

PROPOSED CONDITIONAL AMENDED CLAIMS

1. A substantially homogenous preparation of N-terminally monopegylated G-CSF or analog thereof, optionally in a pharmaceutically acceptable diluent, carrier of adjuvant.
- 1A. A substantially homogenous preparation of N-terminally monopegylated G-CSF or analog thereof wherein said polyethylene glycol is linked to said G-CSF with an amine linkage, optionally in a pharmaceutically acceptable diluent, carrier of adjuvant.
2. A preparation of claim 1 or claim 1A, where said polyethylene glycol has a molecular weight of between about 2 kDa and 100 kDa.
3. A preparation of claim 2 where said polyethylene glycol has a molecular weight of between about 6 kDa and 25 kDa.
4. A preparation of any one of claims 1 to 3, where said preparation is comprised of at least 90% N-terminally monopegylated G-CSF or analog thereof and at most 10% unpegylated G-CSF or analog thereof.
5. A preparation of claim 4 wherein said preparation is comprised of at least 95% N-terminally monopegylated G-CSF or analog thereof and at most 5% unpegylated G-CSF or analog thereof.
6. A preparation of any one of claims 1 to 5 wherein said G-CSF has the sequence identified in SEQ. ID No. 1.
7. A substantially homogenous preparation of N-terminally monopegylated G-CSF, wherein: (a) said G-CSF has the amino acid sequence identified in SEQ. ID No. 1; (b) said G-CSF is monopegylated with a polyethylene glycol moiety having a molecular weight of about 12kDa.
8. A mixed preparation of G-CSF or analog thereof comprising a preparation of N-terminally monopegylated G-CSF or analog thereof mixed with a preparation of multipegylated G-CSF or analog thereof wherein the proportion of said preparation of monopegylated G-CSF or analog thereof is predetermined.
9. A mixed preparation of claim 8 wherein said preparation of N-terminally monopegylated G-CSF is selected from among those of claims 1 to 7.
10. A pharmaceutical composition comprising an effective amount of a preparation selected from among those according to any of claims 1 to 9, in a pharmaceutically acceptable diluent, adjuvant or carrier.
11. Use of a preparation according to any of claims 1 to 9 for the manufacture of a medicament for the treatment of a disorder characterized by a reduced hematopoietic or immune function.

12. A use according to claim 11 wherein said disorder is a result of chemotherapy, radiation therapy, infectious disease, severe chronic neutropenia, or leukemia.
13. A process for preparing N-terminally monopegylated G-CSF or an analog thereof comprising the steps of
 - (a) reacting in an aqueous medium G-CSF or its analog having an alpha-amino-group at its N-terminus with a water-soluble polyethylene glycol having a single aldehyde group under conditions of a reductive alkylation and at a pH sufficiently acidic to selectively activate the alpha amino-group and
 - (b) obtaining the N-terminally monopegylated G-CSF and
 - ~~(b)~~ optionally separating the N-terminally monopegylated product from the reaction mixture unreacted moieties.
14. A process according to claim 13 wherein the polyethylene glycol used has a molecular weight of between about 2 kDa and 100 kDa.
15. A process according to claim 14, wherein the polyethylene glycol used has a molecular weight of between about 6 kDa and 25 kDa.
16. A process according to any one of claims 13 to 15, wherein the polyethylene glycol used is a methoxy polyethylene glycol aldehyde.
17. A process according to any one of claims 13 to 16 wherein the reducing agent used in the reductive alkylation is sodium borohydride or sodium cyano borohydride.
18. A process according to any one of claims 13 to 17 wherein the G-CSF or its analog used is non-glycosylated.
19. A process according to any one of claims 13 to 18 wherein the G-CSF or its analog used has a methionine moiety at its N-terminus at position - 1.
20. A process according to any one of claims 13 to 19 wherein the separation step (b) comprises a purification.
21. A process according to claim 20, wherein the purification comprises ion exchange chromatography of the reaction mixture and size exclusion chromatography.