proteins. At specific timepoints, culture supernatants were collected & PEG-G-CSF & G-CSF levels measured via ELISA. As human neutrophils do not express the c-mpl, which is the receptor for MGDF, this protein was used as a control.

Results: Neutrophils are able to clear G-CSF & PEG-G-CSF from the supernatant media. The PEG-G-CSF was cleared slower than the unPEGylated protein – possibly due to some type of protection from proteolytic cleavage. Both G-CSF & MGDF were cleared from the supernatant, reflective of an apparent lack of receptor-mediated clearance.

2. Competitive Displacement Binding Studies of SD/01 & G-CSF on Isolated Human Neutrophils

Due to the difficulty of radiolabeling G-CSF, a [redacted] – was iodinated & used to determine the relative affinity of SD/01 & G-CSF for the G-CSF receptor on isolated human neutrophils using competition displacement methods.

Results: The IC_{50} for inhibition of the [redacted] was 232 pM (G-CSF) & 286 pM (SD/01) – thus similar affinities for the same receptor.

G-CSF is cleared via renal filtration & neutrophil-mediated degradation. The Pegylation results in a higher MW molecule, making the protein weight too high for renal clearance.

In vivo

1. Timing of SD/01 Relative to Chemotherapy

Peg-G-CSF (1000 μg/kg) was singly SC injected into BDF1 mice at various times relative to dosing with various chemotherapeutic agents:
Study #1 – Carboplatin (IP) + whole body irradiation
Study #2 – 5FU (IV)
Study #3 – Cyclophosphamide (IP)
Study #4 – Doxorubucin (IP)
Study #5 – Vinorelbine (IP)
The chemo was administered on day 0. The PEG-G-CSF was injected from 5 days pre-chemo to 5 days postchemo. Bld samples were collected on various days & CBCs & differentials were measured.
Note that the number of hrs prior to, or after, chemo administration was not specified.

Results:
5FU – Rx on day +1 had the most beneficial effect on the nadir & duration of neutropenia, which resulted in an accelerated return to baseline ANC. Rx on days –3, -1, or 0 resulted in high mortality.

Carboplatin + whole body irradiation - Rx on day 0 minimized the depth of neutropenia & with Rx on day +1, ANC counts were maximally accelerated & the duration of neutropenia was reduced.

Doxorubicin – Rx on day –1, day 0, day +1, day +3, or day +5 reduced the duration of severe neutropenia, improved the degree of neutropenia. There was no specific day that appeared to be notably better over the others

Vinorelbine – Rx on day –1, day 0, or day +1 minimized the neutropenia

Cyclophosphamide – Rx on day +1, day +3, or day +5 minimized the depth of neutropenia & accelerated the return to baseline ANC.

General Conclusion: The optimum timing of PEG-G-CSF administration was primarily shortly (i.e., day +1) after chemo

2. Dose Response to SC Injection of SD/01 or Neupogen

Study #1 - Normal BDF1 mice were singly SC injected with 200, 500, or 1000 μg/kg of G-CSF or with 50, 100, or 500 μg/kg of PEG-G-CSF

Study #2 - Normal BDF1 mice were singly SC injected with 50, 100, 200, or 500 μg/kg of PEG-G-CSF or daily x 5 with 100, 200, or 500 μg/kg/day of G-CSF.

Results: Study #1 – Single injections of G-CSF raised the ANC by no more than 2-fold baseline, while a single injection of PEG-G-CSF raised the ANC by 10-fold baseline, with elevated ANC levels sustained over the 4-day interval.

Study #2 – Repeat injections of G-CSF resulted in increasing ANC levels through the injection period (still increasing on day 5), while a single injection of PEG-G-CSF resulted in peak ANCs by day 3, followed by a decline.

3. Continuous Infusion of SD/01

Male splenectomized mice (n=60) were SC implanted with [redacted] micro osmotic pumps, designed to deliver protein at a rate of 0.5 μL/hr or 12 μL/day for about 7-8 days. The mice were dosed with SD/01 at 50, 100, 200, 500, 1000 μg/kg/day, with blood collected at protocol-specified intervals.

Results: The ANC levels peaked at days 5-6, approaching baseline by day 9.

4. IV vs. SC SD/01 in Normal Mice & Rats

Female BDF1 mice were singly SC or IV injected (200 μL volume) with 1 mg/kg of SD/01 [study #1]. Other mice were rendered neutropenic (via 5FU), followed (two days later) by a single IV or SC injection of 0.5 or 1 mg/kg of SD/01 [study #2]. Another grp of rats were given cyclophosphamide (day 1), followed (on day 5) by a single IV or SC injection of 0.1 or 0.5 mg/kg of SD/01 [study #3].

Results:
Study #1 – No apparent difference in the ANC counts for the two ROAs [even though the bioavailibility of SD/01 via the SC ROA has been reported to be <100%].

Study #2 – All SD/01 grps displayed a transient increase in ANC one day post-SD/01 injection, followed by a notable nadir at days 5-6, returning to baseline by days 7-11 (depending o the dose level), compared to day 14 for control.

Study #3 – similar pattern as for study #2

Study Conclusion: Although the reported bioavailability of PEG-G-CSF via the SC ROA is <100%, the PD effect does not delineate between the different routes.

5. Diurnal Fluctuations in Neutrophils During Daily Neupogen or a Single Injection of SD/01
Female BDF1 mice (n=20/grp) were singly injected (route unknown) with 1000 μg/kg of SD/01 or with 200 μg/kg/day x 6 days of G-CSF, and then bled every six hrs.

Results: A single injection of SD/01 minimized the daily fluctuations in neutrophil counts that occurred with repeat dosing with G-CSF.

6. Mobilization & Transplant of PBPC by SD/01 or Neupogen
Splenectomized BDF1 mice were singly SC injected with 1000 μg/kg of SD/01 or SC injected with 200 μg/kg/ day x 5 days of G-CSF, followed by evaluation of GM-CFC [study #1]. Blood from donor mice, mobilized with SD/01 or G-CSF, was IV injected into lethally irradiated recipient mice (5, 10, 20 μL), & the mice evaluated fro survival [study #2].

Results:
Study #1 – Mice singly injected with SD/01 mobilized similar numbers of hematopoietic progenitor cells to mice that were given repeat injection of G-CSF

Study #2 – Protection was highest at 20 μL of bld from the PEG-G-CSF donor mice, followed by 10 μL of bld from the G-CSF donor mice.

7. Immunological Response to rhuG-CSF or Filgrastim SD/01 in Dogs
Three female purebred hounds (, were SC injected with 100 μg/kg/dose PEG-G-CSF (2/3 dogs), qiw. The third dog was SC injected with G-CSF at 10 μg/kg/dose, 5x/wk. The dogs were dosed for about 10 wks (days 104-117). Bld was collected at various intervals for CBCs. Sera Ab levels were not determined.

Results: One dog injected with PEG-G-CSF & the dog injected with G-CSF displayed neutropenia by the end of dosing, while the second PEG-G-CSF dog was at baseline levels. No antibody data were presented, thus no conclusion can be made regarding a potential correlation with some type of neutralizing Ab response.

8. The Efficacy & Immunogenicity of Filgrastim SD/01 Relative to Filgrastim in Chimps
Chimps (4/grp) were SC injected with 10 µg/kg/day of G-CSF for 28 days or 70 µg/kg/dose of SD/01, on days 0, 7, 14, 21. Bld was collected at various intervals & WBC & ANC levels & antibodies determined [via a [redacted] ELISA].

Results: Daily injections of G-CSF resulted in a 3-5-fold increase in WBC & ANC levels during the dosing interval. By one wk post-last injection, the ANC levels had returned to baseline. Weekly injection of SD/01 also resulted in increased WBCs & neutrophils, but the pattern was much more variable compared to the G-CSF chimps, possibly due to the dosing regimen.
Ab determination [up to 56 days after dose #1] showed that titers of IgM or IgG using G-CSF as the capture antigen were not changed for the chimps dosed with SD/01. Two of four chimps dosed with G-CSF displayed IgM responses, using G-CSF as the capture antigen, at day 42/49/56 – considered to be due to cross-rxtivity with irrelevant proteins (like IL-1ra). One of these two chimps also showed an IgM titer on day 49, when SD/01 was used as the capture antigen.
No definitive conclusion regarding Ab production & any relative correlation to an altered PD response could be drawn from the data generated from this small grp of animals.

PK/ADME Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. PK & PD of PEG (20K) G-CSF Given at Two Dose levels IV & SC to Rhesus Monkeys; study #PK94006; performed at [redacted] Amgen (per GLP); lot # unknown; 11/00

2. PK/PD Dose Ranging Study of Filgrastim SD/01 in Male SD Rats; study #PK97005; performed at Amgen (non-GLP); lot #0201077 (w/ 0.25% HSA added as a carrier); 10/97

3. PK & PD Dose Ranging Study of Single IV Doses of 20 KD PEG –GCSF in Male CD-1 Mice; study #PK97010; performed at Amgen (non-GLP); lot #0201077 (w/ 0.25% HSA added as a carrier); 4/98

4. PK & PD of Different Pegylated Forms of Filgrastim After SC Administration in Cyno Monkeys; study #PK97018/960135; performed at — Amgen (per GLP); lot #6736C6 (G-CSF), #11246 (G-20K Mono PEG-G-CSF), lot # for mono-12K-PEG-G-CSF & multi-20K-PEG-G-CSF unknown; 10/00

5. PK, PD, & Placental Transfer of r-metHuG-CSF-SD/01 in Pregnant & Nonpregnant Rats via SC Administration; study #100338; performed at — (per GLP); lot #1104098; 10/00

6. A PK Study of Filgrastim & Filgrastim-SD/01 Following IV Administration in Bilateral Nephrectomized Male SD Rats; study #100626; performed at Amgen/ — non-GLP); lot #2012048M8B; 9/00

7. PK of Filgrastim-SD/01 After a SC Injection of two Different Concentrations of Dose Solution to Male SD Rats; study #101170; performed at Amgen/ — non-GLP); lot #2012048M8B; 11/00

8. PK & PD of Filgrastim-SD/01 in Myeloablated Rhesus Monkeys Followed by BM Transplantation; study #100841; performed at Amgen/ — non-GLP); lot #2012048M8B; 10/01 [summary included in BLA submission; complete report submitted on 10/31/01, per CBER request]

PK/ADME Studies

1. PK & PD of PEG (20K) G-CSF Given at Two Dose levels IV & SC to Rhesus Monkeys
Species: rhesus monks (1 male & 2 females/grp)
Dose Level: 10, 100 μg/kg
Route/Duration: IV or SC/single dose; cross-over study

Methods: Animals were injected via one route, followed by blood collection at protocol-specified intervals (out to 72 hrs postRx), followed by injection via the second route after a 2-wk wash out interval. Hematology parameters were also evaluated at the same time intervals. Other evaluations included clin signs, BWs, & appetite.
Sera were analyzed via ELISA using a commercial kit for G-CSF with a lower limit of quantification of —

Findings: ↑ neutrophils, WBCs, monocytes, banded neutrophils – both routes (dose-related)
No effect on red cell parameters was noted

The PK data were nonlinear, with $AUC_{(0\text{-last})}$ increasing more than in a dose-proportional manner for both routes. In addition, median C_{max} did not increase in a dose proportional pattern for the SC route. The report notes that the decrease in C_{max} & AUC after the second injection at 100 µg/kg may be a result of the expansion of neutrophil & neutrophil mass after dosing.
The bioavailability of the product was 68% (10 µg/kg) & 49% (100 µg/kg). These values were considered to be low due to the nonlinear clearance exhibited by PEG-G-CSF. The report postulates that this is because the absorption process lowers the concentrations that reach the systemic circulation after SC injection, resulting in a lesser degree of saturation of the clearance pathway.
The increase in ANCs, similar for both routes, was dose-related, but less than dose-proportional. The ANC increase was higher after the second injection, possibly a reflection of the maturation of neutrophil progenitor cells after the first dose.

