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— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
9 February 2017

(54) Title: PEGYLATED GRANULOCYTE COLONY STIMULATING FACTOR (GCSF)

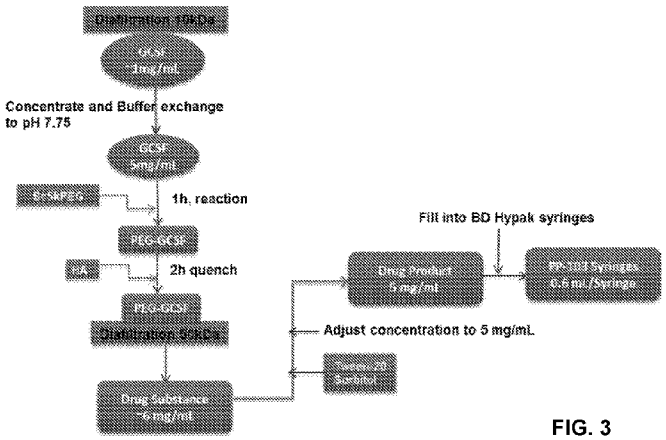

FIG. 3

(57) Abstract: This invention relates to novel PEGx-GCSF conjugates, wherein x is the amount of PEG per GCSF and ranges from 4 to 8. The invention also relates to PEG[x]-GCSF populations of individual PEGx-GCSF conjugates, wherein [x] is the average amount of PEG per GCSF of the population and is 4 or greater. The inventive compositions have unexpected therapeutic efficacy, while avoiding or substantially reducing the likelihood of adverse side effects.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/37278

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 1/00,14/53, 14/535; C12P 21/06 (2016.01)
CPC - A61K 38/193; C07K 1/00, 14/53, 14/535
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61P 31/00; C07K 1/00,14/00, 14/53, 14/535; C12P 21/06 (2016.01)
CPC: A61K 38/193; C07K 1/00, 14/53, 14/535

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO Discovery; PubMed; Google; Google Scholar; Google Patents; The Lens; ENA; NCBI Blast; KEYWORDS: PEG, Polyethylene glycol, GCSF, G-CSF, Granulocyte colony stimulating factor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2007/0254836 A1 (DEFREES, S et al.) November 01, 2007; abstract, paragraphs [0015], [0023], [0024], [0051], [0080], [0115], [0116], [0118], [0123], [0205], [0222], [0232], [0320], [0323], [0372]	1-7, 11-26, 30-31, 33-36, 38, 40-42 --- 8-10, 27-29, 37, 39, 43-63
Y	US 2003/0171559 A1 (OSSLUND, TD) September 11, 2003; figure 1; paragraphs [0066], [0068], [0073]	8-10, 27-29, 37, 43,
Y	WO 2011/041376 A1 (PROLONG PHARMACEUTICALS) September 29, 2010; claim 26	39
Y	US 2005/0288254 A1 (KIM, Y et al.) December 29, 2005; abstract; paragraphs [0037], [0039], [0093]	45
Y	US 2011/0104100 A1 (RIORDAN, N et al.) May 05, 2011; abstract; paragraph [0027]	44
Y	EP 1037657 B1 (ENZON, Inc.) March 12, 2008; paragraphs [0010], [0018], [0062]	46-63
A	WO 2011/075606 A2 (ALIOS BIOPHARMA, INC.) June 23, 2011; paragraph [0125]	8, 27
A	US 2011/0306518 A1 (WOHLGEMUTH, J et al.) December 15, 2011; paragraph [0037]	8, 27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 November 2016 (15.11.2016)	09 DEC 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US16/37278

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-***-Please See Supplemental Page-***-

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US16/37278

-***-Continued from Box III -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups 1+, Claims 1-63 and SEQ ID NO: 1 are directed toward a PEG[x]-GCSF comprising a population of PEGx-GCSF, wherein [x] is an average value of x, and wherein [x] is greater than or equal to about 4, a pharmaceutical composition comprising the PEG[x]-GCSF; a method of increasing white blood cell count, and treating severe sepsis with said composistion, and a method of preparing said PEG [x]-GCSF.

The PEG-GCSF compounds, compositions and methods will be searched to the extent that the GCSF encompasses SEQ ID NO: 1 (first exemplary GCSF sequence). Applicant is invited to elect additional GCSF sequence(s), with specified SEQ ID NO: for each, to be searched. Additional GCSF sequences will be searched upon the payment of additional fees. It is believed that claims 1-7, 8 (in-part), 9 (in-part), 10 (in-part), 11-26, 27 (in-part), 28 (in-part), 29 (in-part) and 30-63 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (GCSF sequence). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a GCSF sequence encompassing SEQ ID NO: 3 (first exemplary elected GCSF sequence).

No technical features are shared between the GCSF sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a PEGx-GCSF, wherein x is an integer from 4 to 8; a PEG[x]-GCSF comprising a population of PEGx-GCSF, wherein [x] is an average value of x, and wherein [x] is greater than or equal to about 4; a pharmaceutical formulation comprising a pharmaceutically active amount of PEG[x]-GCSF and a protein-free carrier; a method of increasing the white blood cell count, and treating severe sepsis or septic shock, in a patient in need thereof, comprising administering to said patient a therapeutically effective amount of the pharmaceutical formulation; and a method for preparing PEGx-GCSF or PEG[x]-GCSF, said method comprising the steps of: (a) obtaining a GCSF solution having a concentration of about 5.0 mg/ml; (b) combining the GCSF solution with PEG, wherein PEG is present in a molar amount of about 65 to about 75 times the molar amount of the GCSF; (c) allowing sufficient time for the GCSF and PEG to react to produce PEGxGCSF; (d) adding hydroxylamine in an amount sufficient to react with residual PEG; and (e) isolating PEGx-GCSF from unreacted PEG, N-hydroxysuccinimide and hydroxylamine and (f) isolating PEGx-GCSF.

