(54) Title: FVIII-INDEPENDENT FIX-MUTANT PROTEINS FOR HEMOPHILIA A TREATMENT

(57) Abstract: The present invention relates to recombinant blood coagulation factor IX (rFIX) mutants having factor VIII (FVIII) independent factor X (FX) activation potential. Five full length FIX proteins with combinations of mutations of amino acids important for functional activity of FIX and FIX wild type were cloned and expressed in HEK 293 cells. The proteins were tested by an activated partial thromboplastin time (aPTT) assay in FVIII-depleted plasma as well as in FVIII-inhibited patient plasma. In FVIII-depleted plasma functional activity of the FIX mutants was calculated as increased FVIII equivalent activity. The mutant proteins had increased FVIII equivalent activity. In FVIII-inhibited patient plasma the FEIBA equivalent activity was calculated for analysis of FVIII independent FX activation potential. The proteins had also increased FEIBA equivalent activity. Furthermore, the pre-activated FIX proteins had an increased activity in FIX-depleted plasma containing FVIII inhibitors. Therefore these FIX mutants are alternatives as bypassing agents for treatment of FVIII inhibitor patients.
INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/000685

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N9/64 A61K38/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. REDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K C07K

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 practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, Sequence Search, WPI Data

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>
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<tbody>
<tr>
<td>X</td>
<td>KOLKMAN JOOST A ET AL: &quot;Insertion loop 256-268 in coagulation factor IX restricts enzymatic activity in the absence but not in the presence of factor VIII&quot; BIOCHEMISTRY, vol. 39, no. 25, 27 June 2000 (2000-06-27), pages 7398-7405, XP002487244 ISSN: 0006-2960 abstract page 7401 page 7403, left-hand column, paragraph 3 figure 1</td>
<td>1,6-9, 13-15</td>
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D. See patent family annex.

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

X Special categories of cited documents:

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P document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search
11 August 2008

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Authorized officer
Surdej, Patrick

Date of mailing of the international search report
25/08/2008

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<tr>
<td>X</td>
<td>CHRISTOPHE OLIVIER D ET AL: &quot;Blood coagulation factor IX residues glu78 and Arg94 provide a link between both epidermal growth factor-like domains that is crucial in the interaction with factor VIII light chain&quot; JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 1, 2 January 1998 (1998-01-02), pages 222-227, XP002487245 ISSN: 0021-9258 abstract page 224 - page 225</td>
<td>1,6-9, 13-15</td>
</tr>
<tr>
<td>Y</td>
<td>SICHLER KATRIN ET AL: &quot;Physiological fIXa activation involves a cooperative conformational rearrangement of the 99-1 oop.&quot; JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 6, 7 February 2003 (2003-02-07), pages 4121-4126, XP002487246 ISSN: 0021-9258 abstract page 4125 - page 4126</td>
<td>2-5, 10-12</td>
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