PK data are presented below:

Table 5-14. Mean (SD) Pharmacokinetic and ANC Parameter Values in Rhesus Monkeys After a IV and SC Administration of 10 and 100 µg/kg Filgrastim-SD/01

Parameter	10 µg/kg, IV	10 µg /kg, SC	100 µg /kg, IV	100 µg /kg, SC
		Period 1		
N	3	3	3	3
C_{max} (ng/mL)	377 (76)	30.5 (9.2)	3930 (380)	618 (186)
T_{max} (h)	0.0 (0.0)	4.0 (0.0)	0.0 (0.0)	6.7 (2.3)
$AUC_{(0\text{-last})}$ (ng·h/mL)	1640 (530)	196 (47)	29,100 (3100)	10,500 (1990)
V_c (mL/kg)	27.4 (6.2)	NA	25.6 (2.5)	NA
C_{max}_ANC (cells x 10^9/L)	24.2 (3.0)	27.8 (4.5)	31.7 (4.7)	30.8 (5.5)
T_{max}_ANC (h)	5.3 (2.3)	6.7 (1.2)	30.7 (20.5)	16.0 (17.4)
AUC_ANC (cells x 10^9·h/L)	762 (38)	888 (83)	1650 (280)	1410 (510)
		Period 2		
N	3	3	3	3
C_{max} (ng/mL)	364 (47)	31.9 (19.5)	3210 (260)	486 (175)
T_{max} (h)	0.0 (0.0)	6.0 (0.0)	0.0 (0.0)	6.0 (2.0)
$AUC_{(0\text{-last})}$ (ng·h/mL)	1390 (270)	268 (144)	24,800 (3400)	6400 (2640)
C_{max}_ANC (cells x 10^9/L)	34.3 (12.5)	27.9 (1.8)	56.4 (8.1)	56.6 (7.6)
T_{max}_ANC (h)	8.0 (3.5)	9.3 (2.3)	18.0 (10.4)	8.7 (3.1)
AUC_ANC (cells x 10^9·h/L)	811 (153)	1090 (120)	2300 (440)	2880 (780)

NA = Not applicable

Table 12.1. Median (Range) Pharmacokinetic and ANC Parameter Values in Rhesus Monkeys After a Single Intravenous and Subcutaneous Administration of 10 and 100 µg/kg Filgrastim-SD/01

Parameter	10 µg/kg, IV (n = 3)	10 µg/kg, SC (n = 3)	100 µg/kg, IV (n = 3)	100 µg/kg, SC (n = 3)
		Period 1		
C_{max} (ng/mL)	408 (290-432)	30.9 (21.1-39.5)	3970 (3530-4280)	542 (482-830)
T_{max} (h)	0.00 (0.00-0.00)	4.00 (4.00-4.00)	0.00 (0.00-0.00)	8.00 (4.00-8.00)
$AUC_{(0\text{-last})}$ (ng·h/mL)	1830 (1040-2060)	190 (152-245)	30500 (25500-31300)	9530 (9200-12800)
C_{max}_ANC (cells x 10^9/L)	24.5 (21.0-27.0)	26.6 (24.0-32.8)	32.8 (26.6-35.8)	27.7 (27.6-37.1)
T_{max}_ANC (h)	4.00 (4.00-8.00)	6.00 (6.00-8.00)	36.0 (8.00-48.0)	8.00 (4.00-36.0)
AUC_ANC (cells x 10^9·h/L)	766 (722-797)	851 (831-983)	1790 (1340-1840)	1430 (895-1910)
		Period 2		
C_{max} (ng/mL)	344 (330-417)	41.8 (9.45-44.5)	3190 (2950-3480)	507 (301-650)
T_{max} (h)	0.00 (0.00-0.00)	6.00 (6.00-6.00)	0.00 (0.00-0.00)	6.00 (4.00-8.00)
$AUC_{(0\text{-last})}$ (ng·h/mL)	1440 (1100-1630)	347 (102-354)	26600 (20800-26800)	7160 (3460-8580)
C_{max}_ANC (cells x 10^9/L)	33.8 (22.0-47.1)	28.8 (25.8-29.2)	55.9 (48.6-64.8)	56.8 (48.9-64.1)
T_{max}_ANC (h)	6.00 (6.00-12.0)	8.00 (8.00-12.0)	24.0 (6.00-24.0)	8.00 (6.00-12.0)
AUC_ANC (cells x 10^9·h/L)	867 (639-928)	1160 (950-1170)	2130 (1980-2800)	3040 (2020-3570)

C_{max} = Maximum serum concentration estimated by fitting a log-linear line to the first 2 data points after intravenous administration or from observation after subcutaneous administration
T_{max} = Time of C_{max}
$AUC_{(0\text{-last})}$ = Area under serum concentration-time curve from time 0 to the time of the last detectable concentration
C_{max}_ANC = Maximum increase in ANC from the baseline value
T_{max}_ANC = Area under the absolute neutrophil count-time curve
AUC_ANC = Area under the absolute neutrophil count-time curve from time 0 to the time of the last ANC measurement

2. PK/PD Dose Ranging Study of Filgrastim SD/01 in Male SD Rats
Species: SD rats (3 males/grp)
Dose Level: 0, 1, 7, 14, 70, 140, 700, 1400 µg/kg (IV & SC); 50, 100, 500, 1000 µg/kg (SC)
Route/Duration: IV or SC/ single dose

Methods: PEG-G-CSF was injected & blood collected at protocol-specified time intervals (out to 216 hrs) postRx, and the plasma analyzed via ELISA using a commercial kit for G-CSF with a lower limit of quantification of [redacted] Neutrophil counts were also evaluated at the same time intervals.

Findings: The clearance rate of PEG-G-CSF was nonlinear, which was attributed to saturation of G-CSF receptor-mediated endocytosis (or receptor mediated clearance, RMC). Thus, the bioavailabiltiy of PEG-G-CSF increased with dose due to saturation of RMC.

Via the IV route - the ANC levels were increased from 1.8 to 11.8-fold above baseline at 1-1400 µg/kg in a dose dependent manner. The duration of response was also dose-dependent. Neutrophil levels were similar to baseline by about 4 days (1 µg/kg) & 7 days (1400 µg/kg) – correlating to the drop in PEG-G-CSF levels.

Via the SC route – the time to peak neutrophil response was 24 hrs (1-100 µg/kg), 72 hrs (700, 1400 µg/kg). The ANC levels were increased from 1.6 to 9.5-fold above baseline at 1-1400 µg/kg.

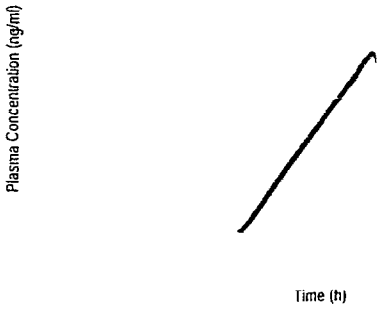
Per the report, plasma levels of PEG-G-CSF declined as these levels approached the k_M [Michaelis constant for nonlinear clearance] for RMC (14.4 ng/mL). Relative exposure [AUC-SC/AUC-IV] increased with dose.

PK data are presented below:

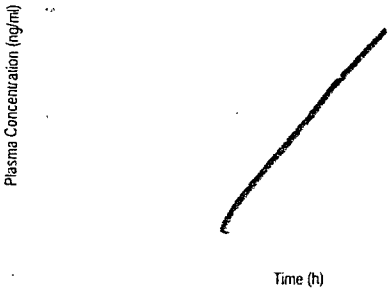
Dose (µg/kg)	C_{max}		AUC (0-∞) (µg*hr/ml)		CL/F or CL (ml/h/kg)		$t_{1/2}$ (h)	T_{max} (h)
	IV(µg/mL)	SC(ng/mL)	IV	SC	IV	SC	IV	SC
1400			478.60	112.00	2.97	12.57	6.23	16.00
1000			ND[1]	84.94	ND[1]	12.00	ND[1]	12.00
700			191.60	60.27	3.70	12.20	7.49	16.00
500			ND[1]	37.02	ND[1]	14.17	ND[1]	16.00
140			37.74	4.14	3.73	36.90	3.33	10.67
100			ND[1]	1.60	ND[1]	69.80	ND[1]	12.00
70			15.66	0.981	4.53	79.57	4.05	12.00
50			ND[1]	0.801	ND[1]	63.37	ND[1]	9.33
14			1.99	0.104	7.13	136.50	2.63	6.67
7			0.71	0.035	9.93	202.03	1.96	5.39
1			.069	ND[1]	14.63	ND[1]	1.34	ND[1]

[1] ND = not determined

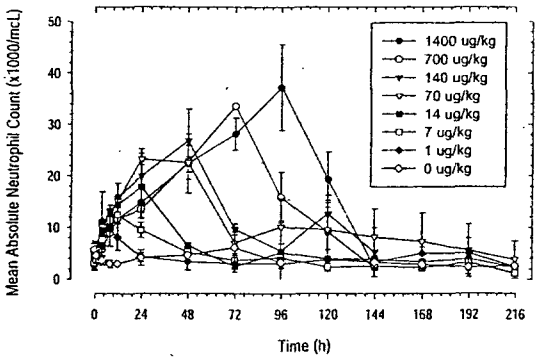
Plasma Concentration from Rats Given a IV Dose of Filgrastim SD/01



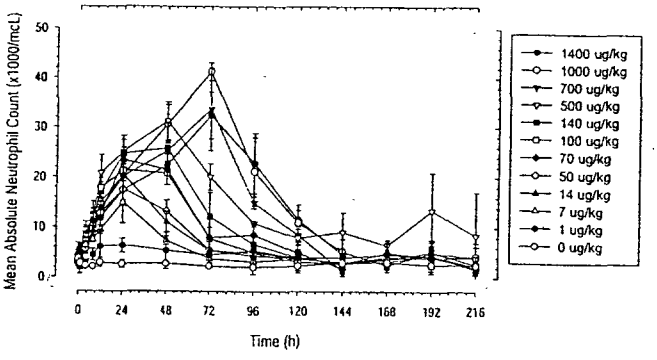
Plasma Concentration from Rats Given a SC Dose of Filgrastim SD/01



Neutrophil Counts from Rats Given a IV Dose of Filgrastim SD/01



Neutrophil Counts from Rats Given a SC Dose of Filgrastim SD/01



3. PK & PD Dose Ranging Study of Single IV Doses of 20 KD PEG –GCSF in Male CD-1 Mice

Species: CD-1 mice (3 males/grp/timepoint)
Dose Level: 10, 100, 1000 µg/kg
Route/Duration: IV/single dose

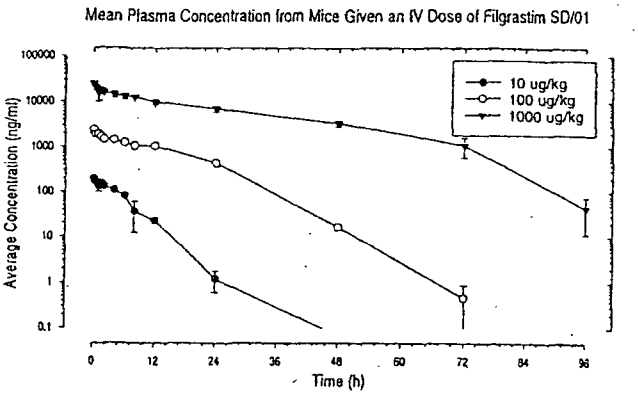
Methods: PEG-G-CSF was injected & blood collected at protocol-specified time intervals (out to 96 hrs) postRx, and the plasma analyzed via ELISA using a commercial kit for G-CSF with a lower limit of quantification of — Neutrophil counts were also evaluated at the same time intervals.