However, these shared technical features are previously disclosed by US 2007/0254836 A1 to Defrees et al. (hereinafter 'Defrees') in view of EP 1 037 657 B1 to Enzon, Inc. (hereinafter 'Enzon') and US 7,381,804 B2 (OSSLUND).

Defrees discloses a PEGx-GCSF, wherein x is an integer from 4 to 8 (pegylated G-CSF, having four functionalized residues with a PEG-modified sialic acid (a PEGx-GCSF); abstract, paragraphs [0115], [0116]); a PEG[x]-GCSF comprising a population of PEGx-GCSF (glycopegylated G-CSF molecules (a PEG[x]-GCSF comprising a population of PEGx-GCSF); paragraph [0015]), wherein [x] is an average value of x, and wherein [x] is greater than or equal to about 4 (wherein X is four; paragraphs [0115], [0116]); a pharmaceutical formulation comprising a pharmaceutically active amount of PEG[x]-GCSF and a protein-free carrier (a pharmaceutical formulation comprising a pharmaceutically active amount of PEG[x]-GCSF and a protein-free carrier; paragraphs [0024], [0051]); a method of increasing white blood cell count (a method of treating leukopenia (a method of increasing white blood cell count); paragraph [0080]), comprising administering to said patient a therapeutically effective amount of the pharmaceutical formulation (comprising administering to said patient a therapeutically effective amount of the pharmaceutical formulation; paragraphs [0024], [0051], [0080]) and a method for preparing PEGx-GCSF or PEG[x]-GCSF (a method for preparing PEGx-GCSF or PEG[x]-GCSF; abstract, paragraphs [0015], [0115], [0116]).

Defrees does not disclose a method of treating severe sepsis or septic shock, in a patient in need thereof, comprising administering to said patient a therapeutically effective amount of the pharmaceutical formulation; said method comprising the steps of: (a) obtaining a GCSF solution having a concentration of about 5.0 mg/ml; (b) combining the GCSF solution with PEG, wherein PEG is present in a molar amount of about 65 to about 75 times the molar amount of the GCSF; (c) allowing sufficient time for the GCSF and PEG to react to produce PEGxGCSF; (d) adding hydroxylamine in an amount sufficient to react with residual PEG; and (e) isolating PEGx-GCSF from unreacted PEG, N-hydroxysuccinimide and hydroxylamine and (f) isolating PEGx-GCSF.

Enzon discloses a method of preparing a G-CSF conjugate with PEG, including the use of a carboxymethyl-N-hydroxysuccinimidyl ester of PEG (a method of preparing a G-CSF conjugate with PEG, including the use of a carboxymethyl-N-hydroxysuccinimidyl ester of PEG; paragraph [0010]), and treating the conjugate with hydroxylamine to remove unstable linkers (treating the conjugate with hydroxylamine to remove unstable linkers; paragraph [0010]), and isolating the product of a conjugation reaction from solution (isolating the product of a conjugation reaction from solution; paragraph [0018]).

-***-Continued on Next Supplemental Page-***-

-***-Continued from Previous Supplemental Page:

Osslund discloses a method of treating severe sepsis or septic shock (a method of treatment of septicemia (septic shock) in a patient in need thereof; column 10, line 53 - column 11, line 3), comprising administering to said patient a therapeutically effective amount (comprising administering to said patient a therapeutically effective amount; column 5, lines 6-17) of a pharmaceutical formulation comprising PEG-linked GCSF (a pharmaceutical composition (formulation) comprising a PEG-linked GCSF; column 4, line 60 - column 5, line 2).

It would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have modified the disclosure of Defrees to have utilized a hydroxysuccinimidyl ester of PEG to form a conjugate with G-CSF, as disclosed by Enzon, using at least one reactive site, such as the n-terminal amine, or a reactive lysyl side chain, using a sufficient amount of GCSF, such as a GCSF solution having a concentration of about 5.0 mg/ml, by combining the GCSF with the hydroxysuccinimidyl PEG, wherein PEG is present in a molar amount of about 65 to about 75 times the molar amount of the GCSF, and allowing sufficient time for the GCSF and PEG to react to produce PEGxGCSF to ensure the conjugation of as many of the reactive groups on the GCSF as possible, in order to produce a conjugate having at least 4 PEG functionalizations, as disclosed by Defrees, then adding hydroxylamine, as disclosed by Enzon, in an amount sufficient to react with residual PEG and unstable linkers; and (e) isolating PEGx-GCSF from unreacted PEG, N-hydroxysuccinimide and hydroxylamine and (f) isolating PEGx-GCSF to produce a purified PEGylated GCSF product having functionalizations at natural reactive positions, such as the amino terminus, and at lysyl side chains, in addition to, or as an alternative to the method disclosed by Defrees, in order to enable the selection of modified forms of GCSF with the most desirable properties, such as specific activity and/or shelf life. It further would have been obvious to a person of ordinary skill in the art at the time of the invention was made to have used a PEG-linked GCSF molecule or composition comprising said molecule, as disclosed by Defrees, for any known medical applications, such as the treatment of sepsis, as disclosed by Osslund, in order to provide a more effective treatment therefor, and to provide a compound with greater specific activity or longer shelf or in-vivo lifetime.

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by a combination of the Defrees, Enzon and Osslund references, unity of invention is lacking.