The present invention relates to the characterization and production of denosumab.

Denosumab HC sequence (SEP ID NO:1):

EVQLLESGGGLVQPGSSGLRLSCAASGFTSFSGDMSWVRQAPGKGLSEWVTGSGGSTYADSV
KGRTTIQRNSKNTLYQMNSSLRAEDTAVYYCARKDSPATVLMWFWPDWQGTLLTVTSSASTKGP
SVVLPAPCSRTSSTEAALGLKVQKYPFEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTV
VPSNFGTQYTCTNVDKPSNTKVDKTERKCCVECPFPCAPFVAGPSVLFLPPKPKDTLMISR
TEPVTCVVDVSHEDEPEVQFNYVVDQVEVHNAKTPREEQFNSTRFRWSVSLTVHQDWLNGKEY
KCQVSNKGLAPAEKTISTKKGQPREPQVYTLPSREEMTKQVSLTCLVKGFYPSDIAYVEWES
NGQPENNYKTTPMGLDSGSFFLYSKLTVDKSRWQQGZNVFSVCNMSEALHNHYTQKSLSPGK
### A. CLASSIFICATION OF SUBJECT MATTER

**IPC(8) -** A61K 39/00, 39/395 (2014.01)

**USPC -** 424/133.1, 130.1

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) -** A61K 39/00, 39/395 (2014.01)

**USPC -** 424/133.1, 130.1

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)


### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 20110280873 A1 (PRESTA, LG et al.) November 17, 2011; paragraphs [0005], [0006], [0026], [0057], [0072]; SEQ ID NOS: 21, 22</td>
<td>1-6, 8/6, 9, 10, 11/8/6, 12/10, 13, 22/1, 22/2, 22/4, 22/9, 22/13, 24/1, 24/2, 24/4, 24/9, 24/13, 25/1, 25/2, 25/4, 25/9, 25/13, 26/1, 26/2, 26/4, 26/9, 26/13, 27/1, 27/2, 27/4, 27/9, 27/13, 28/1, 28/2, 28/4, 28/9, 28/13, 28/15, 28/17</td>
</tr>
</tbody>
</table>

X 1 Further documents are listed in the continuation of Box C.

* 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

04 January 2014 (04.01.2014)

Date of mailing of the international search report

15 JAN 2014

Name and mailing address of the ISA/US

Mai Stop PCT, Attn: ISA/US, Commissioner of Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer: Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774
<table>
<thead>
<tr>
<th>Box No. I</th>
<th>Nucleotide and/or amino acid sequence(s) (Continuation of item 1c of the first sheet)</th>
</tr>
</thead>
</table>

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)
      - [x] on paper
      - [ ] in electronic form

   b. (time)
      - [ ] in the international application as filed
      - [ ] together with the international application in electronic form
      - [x] subsequently to this Authority for the purposes of search

2. [x] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:
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.: 1-18, 22, 24-28, denosumab reference parameters 1 and 2; SEQ ID Nos: 1, 2
   because they relate to subject matter not required to be searched by this Authority, namely: ____________________________

2. □ Claims Nos.: 19-21, 23
   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.: 19-21, 23
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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.: ____________________________
   Groups i-: Claims 1-18, 22, 24-28, denosumab reference parameters 1 and 2; SEQ ID Nos: 1, 2