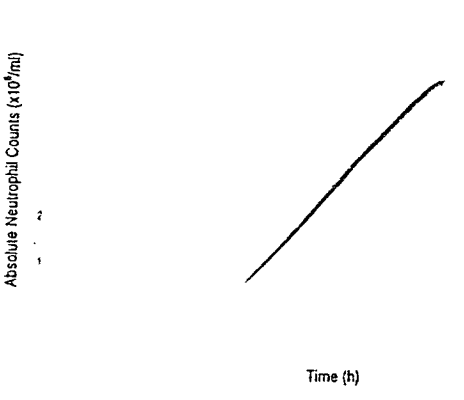
Findings: The clearance rate of PEG-G-CSF was nonlinear w/ dose [4-fold decrease from 10 to 1000 μg/kg], which was attributed to saturation of G-CSF receptor-mediated endocytosis. Thus, the bioavailability of PEG-G-CSF increased with dose due to saturation of RMC.

The ANC levels were increased from 7 to 54-fold above baseline at 1-1000 μg/kg in a dose dependent manner. The duration of response was also dose-dependent. Neutrophil levels were similar to baseline by about 4 days (≤100 μg/kg), while ANCs peaked on day 4 at 1000 μg/kg (last timepoint obtained). The drop in ANCs correlated to the drop in PEG-G-CSF levels. When plasma PEG-G-CSF levels were above k_M, ANC levels remained high. Plasma levels declined as PEG-G-CSF levels approached the k_M [Michaelis constant for nonlinear clearance] for RMC (between 2 & 20 ng/mL).

PK data are presented below:

	Dose		
	10 μg/kg	100 μg/kg	1000 μg/kg
AUC (0-∞) (ng-h/ml)	1119.5	30343.6	448644.6
AUC (0-∞)/ dose (kg-h/ml)	0.112	0.303	0.449
Cmax (ng/ml)		—	
CL (ml/h/kg)	8.93	3.30	2.23
$T_{1/2}$ (h)	2.8	4.9	5.2
Vc (ml/kg)	46.9	33.8	37.4
MRT (h)	5.2	12.1	22.7





4. PK & PD of Different Pegylated Forms of Filgrastim After SC Administration in Cyno Monkeys

Species: cyno monks (3-4/grp)

Dose Level: 0, 100, 300, 1000 μg/kg (mono-12K, mono-20K, & multi-20K-PEG-G-CSF - day 1 only); 10, 100 μg/kg/day (G-CSF – days 1-9)

Route/Duration: SC/single/multiple dose

5. PK, PD, & Placental Transfer of r-metHuG-CSF-SD/01 in Pregnant & Nonpregnant Rats via SC Administration
Species: SD rats (4 females/timepoint)
Dose Level: 1000 µg/kg
Route/Duration: SC/single or repeat doses

Methods:
Pregnant Rats –
PEG-G-CSF was SC injected into one grp of pregnant rats on GD 18 & blood collected at protocol-specified time intervals (out to 96 hrs) postRx. Another grp of pregnant rats was SC injected on GD 6, 8, 10, 12, 14, 16, & 18 & blood collected at protocol-specified time intervals (out to 96 hrs) postRx. This second grp also had amniotic fluid & fetal blood evaluated for ANC & WBC levels, as well as PK analysis.

Nonpregnant Rats – two grps of nonpregnant rats were dosed on day 1 or q2d x 7 doses, & blood collected at protocol-specified time intervals (out to 96 hrs) postRx.

The plasma was analyzed via ELISA with a lower limit of quantification of [redacted]

Findings: After both single & repeat dosing, plasma levels of PEG-G-CSF & ANC levels were higher with in the nonpregnant rats than the pregnant rats. Following repeat dosing, a decrease in AUC/increased clearance was seen – likely reflective of the expansion of neutrophil & neutrophil precursor mass (per the report). Levels of PEG-G-CSF in the amniotic fluid & fetal plasma were minimal, at <0.5%, compared to maternal plasma levels.

	Nonpregnant Plasma		Pregnant Plasma			
Parameter	Single Dose (Group 3)	Multiple Dose (Group 4)	Single Dose (Group 1)	Multiple Dose (Group 2)	Amniotic Fluid (Group 2)	Fetal Plasma (Group 2)
C_{max} (ng/mL)						
T_{max} (h)	24.0	24.0	12.0	24.0	24.0	24.0
$AUC_{(0\text{-last})}$ (ng·h/mL)	134000	56800	60400	23500	79.5	17.9

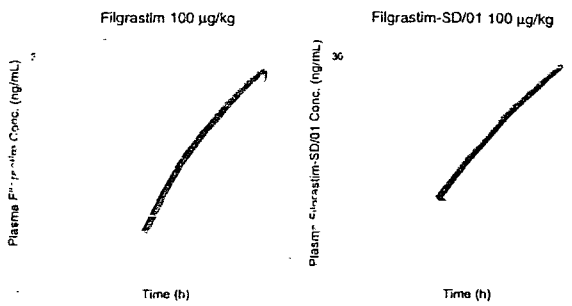
	Nonpregnant Plasma		Pregnant Plasma	
Parameter	Single Dose (Group 3)	Multiple Dose (Group 4)	Single Dose (Group 1)	Multiple Dose (Group 2)
C_{max}_ANC (cells x 10^9/L)				
T_{max}_ANC (h)	96.0	24.0	72.0	3.00
AUC_ANC (cells x 10^9·h/L)	1870	16300	1100	9580

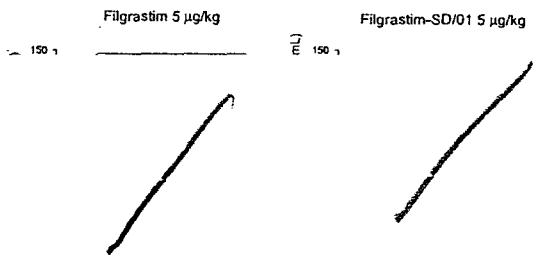
6. A PK Study of Filgrastim & Filgrastim-SD/01 Following IV Administration in Bilateral Nephrectomized Male SD Rats
Species: SD rats (4 males/grp)
Dose Level: 5, 100 μg/kg
Route/Duration: IV/single dose

Methods: Rats received a sham (opened-up only) or bilateral nephrectomy, followed (within 2 hrs post-surgery) by IV injection of PEG-G-CSF or G-CSF. Blood collected at protocol-specified time intervals (out to 18 hrs) postRx, and the plasma analyzed via ELISA, with a lower limit of quantification of [redacted] ng/mL for G-CSF & from [redacted] ng/ml for PEG-G-CSF.

Findings: G-CSF - Approximately 60% of 5 μg/kg & 75% of 100 μg/kg were cleared by the kidney [the mean clearance was about 40%/25% of the values of the sham rats]. It has been documented that G-CSF is partly cleared by receptor-mediated clearance (RMC), which is saturable at high G-CSF concentrations. Upon saturation of RMC, the contribution of nonsaturable & high-capacity clearance pathways (i.e., the renal pathway) to total clearance increases.

PEG-G-CSF – Although the mean concentration-time profiles were slightly higher for the nephrectomized rats given 5 μg/kg, the mean clearance was similar for both this grp & the sham rats were similar. At 100 μg/kg, the mean concentration-time profiles were superimposible for the sham & nephrectomized rats. Thus the kidney does not participate in any significant way to the elimination of PEG-G-CSF. The clearance of PEG-G-CSF was lower compared to G-CSF – likely due to Pegylation. The Pegylation results in a protein that is increased in MW, thus making glomerular filtration harder, resulting in decreased renal clearance and instead, handled via neutrophil-mediated clearance.





Pharmacokinetic parameter values are summarized in the following table.

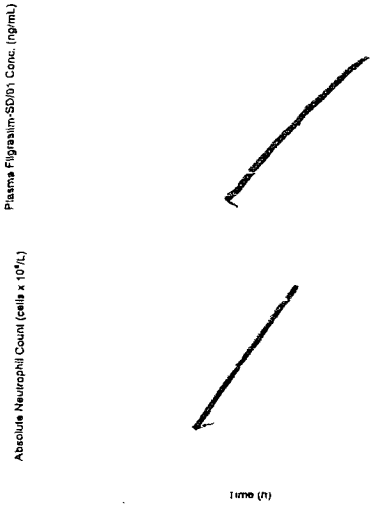
Parameter	Filgrastim 5 μg/kg	Filgrastim 100 μg/kg	Filgrastim-SD/01 5 μg/kg	Filgrastim-SD/01 100 μg/kg
		Sham		
C_{max} (ng/mL)	112 (6)	2470 (130)	106 (20)	2140 (110)
$T_{½}$ (h)	0.71 (0.05)	1.22 (0.18)	1.22 (0.21)	9.43 (0.97)[a]
$AUC_{(0\text{-last})}$ (ng·h/mL)	111 (6)	2670 (250)	447 (75)	17400 (1900)
CL (mL/h/kg)	45.1 (2.4)	37.7 (3.8)	11.4 (1.8)	4.33 (0.48)[a]
		Nephrectomy		
C_{max} (ng/mL)	118 (16)	2390 (240)	104 (5)	2110 (270)
$T_{½}$ (h)	1.19 (0.25)[b]	5.23 (0.73)[b]	2.40 (1.05)	15.8 (7.2)[a]
$AUC_{(0\text{-last})}$ (ng·h/mL)	293 (27)[b]	10000 (1300)[b]	574 (160)	16000 (4300)
CL (mL/h/kg)	17.2 (1.7)[b]	9.28 (1.50)[b]	9.16 (2.74)	3.47 (0.91)[a]

[a]This parameter could not be accurately estimated because a significant amount of drug was left in the body at 18 hours postdose.
[b]Statistically significant difference between the sham and nephrectomy groups receiving the same test material at the same dose (ANOVA, $p<0.05$).

7. PK of Filgrastim-SD/01 After a SC Injection of two Different Concentrations of Dose Solution to Male SD Rats
Species: SD rats (6 males/grp)
Dose Level: 1000 μg/kg of concentrations of 2.5 & 10 mg/mL
Route/Duration: SC/single dose

Methods: Rats were SC injected with PEG-G-CSF, followed by blood collection at protocol-specified time intervals (out to 120 hrs) postRx, and the plasma was analyzed via ELISA [lower limit of quantification of — ng/ml]. In addition, blood was collected with the PK sampling for determination of ANC levels.

Findings: The mean Cmax & AUC values were about 28% higher for the 2.5 mg/mL soln, but not statistically significantly different. The mean ANC levels were similar through day 2, however, the levels noted from the 2.5 mg/mL soln were higher than the ANC levels for the 10 mg/mL soln for the remaining postRx hrs – Cmax & AUC for the ANCs were about 11% higher for the 2.5 mg/mL soln [not statistically significant]. This was also the trend in the PK profile.



Mean (SD) Pharmacokinetic and ANC parameter values are summarized in the following table.

Parameter	2.5 mg/mL	10 mg/mL	p value
n	6	6	-
C_{max} (ng/mL)	2500 (570)	1940 (530)	0.1060
T_{max} (h)	14.7 (2.1)	13.5 (3.0)	0.4458
$AUC_{(0\text{-last})}$ (ng·h/mL)	95300 (28100)	74800 (21500)	0.1858
C_{max}_ANC (cells x10^9/L)	38.9 (8.6)	34.9 (10.5)	0.4795
T_{max}_ANC (h)	72.0 (0.0)	72.0 (15.2)	1.0000
AUC_ANC (cells x10^9·h/L)	3220 (500)	2890 (790)	0.4073

8. PK & PD of Filgrastim-SD/01 in Myeloablated Rhesus Monkeys Followed by BM Transplantation
Species: rhesus monks (3-4 males/grp)
Dose Level: 100 or 300 μg/kg (IV/SC) of PEG-G-CSF; 300 μg/kg (IV/SC) of G-CSF
Route/Duration: SC or IV/single dose

Methods: Rhesus monks were myeloablated by total body irradiation (920 cGy) on day 0, followed by transfusion of about 1 x 10e8 Au BM mononuclear cells/kg. On day 1 (about 20 hrs after AuBMT), the monks were singly SC or IV injected with 100 or 300 μg/kg of PEG-G-CSF or 300 μg/kg of G-CSF. Controls were either SC injected with 0.1% Au serum from days 1-18 (n=9). Control monkeys were SC injected with 0.1% Au serum on days 1-18 (n=9) or IV injected with placebo on day 1 (n=4). Blood was collected at protocol-specified time intervals (out to day 11). Plasma was analyzed via ELISA for PEG-G-CSF [LLQ = [redacted] ng/mL] and G-CSF [LLQ = [redacted] ng/mL] levels. In addition, blood was collected with the PK sampling for determination of CBCs up to day 97.

Findings: Injection of 300 μg/kg of PEG-G-CSF (IV/SC) notably shortened the duration of neutropenia & reduced the time to ANC recovery compared to controls & to G-CSF. At this dose level, the duration of neutropenia & the time to ANC recovery were shortened by about 2-fold. Plasma clearance was about 20-fold higher with G-CSF, thus the systemic exposure (IV) of PEG-G-CSF was higher compared to G-CSF (about 25-fold).

When data from a single IV injection of 100 μg/kg of PEG-G-CSF in normal rhesus monks (report #1 of this section) were compared to the myeloablated monks from this study, the following was noted:
Mean C_{max} = 3930 ±380 ng/mL (normal) vs. 2200 ±310 ng/mL (ablated)
Mean AUC = 29,100 ±3100 ng.h/mL (normal) vs. 46,300 ±9200 ng.h/mL (ablated)
Mean T_{max} = 0 ±0 h (normal) vs. 1.19 ±0.94 h (ablated)

The PEG-G-CSF was cleared from circulation by 2 days in the normal monks & by 8 days in the myeloablated monks. The report postulates that the lower clearance seen in the ablated monks is likely due to a decrease in G-CSF receptor-mediated clearance secondary to the neutropenic condition of the animals.

Table 1-2. Mean ANC Parameter Values in Rhesus Monkeys After AuBMT and Treatment with Cytokines

Treatment	n	Duration of Neutropenia (day)	ANC at Nadir (cells x 10^9/L)	Time to Recovery (day)
		IV		
Placebo	13	11.5	0.063	16.8
SD/01 100 μg/kg	4	5.0	0.192	9.0
SD/01 300 μg/kg	4	2.5	0.333	8.0
Filgrastim 300 μg/kg	4	12.5	0.064	16.3
		SC		
SD/01 100 μg/kg	3	7.0	0.301	12.3
SD/01 300 μg/kg	4	3.5	0.398	6.5
Filgrastim 300 μg/kg	4	10.8	0.132	15.5

Duration of neutropenia: days of ANC < 0.5 x 10^9/L
Time to recovery: days required for ANC to reach ≥ 0.5 x 10^9/L

Figure 1-1. Mean (SD) ANC-time Profiles for Myeloablated Rhesus Monkeys After a Single Administration of the Cytokine

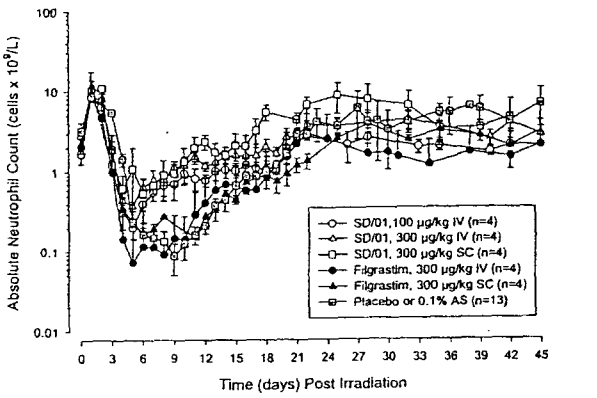
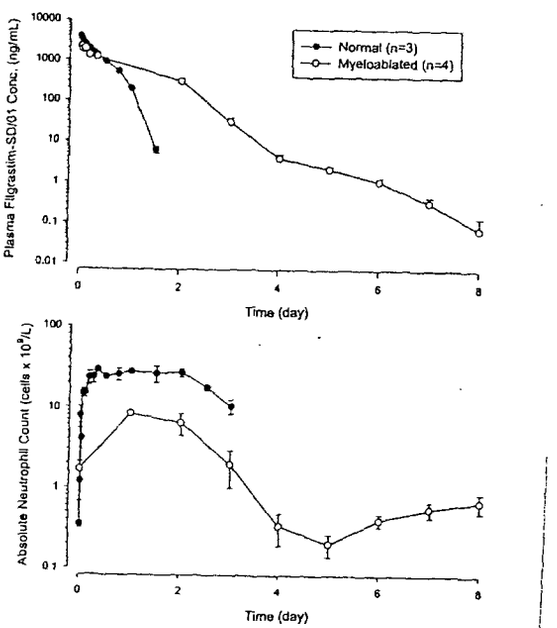


Table 1-1. Mean (SD) Pharmacokinetic Parameter Values in Rhesus Monkeys After AuBMT and Treatment with Intravenous Cytokines

Parameter	SD/01 100 µg/kg	SD/01 300 µg/kg	Filgrastim 300 µg/kg
n	4	4	4
C_{max} (ng/mL)	2200 (310)	6180 (480)	3570 (990)
T_{max} (h)	1.19 (0.94)	0.44 (0.38)	0.31 (0.13)
$T_{½}$ (h)	22.2 (4.6)	16.1 (4.4)	NC
$AUC_{(0\text{-}last)}$ (ng·h/mL)	46300 (9200)	171000 (31000)	6340 (940)
V_c (mL/kg)	46.2 (6.5)	48.8 (3.9)	NC
CL (mL/h/kg)	2.22 (0.44)	1.80 (0.32)	NC

NC = Not calculated

Figure 1-2. Mean (SD) Plasma Filgrastim-SD/01 Concentration- and ANC-time Profiles in Normal and Myeloablated Rhesus Monkeys After a Single Administration of 100 µg/kg Filgrastim-SD/01



Top panels: Filgrastim-SD/01 conc. (semilogarithmic); bottom panels: ANC (semilogarithmic).

Preclinical Toxicology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. Single Dose IV Two-Week Toxicity Study of Filgrastim-SD/01 in Rats; study #100965; performed by — . (per GLP); lot #2012048M8A; 9/00

2.A 9-Day Screening Toxicology Study of SC Filgrastim-SD/01 & Filgrastim-SD/02-157 in SD Rats; study #100204; performed by Amgen (non-GLP); lot #unknown; 6/98

3. A Two-Week SC Toxicity Study of G-20K in Rats; study #970002; performed by — — . (per GLP) & PK analysis by Amgen (study #PK97019, non-GLP; 12/00); lot #0201077A7; 12/00

4. A One-Month Repeat Dose SC Toxicity Study of Filgrastim-SD/01 in Cynomolgus Monkeys with a One-Month Recovery Period; study #100298; performed by — per GLP); lot #1104098; 6/00

5. 3/6 Month SC/IV Toxicity Study of Filgrastim-SD/01 in Rats with Recovery; study #100062; performed by [redacted] (per GLP); lot #1112037A8; 7/99

Acute Toxicity Studies

1. Single Dose IV Two-Week Toxicity Study of Filgrastim-SD/01 in Rats
Species: CD®(SD)IGS BR rats (5/sex/grp)
Dose Levels: 0,100, 1000, 3000, 10,000 μg/kg/dose
Route/Duration: IV/single dose + kill on day 15

Methods: Clinical signs, BWs, clin path (days 2 & 15), organ weights, and gross & histopathology (only tissues with gross findings)

Findings: No Rx-related abnormalities in clin signs

↓ BWs - ≥3000 μg/kg

↑ neutrophils – day 2 [within 24 hrs of dosing] – Rx grps – 12 to 18-fold higher than controls; then ≤2-fold higher than controls on day 15 for ≥1000 μg/kg

↑WBCs – day 2 [74-124% over controls] – Rx grps – recovery

↑PLTs – day 15 [10-21% over controls] – ≥1000 μg/kg males
↑PLTs – day 15 [16-31% over controls] – Rx females

↓RBCs, HCT – day 15 [≤8%] - 10,000 μg/kg males

↑ spleen wts - ≥1000 μg/kg

Spleen - extramedullary hematopoiesis – in one 10,000 μg/kg male [only animal w/ gross notations, thus only animal examined]

2.A 9-Day Screening Toxicology Study of SC Filgrastim-SD/01 & Filgrastim-SD/02-157 in SD Rats
Species: CD®(SD) rats (5 males/grp)
Dose Levels: 0,200, 1000 μg/kg/dose of each agent
Route/Duration: SC/q2days x 7 days (total of 4 injections) + kill in day 8

Methods: Clinical signs, clin path*, organ wts [liver, spleen, kidneys], and gross & histopathology [liver, spleen, kidneys]
*WBCs, RBCs, PLTs, AlkP, AST, BUN, creatinine

Findings: ↑ AlkP – both agents; dose-related for SD/01
↑ WBCs, ANCs – both agents; dose-related for SD/01
↓ RBCs – SD/02-157 (1000 μg/kg)

↑ spleen wts – both agents
Splenomegaly – both agents; dose-related for SD/01

Liver – extramedullary hematopoiesis (mainly of myeloid origin) – both agents; dose-related for SD/01
↑ mitotic figures – both agents
Areas of coagulation necrosis – hepatocytes – SD/02-157, 1/5 rats at each dose

Spleen – extramedullary hematopoiesis – both agents; dose-related for SD/01

Both pegylated forms resulted in similar effect, with SD/02-157 being the more potent form.

Multidose Toxicity Studies

3. A Two-Week SC Toxicity Study of G-20K in Rats
Species: SD CD®(SD)BR rats (10/sex/grp)
Dose Levels: 0, 50, 100, 500, 1000 μg/kg/dose
Route/Duration: SC/q2d x 2 wks (total of 7 doses) + kill on day 14

Methods: Clinical signs, BWs, food consumption, clin path (at kill), neutrophil levels (days 1, 3, 5, 7, 9, 11), PK samples, Abs, organ weights, and gross & histopathology

Findings: No Rx-related abnormalities in clin signs, BWs, food consumption, urinalysis

↑ neutrophils – within 24 hrs of dose #1 – Rx grps – dose-related, at 3 to 10-fold above baseline
The levels remained elevated throut the study, being ~7 to 10-fold higher than controls at study termination (50 μg/kg) & ~60-to 90-fold higher at 1000 μg/kg

↑ WBCs – paralleled the neutrophils
↑ lymphocytes (2-4-fold the controls at kill) - ≥500 μg/kg

Hypersegmentation of neutrophils, macroneutrophils, ↓ neutrophilic cytoplasm – Rx grps – dose-related

↓ red cell mass, MCHC; ↑ MCV, retics – Rx grps
↓ M/E ratio = ↑ myeloid cells [proliferating & nonproliferating pools]; ↓ erythroid - ≥500 µg/kg

↑ AlkP (1.5-10-fold the controls) – Rx grps – correlated w/ ↑ osteoblastic activity in bone

↑ AST, ALT, Ca (minimal); ↓ K (minimal) - ≥500 µg/kg

Abs – seen in 1/20, 2/20, 5/20, 2/20 rats at 50, 100, 500, 1000 µg/kg

PK – (via commercial ELISA kit) – Increased exposure was not dose-proportional: a 20-fold dose increase = 90 (day 1, preRx)/120 (day 13)-fold increase in AUC & a 92 (day 1, preRx)/104 (day 13)-fold increase in C_{max}. No accumulation of PEG-G-CSF occurred.
Per the sponsor, the nonlinear increases in AUC were due to saturation of RMC, induced by ↑ neutrophil mass, at the higher doses [i.e., G-CSF receptor-mediated endocytosis]. The drop in plasma levels & in AUCs on day 13 were due to the induction of RMC [which is capacity-limited by saturation of receptor binding sites]. The increased clearance was not as notable at ≥500 µg/kg because the RMA became saturated.