**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.
<table>
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</thead>
<tbody>
<tr>
<td>Y</td>
<td>WO 2011/127322 A1 (COLLINS, BE et al.) October 13, 2011; abstract; paragraphs [0002], [00018], [00019], [00020], [00066]; Claims 1, 7, 20; figure 1</td>
<td>1-6, 8/6, 9, 10, 11/8/6, 12/10, 13, 22/1, 22/2, 22/4, 22/9, 22/13, 24/1, 24/2, 24/4, 24/9, 24/13, 25/1, 25/2, 25/4, 25/9, 25/13, 26/1, 26/2, 26/4, 26/9, 26/13, 27/1, 27/2, 27/4, 27/9, 27/13, 28/1, 28/2, 28/4, 28/9, 28/13, 7, 8/7, 11/8/7, 12/1 1/8/6, 12/1 1/8/7, 14, 17, 18, 22/14, 22/15, 22/17, 22/16, 24/14, 24/15, 24/17, 25/14, 25/15, 25/17, 26/14, 26/15, 26/17, 27/14, 27/15, 27/17, 28/14, 28/15, 28/17</td>
</tr>
</tbody>
</table>
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 I: Claims 1-18, 22, 24-28, denosumab reference parameter numbers 1, 2 and SEQ ID NOs: 1, 2 (recombinant antibody amino acid sequences) are directed toward a method of manufacturing a denosumab drug product, comprising: providing or obtaining a test glycoprotein preparation; acquiring at least one value for a denosumab parameter listed in Table 1 for the test glycoprotein preparation; and processing at least a portion of the test glycoprotein preparation as denosumab drug product if the at least one value for the test glycoprotein preparation meets a reference criterion shown in Table 1 for the parameter, thereby manufacturing an denosumab drug product; a method of manufacturing an denosumab drug product, comprising: providing or obtaining a test glycoprotein preparation; acquiring a value for each parameter listed in Table 1 for the test glycoprotein preparation; and processing at least a portion of the test glycoprotein preparation as denosumab drug product if the value for each parameter listed in Table 1 for the test glycoprotein preparation meets the reference criterion show in Table 1, thereby manufacturing an denosumab drug product; a method of manufacturing an denosumab drug product, comprising: providing a host cell that is genetically engineered to express a first amino acid sequence having a sequence with at least about 85% identity to SEQ ID NO:1 (e.g., 90, 95, 96, or 100% identity to SEQ ID NO:1) and a second amino acid sequence having a sequence with at least about 85% identity to SEQ ID NO:2 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:2), wherein the expressed amino acid sequences form a recombinant antibody composition, culturing the host cell under conditions whereby the cell expresses the first and second amino acid sequences, wherein the expressed first and second amino acid sequences form recombinant antibodies, harvesting the recombinant antibodies from the host cell culture to produce an antibody preparation, acquiring a value for each parameter listed in Table 1 for the antibody preparation; and processing at least a portion of the antibody preparation into denosumab drug product if the value for each parameter listed in Table 1 for the antibody preparation meets the reference criterion shown in Table 1, thereby manufacturing an denosumab drug product; and a method of manufacturing an denosumab drug product, comprising: providing a host cell that is genetically engineered to express a first amino acid sequence having the sequence of SEQ ID NO: 1 and a second amino acid sequence having the sequence of SEQ ID NO: 2, wherein the expressed amino acid sequences form a recombinant antibody composition, culturing the host cell under conditions whereby the cell expresses the first and second amino acid sequences, wherein the expressed first and second amino acid sequences form recombinant antibodies, harvesting the recombinant antibodies from the host cell culture to produce an antibody preparation, acquiring at least one value for an denosumab parameter listed in Table 1 for the antibody preparation; and processing or directing the processing of at least a portion of the antibody preparation as denosumab drug product if the at least one value for the antibody preparation meets a reference criterion shown in Table 1, thereby manufacturing an denosumab drug product.

The method of manufacturing an denosumab drug product of Groups I will be searched to the extent that they encompass denosumab parameters 1 and 2. It is believed that Claims 1-18, 22, and 24-28 encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass denosumab parameters 1 and 2. Applicants must indicate, if applicable, the claims which encompass the first named invention if different than what was indicated above for this group. 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. Additional denosumab parameters can be searched upon the payment of additional fees. An Exemplary Election would be: denosumab parameter number 3.

Groups I share the technical features including a method of manufacturing a denosumab drug product, comprising: providing or obtaining a test glycoprotein preparation; acquiring at least one value for a denosumab parameter listed in Table 1 for the test glycoprotein preparation; and processing at least a portion of the test glycoprotein preparation as denosumab drug product if the at least one value for the test glycoprotein preparation meets a reference criterion shown in Table 1 for the parameter, thereby manufacturing an denosumab drug product; and a method of manufacturing a denosumab drug product, comprising: providing a host cell that is genetically engineered to express a first amino acid sequence having a sequence with at least about 85% identity to SEQ ID NO:1 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:1) and a second amino acid sequence having a sequence with at least about 85% identity to SEQ ID NO:2 (e.g., 90, 95, 98, or 100% identity to SEQ ID NO:2), wherein the expressed amino acid sequences form a recombinant antibody composition, culturing the host cell under conditions whereby the cell expresses the first and second amino acid sequences, wherein the expressed first and second amino acid sequences form recombinant antibodies, harvesting the recombinant antibodies from the host cell culture to produce an antibody preparation, acquiring at least one value for an denosumab parameter listed in Table 1 for the antibody preparation; and processing or directing the processing of at least a portion of the antibody preparation as denosumab drug product if the at least one value for the antibody preparation meets a reference criterion shown in Table 1, thereby manufacturing an denosumab drug product.
However, these shared technical features are previously disclosed by US 2010/01 13294 A 1 to Venkataraman, et al. (hereinafter Venkataraman) in view of the article entitled A Study Of The Biological Receptor Activator Of Nuclear Factor-kappaB Ligand Inhibitor, Denosumab, In Patients With Multiple Myeloma Or Bone Metastases From Breast Cancer” by Body, et al. (hereinafter Body).