Dose = 50 µg/kg

	Day 1 (1st dose)	Day 13 (7th dose)	Ratio (Day13/Day1)
AUC(0-∞) (µg-h/ml)	0.791	0.105	0.13
Cmax (ng/ml)		[redacted]	
Tmax (h)	0	0	1.00
CL/F (L/h/kg)	0.0632	0.476	7.53

Dose = 500 µg/kg

	Day 1 (1st dose)	Day 13 (7th dose)	Ratio (Day13/Day1)
AUC(0-∞) (µg-h/ml)	30.5	6.37	0.21
Cmax (ng/ml)		[redacted]	
Tmax (h)	24	12	0.50
CL/F (L/h/kg)	0.0164	0.0785	4.79

Dose = 100 µg/kg

	Day 1 (1st dose)	Day 13 (7th dose)	Ratio (Day13/Day1)
AUC(0-∞) (µg-h/ml)	9.155	0.207	0.02
Cmax (ng/ml)		[redacted]	
Tmax (h)	12	6	0.5
CL/F (L/h/kg)	0.0109	0.483	44.31

Dose = 1000 µg/kg

	Day 1 (1st dose)	Day 13 (7th dose)	Ratio (Day13/Day1)
AUC(0-∞) (µg-h/ml)	77.6	48.7	0.63
Cmax (ng/ml)		[redacted]	
Tmax (h)	24	8	0.33
CL/F (L/h/kg)	0.0138	0.0205	1.49

*Pharmacokinetic parameters were derived by non compartmental analysis using WINNONLIN v1.1

↑ spleen wt - ≥100 µg/kg
↑ liver wt – 1000 µg/kg
↑ prostate wt – ≥100 µg/kg

Gross Path – enlarged spleens – Rx grps – dose-related

Histopath –
BM – hyperplasia of myeloid component – neutrophilic granulopoiesis [maturing neutrophils & neutrophil precursors] – Rx grps - dose-related
Focal areas of myelofibrosis - ≥500 µg/kg

Spleen, liver – extramedullary granulopoiesis/hematopoiesis – Rx grps – dose-related

Bone (femur, sternum) –↑ [minimal/mild] osteoblastic + osteoclastic activity [focal areas of osseous hyperplasia w/ ↑ deposition of immature osteoid matrix] - ≥500 µg/kg

Lung - ↑ incidence of focal hemorrhage in alveolar spaces, acute inflammation - 1000µg/kg

Mandibular LN – lymphocytic/plasmacytic hyperplasia - acute inflammation - 1000µg/kg

The NOAEL was 100 µg/kg/dose.

4. A One-Month Repeat Dose SC Toxicity Study of Filgrastim-SD/01 in Cynomolgus Monkeys with a One-Month Recovery Period
Species: cyno monks (3-5/sex/grp)
Dose Levels: 0, 75, 250, 750 µg/kg/dose
Route/Duration: SC/qiw x 5 [days 1, 8, 15, 22, 29] + kills on day 30 (3/sex/grp) + recovery kill on day 57 (2/sex/grp for control & 750 µg/kg)

Methods: Clinical signs, BWs, appetite, ophthalmology, ECGs, clin path (baseline & days 16, 30, 57), plasma glucose (day 30), TK samples (days 1, 22), Abs, organ weights, and gross & histopathology
WBCs also evaluated from TK samples

Findings: No abnormalities in clinical signs, BWs, appetite, ophthalmology, ECGs

↑ WBCs - Rx grps - days 16, 30 - recovery
[TK] WBCs began to increase by 12 hrs postRx, peaked at 24-72 hrs, then decreased (dose-dependent response)
↑ neutrophils, band neutrophils - Rx grps - paralleled the WBC changes - recovery

↑ lymphocytes, monocytes - Rx grps - peak levels at 12-48 hrs postRx - recovery

WBC & neutrophil peaks in Rx females were slightly higher & of longer duration compared to males

Neutrophils - cytoplasmic basophilia & vacuolation, nuclear swelling, Dohle bodies, enlarged size - Rx grps

↓ red cell mass; ↑ retics - all grps - days 16, 30 - greater in severity in Rx grps & in Rx females vs. Rx males - recovery

↑ M/E ratios - Rx grps (minimal)

↑ AlkP - 750 µg/kg males - day 16; Rx females - days 16, 30 - recovery
↓ cholesterol - Rx grps - days 16, 30 - recovery

↓ K, glucose (serum) - Rx grps - days 16, 30 - recovery
No change in day 30 plasma glucose - thus the drop in sera levels is from continued glucose uptake by WBCs in blood containing very high WBC levels

Positive occult blood - 75 µg/kg - day 30

Abs -
Day 16 - Abs noted in 2/10 monks (control), 1/6 monks (75 µg/kg), 2/6 monks (250 µg/kg), 5/10 monks (750 µg/kg)

Day 30 - Abs noted in 1/10 monks (control), 4/6 monks (75 µg/kg), 5/6 monks (250 µg/kg), 10/10 monks (750 µg/kg)

Day 57 - Abs noted in 1/4 monks (control), 2/4 monks (750 µg/kg)

One 75 µg/kg monk that was seropositive on days 16 & 30 did display a notable decline in neutrophil levels, while all other Rx monks continued to display elevated neutrophil levels

One 250 µg/kg monk was minimally positive for neutralizing Abs on day 22. This animal showed a decrease in neutrophil counts between days 16-30, as did others, however, the neutrophil levels remained notably elevated compared to baseline.

TK - Increased exposure was not dose-proportional: a 10-fold dose increase resulted in ~70/72-fold increase in AUC on day 1/day 22. A decrease in AUC over time occurred. This was hypothesized to be due to expansion of neutrophil & neutrophil precursor mass, thus saturation of RMC. ANC levels were higher on day 22 compared to day 1.

Parameter	75 µg/kg Day 1	75 µg/kg Day 22	250 µg/kg Day 1	250 µg/kg Day 22	750 µg/kg Day 1	750 µg/kg Day 22
Cmax						
nCmax	113	34.1	213	49.9	321	151
Tmax	12.0	12.0	12.0	12.0	12.0	12.0
AUC(0-last)	1810	635	14200	4050	136000	47300
nAUC(0-last)	1810	635	4270	1220	13600	4730
AR	NA	0.377	NA	0.219	NA	0.280

Cmax = Maximum observed plasma concentration (ng/mL).
nCmax = Cmax normalized to a 75 µg/kg dose (ng/mL/75 µg/kg).
Tmax = Time of Cmax (h).
AUC(0-last) = Area under the plasma concentration-time curve from time zero to the time of the last detectable concentration (ng·h/mL).
nAUC(0-last) = AUC(0-last) normalized to a 75 µg/kg dose (ng·h/mL/75 µg/kg).
AR = Accumulation ratio.
NA = Not applicable.

↑ spleen wt - Rx grps - trend toward recovery
↑ liver wt - Rx grps - recovery
↑ adrenal wt - Rx grps - recovery

Gross Path - Terminal Kill
Pale BM - Rx grps
Enlarged spleens - Rx grps
Red discoloration of injection site - Rx grps

Histopath
Spleen - leukocytosis, red pulp (dose-related); foci of extramedullary hematopoiesis (erythroid precursors/megakaryocytes) - Rx grps

Liver - extramedullary granulopoiesis/hematopoiesis; vacuolization of hepatocyte cytoplasm - Rx grps

BM - myeloid hyperplasia - neutrophil precursors; ↓ granulocytes - Rx grps

LN - foci of extramedullary hematopoiesis; engorgement of medullary chords w/ granulocytes - ≥250 µg/kg

Injection site - inflammation, mononuclear cell infiltration - Rx grps

General recovery by day 57

The NOAEL was 750 µg/kg/dose.

5. 3/6 Month SC/IV Toxicity Study of Filgrastim-SD/01 in Rats with Recovery
Species: SD ~~CD®~~VAF/Plus rats; Tox study = 20-25/sex/grp; TK = 2-9/sex/grp
Dose Levels: 0 (IV/SC), [SC] 100, 300, 1000 µg/kg/dose; [IV] 300 µg/kg/dose
Route/Duration: SC/IV / qiw x 3/6 months
Kills at 3 months; 5 months [2-month recovery- controls & 1000 µg/kg-SC & 300 µg/kg-IV]; 6 months
3-month dosing - total of 14 doses
6-month dosing - total of 27 doses

Methods: Clinical signs, BWs, food consumption, ophthalmology, hematology (days 1, 15, 23, 30, 72, 3, 5, 6 months), chemistry (day 30, 3, 5, 6 months), urinalysis (3, 5, 6 months), TK samples, Abs, organ weights, and gross & histopathology

Findings:
Deaths - 1/40 rats (100 µg/kg-SC) - neck ulceration
1/50 rats (1000 µg/kg-SC) - nasal bone fractures

No abnormalities in clinical signs, BWs, food consumption, ophthalmology.

↑ neutrophils - Rx grps - dose-related (~20-50-fold for neutrophils) - days 23, 30, 72 & 3, 6 months (~24 hrs postRx)

300 µg/kg-SC value = 300 µg/kg-IV value
Note - Not seen on day 1 or 15 (sample collected immediately postRx)

↑ WBCs - paralleled the neutrophils
↑ band neutrophils - Rx grps
↑ lymphocytes - Rx grps (~2-fold) - day 72; 3/6 months
↑ monocytes - Rx grps - day 23 thru 6 months

↓ red cell mass - 300 µg/kg-SC males
↑ MCV - 300 µg/kg-SC; 300 µg/kg-IV - between day 15 & 3 months
↓ MCHC, MCH - Rx grps - between day 23 & 3 months

↑ M/E ratio = 1000 µg/kg - 3, 6 months

↑ AlkP - Rx grps (~1.3 to 4-fold)- day 30, 3, 6 months - no histo correlate in bone

↓ triglycerides – Rx males (SC) - day 30
↓ cholesterol – Rx males (SC) - between day 30 & 6 months
↓ K - 100 µg/kg males (3 months), 300 µg/kg-IV females (day 30, 3 months), 1000 µg/kg (3, 6 months)

↑ urinary bilirubin, ketones, protein - 1000 µg/kg females - 6 months

Abs - none of the samples collected on days 15, 30, 93 were seroreactive

Abs noted in 1/20 rats each at 0 & 100 µg/kg-SC on day 184/185
Abs noted in 1/9 rats at 1000 µg/kg-SC at 6 months (TK rats)

TK - Increased exposure was not dose-proportional: a 10-fold SC dose increase resulted in ~39/47/67-fold increase in AUC on day 1/3 months/6 months. Systemic exposure was higher via the SC route; no sex difference was noted via the IV route. The bioavailability via the SC route was ~15%.

	100 µg/kg, SC		300 µg/kg, SC		1000 µg/kg, SC		300 µg/kg, IV	
Parameter	Male	Female	Male	Female	Male	Female	Male	Female
Day 1								
Cmax								
nCmax	99.7	116	137	192	179	243	1670	1880
Tmax	12.0	12.0	12.0	8.00	12.0	24.0	4.0	2.0
AUC(0-last)	1940	2980	13800	14200	82000	95900	108000	87200
nAUC(0-last)	1940	2980	4620	4730	8200	9590	35800	29100
Relative Exposure	NA	NA	12.9	16.3	NA	NA	NA	NA
Month 3								
Cmax								
nCmax	99.9	202	188	376	302	472	2640	2250
Tmax	12.0	8.00	12.0	12.0	12.0	12.0	2.0	4.0
AUC(0-last)	1550	3580	9990	30800	100000	163000	149000	121000
nAUC(0-last)	1550	3580	3330	10300	10000	16300	49800	40400
AR	0.80	1.20	0.72	2.17	1.23	1.70	1.39	1.39
Relative Exposure	NA	NA	6.69	25.5	NA	NA	NA	NA
Month 6								
Cmax								
nCmax	52.5	127	183	289	207	369	2250	1730
Tmax	4.00	12.0	12.0	12.0	12.0	24.0	2.0	2.0
AUC(0-last)	734	1980	6910	20400	74600	122000	117000	82400
nAUC(0-last)	734	1980	2300	6790	7460	12200	39000	27500
AR	0.38	0.68	0.50	1.43	0.91	1.27	1.09	0.94
Relative Exposure	NA	NA	5.91	24.7	NA	NA	NA	NA

Cmax = Maximum observed plasma concentration (ng/mL).
nCmax = Dose normalized Cmax (ng/mL/100 µg/kg).
Tmax = Time of Cmax (h).
AUC(0-last) = Area under the plasma concentration-time curve from time zero to the time of the last detectable concentration (ng-h/mL).
nAUC(0-last) = Dose normalized AUC(0-last) (ng-h/mL/100 µg/kg).
AR = Accumulation ratio.
Relative Exposure = $(AUC(0\text{-last})_{SC}/AUC(0\text{-last})_{IV}) \cdot 100$ (%).
NA = Not applicable.

↑ spleen wt - Rx grps - 3 & 6 month kill
↑ liver wt -300 µg/kg-IV, 1000 µg/kg - 6 month kill

Gross Path - both kills
Enlarged spleens - ≥300 µg/kg
Red discoloration of injection site - Rx (SC) grps

Histopath - 3-month kill
Spleen - myeloid hyperplasia (dose-related); erythroid hyperplasia; erythroid/megakaryocyte proliferation - Rx grps

Liver - extramedullary granulopoiesis/ hematopoiesis - Rx grps

BM (femur, sternum) - hyperplasia of myeloid component - neutrophilic granulopoiesis [maturing neutrophils & neutrophil precursors]; some erythropoiesis - Rx grps

Injection site - SC hemorrhage/congestion - Rx grps

2-month recovery kill
No notable findings

6-month kill
Spleen - myeloid hyperplasia (dose-related); erythroid hyperplasia; erythroid/megakaryocyte proliferation - Rx grps

Liver - extramedullary granulopoiesis/hematopoiesis - Rx grps

BM (femur, sternum) - hyperplasia of myeloid component - neutrophilic granulopoiesis [maturing neutrophils & neutrophil precursors]; some erythropoiesis - Rx grps

LN (mandibular) - plasmacytosis; lymphoid hyperplasia - sporadic across the grps

The NOAEL was 1000 µg/kg/dose (SC) & 300 µg/kg/dose (IV). No distinct difference in the toxicity profile was seen at 300 µg/kg/dose via either the SC or the IV ROA.

Reproduction/Teratology Studies

List of Studies:
Note that the dates presented with each study are the dates the report was issued, not the date of study completion.

1. A Study of the Effects of Filgrastim-SD/01 Administered SC on Embryo/Fetal Development in Rats; study #100168; performed by [redacted] (per GLP); lot #1104098; 10/00

2. A Range Finding Developmental Toxicity Study in Rabbits with Filgrastim-SD/01 via SC Administration; study #100199; performed by [redacted] (per GLP); lot #1104098; 5/99

3. The Effects on Embryo-Fetal Development of SC Administration of Filgrastim-SD/01 to Rabbits; study #100297; performed by [redacted] (per GLP); lot #1104098; 4/99

4. A Study of the Effects of Filgrastim-SD/01 Administered SC on Pre-& Postnatal Development, Including Maternal Function in the Rat; study #100439; performed by — (per GLP); lot #2012048M8A; 7/00

5. A Study of the Effects of Filgrastim-SD/01 Administered SC on Fertility & Early Embryonic Development to Implantation in Rats; study #100782; performed by — (per GLP); lot #2012048M8B; 10/99

Reproduction/Teratology Studies

1. A Study of the Effects of Filgrastim-SD/01 Administered SC on Embryo/Fetal Development in Rats
Species: — CD(SD)BR rats (22 females/grp)
Dose Levels: 0, 100, 300, 1000 µg/kg/dose
Route/Duration: SC/GD 6, 8, 10, 12, 14, 16, 18 + C-sections on GD 20

Methods: Clinical signs, BWs, food consumption, hematology (10/grp, GD 7, 19 at about 24 hrs postRx), gross evaluation, uterine findings, fetal exams

Findings: No abnormalities in clinical signs, BWs, food consumption

↑ WBCs, neutrophils; ↓ lymphocytes - Rx grps - GD 7, 19
↓ red cell mass - Rx grps - GD 19

Enlarged spleens; ↑ spleen wts - Rx grps

No effect on intrauterine endpoints - number of corpora lutea, pre/postimplantation loss, fetal BWs, viable fetuses

F_1 - No external or visceral variations/malformations

Skeletal malformations - two 100 µg/kg fetuses (from two litters) - vertebral anomalies with/without rib anomalies

↑ incidence of bent ribs (variation) - control (0%/litter), 100 µg/kg (0.3%/litter), 300 µg/kg (3%/litter), 1000 µg/kg (5.3%/litter) - historical controls = 0-4.6%/litter

The NOAEL for maternal toxicity was 1000 µg/kg/dose & the NOEL for embryo-fetal effects was 300 µg/kg/dose.

2. A Range Finding Developmental Toxicity Study in Rabbits with Filgrastim-SD/01 via SC Administration
Species: NZW rabbits (5 females/grp)
Dose Levels: 0, 50, 250, 1000 µg/kg/dose
Route/Duration: SC/GD 6, 8, 10, 12, 14, 16, 18 + C-sections on GD 29

Methods: Clinical signs, BWs, food consumption, hematology (GD 7, 19 at about 24 hrs postRx), gross evaluation, uterine findings, histopathology (lesioned tissues only), fetal exams

Additional satellite animals (4 females) dosed at 250 µg/kg/dose, were bled on GD 6 (preRx), 7, 8 (preRx), 18 (preRx), 19, 20, 22 for determination of neutrophil levels

Findings: Deaths - 1/5 controls - killed on GD 14, showing excessive salivation, motor impairment, dyspnea, hypersensitivity to tactile stimulus, abnormal posture

1/5 rabbits at 50 µg/kg - killed on GD 9 - weight loss, □ food consumption, impaired limb function

1/5 rabbits at 50 µg/kg - dead on GD 14 - exhibited the same signs as the control rabbit - death due to pulmonary edema

Abortions - 3/9 rabbits at 250 µg/kg - GD 21, 22, 26

↓ BWs - Rx grps (89-92% that of the control) - GD 16-29
↓ BW gain - Rx grps (39-44% that of the control) - GD 6-18

Food consumption paralleled the BW changes

↑ neutrophils - Rx grps - GD 7, 8, 18, 19, 20, 22

Extramedullary hematopoiesis - spleen - all grps, slight ↑ incidence in Rx grps

Discolored foci - lungs - 2/5 rabbits each in controls & 250 µg/kg
Alveolar/intra-alveolar macrophages, interstitial inflammation - lungs - 4 controls, 1 rabbit at 50 µg/kg, 2 rabbits at 250 µg/kg
Leukocytosis - lungs - 1 rabbit at 50 µg/kg, 2 rabbits at 250 µg/kg

↑ preimplantation loss - 16.4%/17.4%/14.9%/21.2% - control/50/250/1000 µg/kg

↑ postimplantation loss - 3.8%/4.2%/37%/78% - control/50/250/1000 µg/kg

↑ early resorptions - 0.3/0.0/2.3/5.6 - control/50/250/1000 µg/kg

↑ late resorptions - 0.3/0.3/0.3/1.2 - control/50/250/1000 µg/kg

↓ viable fetuses - 9.3/7.3/5.0/2.2 - control/50/250/1000 µg/kg

↓ fetal BW (g) - 47.1/38.0/36.8/28.8 - control/50/250/1000 µg/kg

F_1 - no external, visceral, skeletal variations/malformations

A NOAEL for maternal & embryo-fetal toxicity was not achieved.

3. The Effects on Embryo-Fetal Development of SC Administration of Filgrastim-SD/01 to Rabbits
Species: NZW rabbits (22 females/grp)
Dose Levels: 0, 10, 50, 200 µg/kg/dose
Route/Duration: SC/GD 6, 8, 10, 12, 14, 16, 18 + C-sections on GD 29

Methods: Clinical signs, BWs, food consumption, hematology (GD 7, 19 at about 24 hrs postRx), gross evaluation, uterine findings, fetal exams

Some (11/22 females/grp) were bled on GD 7 & 19 for determination of neutrophil levels

Findings: Abortions - 13/22 at 200 µg/kg - GD 20 (4 rabbits), GD 21 (4 rabbits), GD 22 (2 rabbits), GD 24 (1 rabbit), GD 28 (2 rabbits)

↓ BW gain - 50 µg/kg - GD 6-19
Food consumption paralleled the BW changes

↑ neutrophils - Rx grps - GD 7, 19

Enlarged spleens; ↑ spleen wt - 200 µg/kg
Discolored foci - lungs - 200 µg/kg - 5/22 rabbits (all aborted)

↑ postimplantation loss - 1.5%/4.8%/9.5%/49.1% - control/10/50/200 µg/kg

↑ early resorptions - 0.1/0.2/0.5/4.4 - control/10/50/200 µg/kg

↓ viable fetuses - 8.9/8.4/8.4/4.9 - control/10/50/200 µg/kg

↓ fetal BW (g) - 43.8/41.8/39.4/36.6 - control/10/50/200 µg/kg

F_1 - no external, visceral variations/malformations
Reduced levels of ossification for various bones – Rx grps

Malformations - fused sternebrae - 6/176 fetuses (3.4%); 5/21 litters (24%) - 50 µg/kg
[Historical control = 20/1373 fetuses (1.5%); 14/162 litters (8.6%)

The NOAEL for maternal & embryo-fetal toxicity was 10 µg/kg/dose.