Venkataraman discloses a method of manufacturing (method for the production (manufacture); paragraphs [0007], [0138]) an antibody (antibodies; paragraph [0219]) drug product (drug product; paragraph [0138]), comprising: providing or obtaining a test glycoprotein preparation (a glycoprotein product (a test glycoprotein preparation); paragraph [0137]); acquiring at least one value for a parameter for the test glycoprotein preparation (acquiring at least one value for a parameter for the test glycoprotein preparation; paragraph [0137]); and processing at least a portion of the test glycoprotein preparation (processing at least a portion of the test glycoprotein preparation; paragraphs [0137], [0138]) as an antibody (antibodies; paragraph [0219]) drug product (drug product; paragraph [0138]). If the at least one value for the test glycoprotein preparation meets a reference criterion for the parameter (if the at least one value for the test glycoprotein preparation meets a reference criterion for the parameter; paragraph [0138]), thereby manufacturing (manufacturing; paragraphs [0007], [0138]) an antibody (antibodies; paragraph [0219]) drug product (drug product; paragraph [0138]); and comprising: providing a host cell (providing a genetically engineered cell that produces the glycoprotein (a host cell); paragraph [0254]) that is genetically engineered, to express a first amino acid sequence (genetically engineered to produce a first protein (amino acid sequence); paragraph [0254]), wherein the expressed amino acid sequence forms a recombinant (recombinant; paragraph [0280]) antibody composition (antibody; paragraph [0219]; Table I), culturing the host cell under conditions whereby the cell expresses the amino acid sequence (culturing the host cell under conditions, whereby the cell expresses the amino acid sequence; paragraph [0283]), wherein the expressed amino acid sequences (expressed glycoproteins (amino acid sequences); paragraph [0254]) form recombinant (recombinant; paragraph [0280]) antibodies (antibodies; paragraphs [0219]), Table I, harvesting the recombinant antibodies from the host cell culture (purifying (harvesting); paragraphs [0417], [0418]) the recombinant (recombinant; paragraph [0280]) antibodies (antibodies; paragraphs [0219], Table I) from the host cell culture (from the host cell culture; paragraphs [0283], [0418]) to produce (produce; paragraph [0138]) an antibody preparation (antibodies; paragraphs [0219], Table I), acquiring at least one value for a parameter for the preparation (acquiring at least one value for a parameter for the test preparation; paragraph [0137]); and processing at least a portion of the preparation (processing at least a portion of the preparation; paragraphs [0137], [0138]) as an antibody (antibodies; paragraph [0219]) drug product (drug product; paragraph [0138]) if the at least one value for the preparation meets a reference criterion for the parameter (if the at least one value for the preparation meets a reference criterion for the parameter; paragraph [0138]), thereby manufacturing (manufacturing; paragraphs [0007], [0138]) an antibody (antibodies; paragraph [0219]) drug product (drug product; paragraph [0138]).

Venkataraman does not disclose the use of the denosumab drug. Body discloses wherein denosumab glycosylation prevents proteolytic breakdown (page 1227, column 1, paragraph 1). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the previous disclosure of Venkataraman, in order to have included the analysis of parameters associated with the production of a well-known therapeutic antibody, such as denosumab, as disclosed by Body, for enabling the production of well-characterized lots or batches of the antibody which met required criteria, as previously disclosed by Venkataraman, for ensuring the resistance of the antibody to proteolysis and, therefore, ensuring its proper dosing and activity in a subject treated with the manufactured antibody.

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 Venkataraman and Body references, unity of invention is lacking.