4. A Study of the Effects of Filgrastim-SD/01 Administered SC on Pre-& Postnatal Development, Including Maternal Function in the Rat
Species: — CD(SD)IGS BR rats (22 females/grp)
Dose Levels: 0, 100, 300, 1000 µg/kg/dose
Route/Duration: SC/GD 6, 13, 20 & LD 4, 11, 18
Kills: F_0 - LD 21 or postmating day 25 (nonpregnant rats)
F_1 - some culled on LD 4; some killed on LD 21; remaining pups [25/sex/grp (1/sex/litter, if possible)] allowed to deliver the F_2 pups & killed on GD 20
F_2 – GD 20

Methods:
F_0 - Clinical signs, BWs, food consumption, clin path (10/grp, GD 6 & LD 18), gross evaluation
F_1 pups - survival, growth & development [vaginal patency, preputial separation], behavioral [using 10/sex/grp - auditory startle, open field — multiple T-water maze]

Mating at 91-96 days old, followed by clin signs, BWs, food consumption, uterine exam, gross evaluation

F_2 pups – external fetal exams

Findings:
E_0 – No abnormalities in clinical signs, BWs, food consumption, duration of gestation

↑ WBCs [2-2.5-fold], neutrophils [7.5-9-fold]; ↓ lymphocytes [2-fold] - Rx grps - GD 7

↑ WBCs [4-6.5-fold], neutrophils [15-26-fold] - Rx grps – LD 18
↓ RBCs [1.2-fold] - Rx grps – LD 18
↓ red cell mass – 1000 µg/kg – LD 18
↑ MCV, MCH - ≥300 µg/kg – LD 18

Enlarged spleens – 1/21 females (300 µg/kg), 1/22 females (1000 µg/kg) – LD 21

No effect on intrauterine endpoints - number of corpora lutea, pre/postimplantation loss, fetal BWs, viable fetuses

E_1 Pups – Deaths/missing – 5/10/10/12 for control/100/300/1000 µg/kg – during lactation
One pup at 1000 µg/kg – had unilateral microphthalmia & mandibular agnathia
No effect on BWs

Sex distribution, live/dead pups - no differences

Pup survival [LD 1-4] – 98.8% (control), 99.7% (100 µg/kg), 99.1% (300 µg/kg), 99.7% (1000 µg/kg)

Pup survival [LD 4-21] - 100% (control), 99.4% (100 µg/kg), 100% (300 µg/kg), 100% (1000 µg/kg)

Unable to interpret auditory startle data due to malfunctioning equipment
No effect on open field, water maze

Vaginal Patency - mean age of 31.9/31.8/31.8/32.0 days for 0/100/300/1000 µg/kg
Preputial Separation - mean age of 42.4/42.4/42.8/43.4 days for 0/100/300/1000 µg/kg

E_1 Parents – Deaths – 1 male each at 300 µg/kg (day 103) & 1000 µg/kg (day 118)
No effect on estrous cycles, BWs, gravid uterine wts

Mating - fertility indices –
Males - 72% (control), 52% (100 µg/kg), 87.5% (300 µg/kg), 91.7%(1000 µg/kg)
Females - 76% (control), 60% (100 µg/kg), 88% (300 µg/kg), 92% (1000 µg/kg)

E_2 Fetuses – No effect on intrauterine endpoints - number of corpora lutea, pre/post-implantation loss, resorptions, fetal BWs, viable fetuses

External –
Bilateral microphthalmia, localized edema (head/thorax) – 1/211 fetuses (100 µg/kg)
Anury – 1/211 fetuses (100 µg/kg)
Bilateral anophthalmia, cleft palate, craniorachischisis – 1/309 fetuses (300 µg/kg)
Filamentous tail – 1/332 fetuses (1000 µg/kg)

The NOAEL was 1000 µg/kg/dose for peri/postnatal developmental toxicity.

5. A Study of the Effects of Filgrastim-SD/01 Administered SC on Fertility & Early Embryonic Development to Implantation in Rats
Species: — CD(SD)IGS BR rats (25/sex/grp)
Dose Level: 0, 100, 300, 1000 µg/kg/dose
Route/Duration: SC
Males - dosed qiw for 4 wks premating, qiw during mating [21 days] & postmating - killed 1 wk postmating
Females- dosed qiw for 2 wks premating, through mating confirmation; to GD 14 - killed on GD 15

Methods
Females - clinical signs, BWs, food consumption, vaginal smears, pregnancy rate, uterine contents, ovary evaluation, gross evaluation, hematology [24 hrs postdose #1 & 24 & 120 hrs postdose #2 – all premating], histopath [spleen]

Males - clinical signs, BWs, food consumption, fertility rate, gross evaluation, testicular/ epididymal wts, sperm evaluation [motility, morphology], hematology [24 hrs postdose #1 & 24 & 120 hrs postdose #4 – all premating], histopath [spleen]

Findings:
Deaths –
1/25 males (1000 µg/kg) – day 32; hypoactivity, hypothermia, dyspnea, wt loss, red material (preputial area) – noted prior to death. Dilated renal pelves; distended, red-fluid-filled U. bladder – death due to renal calculi

1/25 females (300 µg/kg) – day 22

Dried red material around eyes – Rx males, notably at 1000 µg/kg

↓ BWs – 1/1/4/3 males at 0/100/300/1000 µg/kg – days 10-14 - transient

↑ neutrophils (8-fold), WBCs; ↓ lymphocytes – Rx males – 24 hrs postdose #1
↑ neutrophils, WBCs (2-fold); ↓ lymphocytes – Rx females – 24 hrs postdose #1

↑ neutrophils (13.5-21-fold), WBCs (3.6-5-fold) – Rx males – 24 hrs postdose #4
↑ neutrophils (20-33.5-fold), WBCs (3.5-5-fold), lymphocytes – Rx females – 24 hrs postdose #2

↓ red cell mass – Rx males – 24 hrs postdose #4 – dose-related
↓ red cell mass –≥300 µg/kg females – 24 hrs postdose #2
↓ PLTs – 1000 µg/kg females – 24 hrs postdose #2

↑ neutrophils (1.6-3.8-fold) – Rx males – 120 hrs postdose #4
↑ neutrophils – Rx females – 120 hrs postdose #2 – dose-related

↑ WBCs – 1000 µg/kg males – 120 hrs postdose #4
↑ WBCs – ≥300 µg/kg females – 120 hrs postdose #2

↓ lymphocytes – Rx males – 120 hrs postdose #4
↓ lymphocytes – Rx females – 120 hrs postdose #2

↓ PLTs – sporadic in the males – ≤300 µg/kg -120 hrs postdose #4
↓ red cell mass –≥300 µg/kg females – 120 hrs postdose #2
↓ PLTs – 1000 µg/kg females – 120 hrs postdose #2

Enlarged spleens – 2/6/14 males at 100/300/1000 µg/kg; 6/19 females at 300/1000 µg/kg
Swollen spleens – 1/1 males at 300/1000 µg/kg
Capular scarring of spleen – 1 male & 1 female at 1000 µg/kg

↑ splenic extramedullary hematopoiesis; ↑ splenic neutrophilic infiltrate; ↑ severity of myeloid/ megakaryocytic/erythroid hyperplasia, ↑ lymphoid depletion [due to displacement of lymphoid cells due to the hematopoiesis] – Rx males & females – dose-related

Mating indices [males & females] = 100% - all grps
Fertility indices [males & females] = 84%/96%/87.5%/96% for 0/100/300/1000 µg/kg

Mean preimplantation loss per rat = 2.6/2.5/2.7/3.5 at 0/100/300/1000 µg/kg
Mean postimplantation loss/early resorptions per rat = 0.7/0.8/0.9/0.6 at 0/100/300/1000 µg/kg
Mean number of live fetuses per rat = 15.5/14.8/15.2/15.5 at 0/100/300/1000 µg/kg

The NOAEL for parental toxicity, fertility, & reproductive performance & for embryotoxicity was 1000 µg/kg/dose.

Safety Pharmacology Studies

No safety pharmacology studies were performed with this product.

Mutagenicity Studies

No mutagenicity studies were performed with this product. The sponsor states that G-CSF was determined to be nonmutagenic in the *in vitro* Ames assay, in the *in vitro* chromosomal aberration test in mammalian cells, & in the *in vivo* mouse micronucleus test. The 20 kd PEG portion of the product has no structural alerts suggestive of genotoxic potential & the linker between the PEG molecule & the protein is a covalent [redacted] bond.

Carcinogenicity Studies

No carcinogenicity studies were performed with this product. PEG-G-CSF is a monopegylated from of r-met-HuG-CSF. There is a large existing body of evidence based on the large numbers of patients (sponsor estimate of — that have received G-CSF (Filgrastim) in the last ten years in the treatment of neutropenia of various origins – chemo-induced neutropenia, acute myeloid leukemia, bone marrow transplantation, & severe chronic neutropenia - that minimizes this potential concern. No definitive evidence that G-CSF administration results in tumor formation or stimulates the growth of existing tumors has been noted in these patient populations.

The biological activity of G-CSF is directed through binding to a specific, high affinity cell surface receptor, G-CSF-R, which is located primarily on cells in the myeloid lineage. This receptor is most abundant on mature neutrophils, & it is likely also located on PLTS & monocytes (both nonproliferating cells). Through the use of a radiolabeled, — of G-CSF, the relative affinity of PEG-G-CSF & G-CSF for the G-CSF-R on human neutrophils was similar, thus pegylation did not appear to the alter receptor binding affinity. In addition, there is limited tissue distribution of the G-CSF-R.

Data from the 6-month rodent toxicity study revealed the lack of any preneoplastic/neoplastic findings after weekly SC injections of PEG-G-CSF doses up to 1000 µg/kg/dose. Other than stimulating neutrophilic granulopoiesis & extramedullary hematopoiesis in the spleen, liver, and/or LNs, no notable mitogenic effects were observed in other tissues in these two species. The sponsor cites a study in which lethally irradiated mice were injected with a retroviral vector expressing G-CSF. Measurable levels of G-CSF were seen out to 30 weeks, accompanied by neutrophilic granulocytosis in hematopoietic tissues, with no leukemic transformation. Syngeneic recipients transplanted with bone marrow or spleen of the injected mice did not develop tumors [Chang, et. al., 1989]. An extensive literature search (refer to the BLA submission) of both in vitro & in vivo data revealed that there is no conclusive evidence for the potential of tumor-enhancing effects of G-CSF [i.e., a mitogenic effect].

In addition, the proposed label will specify a limited dosing regimen for the treatment of chemo-induced neutropenia, with a single injection per chemotherapy cycle – about once every three weeks for about four to six cycles. The label will also specify that administration of PEG-G-CSF be given no sooner than 24 hrs after chemotherapy, to avoid any potential enhancement of mutagenic damage caused by the chemotherapy.

Additional reasons as to the low carcinogenic potential of PEG-G-CSF in the proposed patient population include: 1) the limited tissue distribution of the G-CSF receptor to which the protein binds; 2) the limited dosing regimen in patients [once per chemo cycle]; 3) the lack of any preneoplastic/neoplastic findings seen in a 6-month rodent toxicity study; and 4) the structural identity to G-CSF, which was nonmutagenic in various assays.

CONCLUSION:

Filgrastim SD/01 is a modified version of Filgrastim that has been engineered via pegylation, to make the hydrodynamic size of the protein greater than the maximal size allowable for renal glomerular filtration [one elimination pathway for G-CSF], resulting in a primary dependence on neutrophil-mediated clearance [also an elimination pathway for G-CSF] as the major route of elimination. This phenomena results in a higher concentration of circulating PEG-G-CSF over an extended interval of time, due to the presence of increased neutrophil levels [which is the intended primary PD response to PEG-G-CSF]. Thus, less injections of PEG-G-CSF are needed to achieve the same PD effect as seen from multiple injections of G-CSF.

The proposed clinical indication [per the package insert] for PEG-G-CSF is to decrease the incidence of infection, as manifested by febrile neutropenia, in patients [redacted]. The PI-recommended dose of PEG-G-CSF for the specified patient population is a single SC injection of 6 mg/human/dose [about 100 µg/kg], administered once per chemotherapy cycle.

The formulation of PEG-G-CSF used in the preclinical studies is identical to that proposed for commercial use in humans. In vitro pharmacodynamic evaluation showed that both G-CSF & PEG-G-CSF were cleared by neutrophils, with slower clearance for PEG-G-CSF. Data generated in mice showed that a single injection of PEG-G-CSF resulted in a 2 to 10-fold increase in neutrophil counts (dose-dependent), compared to repeated injections of G-CSF in order to achieve the same effect. The PD activity of PEG-G-CSF in the setting of chemotherapy-induced neutropenia was evaluated in mice, varying the type of cytotoxic agent used, as well as timing of the SC injection(s) of PEG-G-CSF. Administration of the protein resulted in a reduction in the degree and/or duration of neutropenia that occurred with 5FU, cyclophosphamide, carboplatin, busulfex, or navelbine. In general, the optimum results were seen with injection of PEG-G-CSF within two days post-chemotherapy. A single injection by either the SC or the IV route in normal mice or in myeloablated rhesus monkeys resulted in increases in ANC levels that were route-independent.

Preclinical studies were performed in rats, cynomolgus monkeys, and rabbits, which are species in which PEG-G-CSF is pharmacologically active. In addition, toxicology studies for G-CSF were performed in these species in support of the application for G-CSF. The sponsor used either the SC or the IV route & the dosing regimen in the animals attempted to mimic the proposed human schedule, using an intermittent injection schedule. Based on PK data in rhesus monkeys & in humans, non-neutropenic animals clear PEG-G-CSF from the circulation faster than neutropenic animals, thus the dosing regimen in normal animals was more intensive (i.e., qiw) in order to achieve desired systemic levels that are detected in humans. In some cases dosing occurred every two days in order to better evaluate the toxicity of this protein.

A single IV injection in rats showed expected exaggerated pharmacological effects of PEG-G-CSF at levels from 100 to 10,000 μg/kg, consisting of an increased number of circulating neutrophils, reflected in elevated total WBC levels. Extramedullary hematopoiesis was evident in the enlarged spleen of 1/10 rats at 10,000 μg/kg. [Per the sponsor] Splenic changes were not observed in a single dose study with G-CSF, however the dose administered was one-third the PEG-G-CSF dose. Although a NOEL was not achieved due to the exaggerated pharmacological response seen, PEG-G-CSF was well tolerated at doses up to 10 mg/kg in the rats.

Repeat SC administration of PEG-G-CSF in rats at doses ranging from 50 to 1000 μg/kg/dose, q2d, for a total of 4 to 7 injections, resulted in findings similar to the single dose study. Additional findings included, but were not limited to, morphological changes in the neutrophils (e.g., hypersegmentation, enlargement), decreased M/E ratio (reflective of the hyperplasia of the myeloid component), decreased red cell mass, increased reticulocyte counts, increased AlkP levels (correlating with the stimulation of osteoblastic/osteoclastic activity in the bone), extramedullary hematopoiesis in the liver, and lymphocytic/plasmacytic hyperplasia in the LNs. In addition, an increased incidence of inflammation and focal hemorrhage in the alveolar spaces of the lungs was noted at 1000 μg/kg/dose. The extramedullary hematopoiesis in the spleen, liver, and/or LNs reflect a secondary pharmacological effect, potentially representative of the ability of this protein to mobilize stem cells from the bone marrow. These findings were also seen in animal studies performed in the past with G-CSF.

Note that the desired pharmacological effect – increased neutrophil levels - was notable within 24 hours of the first dose and remained elevated throughout the duration of the studies. The dose-dependent elevation was not a result of protein accumulation, as depicted in the PK profile of this product.

A comparison of the observations noted in animals given either PEG-G-CSF or G-CSF is provided in Table 3-2 [provided by the sponsor in the BLA submission]. The modification of Filgrastim via the PEG molecule did not result in additional pharmacological/toxicological findings, as compared to G-CSF. In fact, the less intensive dosing regimen for PEG-G-CSF likely resulted in reduced toxicities.

Table 3-2. Comparison of Key Effects of Filgrastim-SD/01 in Repeat-dose Toxicity Studies with Known Effects of Filgrastim

Finding	Filgrastim-SD/01	Filgrastim	Comments
Clinical Observations			
Articular and hind limb swelling		√	Rat-specific effect in Filgrastim studies; intermittent dosing may have negated this effect in Filgrastim-SD/01 study
Cerebral hemorrhage		√	Only observed at high, repeated daily doses of Filgrastim in monkeys
Hematology			
Increased neutrophil counts	√	√	
Morphological changes in neutrophils	√	√	Described in rat and human studies with Filgrastim; hypersegmented neutrophils also observed in hamsters treated SC with Filgrastim
Increased band neutrophils	√	√	
Increased monocytes (modest)	√	√	
Increased lymphocytes (modest)	√	√	
Decreased erythrocyte counts, hematocrit, hemoglobin	√	√	
Decreased platelet counts	√[a]	√	[a]Was observed at a dose of 1000 µg/kg/week in Filgrastim-SD/01 treated females in fertility and pre-/post-natal studies in rats
Clinical Chemistry			
Increased serum alkaline phosphatase	√	√	
Decreased serum cholesterol	√	√	
Decreased serum potassium	√	√	Possibly an ex vivo artifact
Decreased serum glucose	√	√	Only in monkeys in the case of Filgrastim-SD/01; demonstrated to be an ex vivo artifact

Table 3-2. Comparison of Key Effects of Filgrastim-SD/01 in Repeat-dose Toxicity Studies with Known Effects of Filgrastim (Continued)

Finding	Filgrastim-SD/01	Filgrastim	Comments
LDH elevations		√	Can be a highly variable parameter in preclinical studies
ALT, AST elevations	√	√	Only with high dose, q.o.d. treatment with Filgrastim-SD/01 in rats; sporadic with Filgrastim treatment in monkeys
Gross Pathology			
Splenomegaly, increased spleen weight	√	√	
Liver weight increases (modest)	√	√	
Microscopic Pathology			
Increased granulopoiesis in bone marrow	√	√	
Myelofibrosis	√	√	Only with high dose, q.o.d. treatment with Filgrastim-SD/01 in rats
Bone marrow necrosis		√	A reversible, minimal to mild finding with Filgrastim only in golden hamsters; Filgrastim-SD/01 was not tested in hamsters
Extramedullary hematopoiesis in spleen, liver, and lymph nodes	√	√	
Leukocytosis in spleen, liver, and/or lymph nodes	√	√	
Inflammation, mononuclear cell infiltration at injection site	√		Observed in Filgrastim-SD/01-treated monkeys only; most repeat-dose toxicity studies of Filgrastim in monkeys were not by SC route
Increased osteoblast/osteoclast activity	√	√	Only with high dose, q.o.d. treatment with Filgrastim-SD/01 in rats; common with daily Filgrastim treatment in rats

Data generated from the 6-month toxicity study in rats and from the 1-month cynomolgus monkey study show the extent of the pharmacological action of PEG-G-CSF in non-neutropenic animals. Rats IV or SC injected at doses from 100 to 1000 μg/kg/dose, once weekly for up to 6 months, displayed findings similar to those exhibited in the short-term repeat dose studies in rats. No distinct difference was noted in the toxicology profile via the different routes. Monkeys SC injected at doses from 75 to 750 μg/kg/dose, once weekly, for five administrations, also showed findings similar to earlier studies performed using G-CSF, as well as to the long-term rat study using PEG-G-CSF. The NOAEL ranged from 750 to 1000 μg/kg/ dose via the SC route for these two studies.

The development of antibodies occurred in some rats upon repeated dosing of PEG-G-CSF, however there was no resultant change in the PK/PD profile or in other toxicology parameters (i.e., neutralization). A dose- and time-related increase in antibody formation was seen in the monkeys upon repeated weekly dosing, however, neutropenia was not evident.

Evaluation of the distribution of PEG-G-CSF in nephrectomized rats revealed a small renal contribution to the overall clearance of the protein. It is postulated that because pegylation of G-CSF results in a protein that is increased in MW, glomerular filtration is decreased. The proposed clearance mechanism of PEG-G-CSF is via the saturation of G-CSF receptor-mediated endocytosis [or receptor-mediated clearance (RMC)]. The RMC is capacity-limited by saturation of receptor binding sites on neutrophils & neutrophil precursors. Thus, upon repeat dosing in animals, plasma levels of PEG-G-CSF were higher post-last-dose compared to the first dose. However, the ANC levels were higher post-last-dose – likely due to the expansion of neutrophil & neutrophil precursor mass, resulting in increased RMC over time.

Following a single IV/SC injection of PEG-G-CSF in myeloablated (via irradiation) rhesus monkeys or in normal monkeys, the protein was cleared from circulation by 2 days in the normal monks and by 8 days in the myeloablated animals. The report postulates that the lower clearance seen in the ablated monks is likely due to a decrease in G-CSF receptor-mediated clearance secondary to the neutropenic condition of the animals.

Administration of PEG-G-CSF via once weekly SC injections to rats from prior to mating to approximately gestation day 14 (Segment I) revealed a NOAEL of 1000μg/kg/dose for F_0 parental toxicity (reduced body weights) and 1000 μg/kg/dose for fertility and reproductive function of the F_0 animals. Both males and females displayed the expected pharmacological response to PEG-G-CSF [i.e., increased neutrophils, & WBCs; decreased red cell mass & PLTs; and enlarged spleens]. The NOEL for embryotoxicity was 1000 μg/kg/dose.

In addition, the SC injection of PEG-G-CSF in pregnant rats, q2d, during gestation (Segment II) resulted in a NOAEL of 1000 μg/kg/day for the dams and 300 μg/kg/dose for the fetuses. The F_1

fetuses exhibited an increased incidence of bent ribs. Low levels (<0.5%) of PEG-G-CSF crossed the placenta when SC injected into pregnant rats, q2d, during gestation. A NOAEL of 1000 μg/kg/dose for the dams and F_1/ F_2 offspring was achieved with once weekly SC injections of PEG-G-CSF into pregnant F_0 rats in a perinatal/postnatal study.

Pregnant rabbits displayed toxicity to the protein, as SC injections, q2d, during gestation resulted in a NOAEL of only 10 μg/kg/dose. Doses from 50 to 1000 μg/kg/dose caused decreased body weights and food consumption in the does and an increased incidence of abortions occurred at doses of 200 and 250 μg/kg/dose. Embryo-fetal toxicity occurred, reflected as increased post-implantation loss due to early resorptions and decreased numbers of live fetuses at ≥200 μg/kg/dose. Decreased fetal body weights were noted at ≥50 μg/kg/dose.

Although mutagenicity studies were not performed with the pegylated protein, no genotoxic potential was exhibited for G-CSF via in vitro or in vivo mammalian systems.

Carcinogenicity studies were not performed with this product. PEG-G-CSF is a monopegylated from of r-met-HuG-CSF. The sponsor is relying on the wealth of clinical data that have been generated with the use of Filgrastim to serve as a replacement for the traditional rodent carcinogenicity study. There is a large existing body of evidence based on the large numbers of patients (sponsor estimate of — that have received G-CSF (Filgrastim) in the last ten years in the treatment of neutropenia of various origins – chemo-induced neutropenia, acute myeloid leukemia, bone marrow transplantation, & severe chronic neutropenia - that minimizes this potential concern. No definitive evidence that G-CSF administration results in tumor formation or stimulates the growth of existing tumors has been noted in these patient populations. Additional reasons as to the low carcinogenic potential of PEG-G-CSF in the proposed patient population include: 1) the limited tissue distribution of the G-CSF receptor to which the protein binds; 2) the limited dosing regimen in patients [once per chemo cycle]; 3) the lack of any preneoplastic/neoplastic findings seen in a 6-month rodent toxicity study; and 4) the structural identity to G-CSF, which was nonmutagenic in mammalian assay systems.

The preclinical data adequately support use of the product, PEG-G-CSF, for the indication specified by the sponsor.

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Key Words: G-CSF; Filgrastim; PEG-G-CSF; recombinant human pegylated granulocyte-colony stimulating factor; Filgrastim-SD/01; Pegfilgrastim; neutrophils; ANC levels; chemotherapy

Concurrences: 151 1/23/02 151 2/5/2002
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