(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 May 2021 (06.05.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/084276 A3

(51) International Patent Classification:
C07K 14/755 (2006.01) *C12N 15/861* (2006.01)

(21) International Application Number:
PCT/GB2020/052761

(22) International Filing Date:
30 October 2020 (30.10.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

1915953.2	01 November 2019 (01.11.2019)	GB
1915955.7	01 November 2019 (01.11.2019)	GB
1915956.5	01 November 2019 (01.11.2019)	GB
1917925.8	06 December 2019 (06.12.2019)	GB
1917926.6	06 December 2019 (06.12.2019)	GB
1917927.4	06 December 2019 (06.12.2019)	GB
2006250.1	28 April 2020 (28.04.2020)	GB

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *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:
22 July 2021 (22.07.2021)

WO 2021/084276 A3

(54) Title: FACTOR VIII CONSTRUCT

(57) Abstract: The present invention relates to a Factor VIII (FVIII) polypeptide, a polynucleotide comprising a Factor VIII nucleotide sequence, and a recombinant AAV construct. The invention further relates to an AAV viral particle comprising the recombinant AAV construct of the invention, and a composition comprising the Factor VIII polypeptide, polynucleotide, recombinant AAV construct or AAV viral particle of the invention. The invention also relates to methods of using, and uses of, the Factor VIII polypeptide, polynucleotide, recombinant AAV construct, AAV viral particle and/or composition of the invention. The invention also relates to uses of the recombinant AAV construct of the invention for the production of AAV viral particles, and methods for producing AAV viral particles using the recombinant AAV constructs of the invention.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/052761

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/755 C12N15/861
ADD.

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)
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 practicable, search terms used)
EPO-Internal, Sequence Search, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/140330 A1 (CASEBIA THERAPEUTICS LTD LIABILITY PARTNERSHIP [US]) 18 July 2019 (2019-07-18)	1-5,8,9, 12-14, 38-42, 44-55,60
Y	abstract; claims 1-11; figures 2,3; example 4b; sequences 197,224 -----	15,42, 56-59
X	US 2017/095538 A1 (COLOSI PETER CAMERON [US] ET AL) 6 April 2017 (2017-04-06)	1-5,9, 12-14, 38-42, 44-60
Y	abstract paragraph [0008] - paragraph [0010] paragraph [0062] paragraph [0088] - paragraph [0089]; claims 9-17; sequences 1,9 paragraph [0153] paragraph [0080] ----- -/--	15,42, 56-59

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

[X] 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
16 June 2021	24/06/2021
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Gurdjian, Didier

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

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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:

see additional sheet

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 an 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.:

1-5, 8, 9, 12-25, 35, 36, 38-60

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

International application No.
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Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

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:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] 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 forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/052761

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017/075619 A1 (SPARK THERAPEUTICS INC [US]) 4 May 2017 (2017-05-04)	1-5, 12-14, 16-24, 28-33, 35,36, 38-42, 44-55,60
Y	abstract paragraph [0056]; claims 1-49; example 10; sequences 1,4,23,25,29	15,42, 56-59

X	WO 2019/028192 A1 (SPARK THERAPEUTICS INC [US]) 7 February 2019 (2019-02-07)	1-5, 16-24, 38-42, 44-55,60
Y	abstract; claims 1-122; example 10; sequences 1,23,25	15,42, 43,56-59

X	WO 2017/180857 A1 (UNIV PENNSYLVANIA [US]) 19 October 2017 (2017-10-19)	1-5, 12-14, 38-42, 44-55,60
Y	abstract page 15, paragraph 2 page 44, paragraph 2 - page 45, paragraph 1 page 49, line 20 - page 50, line 10	15,42, 56-59

X	WO 2017/083762 A1 (BAXALTA INC [US]; BAXALTA GMBH [CH]; LENGLER JOHANNES) 18 May 2017 (2017-05-18)	1-5, 12-14, 38-42, 44-55,60
Y	page 213; claims 1-70; figures 7,10; table 7; sequence 10	15,42, 43,56-59

X	ISHIWATA A ET AL: "Phenotype correction of hemophilia A mice with adeno-associated virus vectors carrying the B domain-deleted canine factor VIII gene", THROMBOSIS RESEARCH, ELSEVIER, AMSTERDAM, NL, vol. 118, no. 5, 1 January 2006 (2006-01-01), pages 627-635, XP027962708, ISSN: 0049-3848 [retrieved on 2006-01-01]	1-5, 12-14
Y	abstract page 628, right-hand column, paragraph 2 - page 629, left-hand column, paragraph 1	15,42, 56-59

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2020/052761

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2009/102085 A1 (MOGAM BIOTECH RES INST [KR]; KOH DAEKYUNG [KR] ET AL.) 20 August 2009 (2009-08-20) abstract page 16, paragraph 3 - page 17, paragraph 2; claims 1-7	42,56-59
Y	WO 2016/126857 A1 (UNIV FLORIDA [US]) 11 August 2016 (2016-08-11) abstract; claims 1-16; sequence 2	42,56-59
A	WO 2017/053677 A1 (BIOMARIN PHARM INC [US]) 30 March 2017 (2017-03-30) cited in the application abstract paragraph [0012] - paragraph [0014]; claims 1-64; figure 2a; sequence 1	1-5,15, 43
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A	WO 2012/007324 A2 (NOVO NORDISK AS [DK]; OESTERGAARD HENRIK [DK] ET AL.) 19 January 2012 (2012-01-19) abstract page 12, paragraph 5 - page 13, paragraph 3 page 15, paragraph 5 page 17, paragraph 5 - page 18, paragraph 1; claims 1-7,11-13; figure 1; tables 3-5; sequence 3	12
X	WO 2018/093766 A1 (BAYER HEALTHCARE LLC [US]) 24 May 2018 (2018-05-24) abstract; claims 1-28; figure 1; example 10; table 24; sequence 121	16-24

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Information on patent family members

International application No
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-5, 8, 9, 12-15, 38-60(all partially)

A recombinant AAV construct which is less than 4900 nucleotides in length and which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, a recombinant AAV construct which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, further comprising a nucleotide sequence encoding a signal peptide, the signal peptide is a wild-type Factor VIII signal peptide; and/or) the signal peptide comprises SEQ ID NO: 53 or wherein the nucleotide sequence encoding the signal peptide comprises SEQ ID NO: 54 or 55,
and corresponding to an AAV viral particle comprising the recombinant AAV construct, a composition comprising the Factor VIII polypeptide, AAV viral particle and a pharmaceutically acceptable excipient, for use in a method of treatment, corresponding use of the recombinant AAV construct for producing a population of AAV viral particles , method for producing a population of AAV viral particles , method for increasing the vector genome yield during the production of a population of AAV viral particles, a method for decreasing the nucleic acid impurity level during the production of a population of AAV viral particles, and a population of AAV viral particles.

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2. claims: 10, 11(completely); 1-9, 12-15, 38-60(partially)

A recombinant AAV construct which is less than 4900 nucleotides in length and which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, a recombinant AAV construct which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, further comprising a nucleotide sequence encoding a signal peptide, wherein the signal peptide comprises SEQ ID NO: 56 or wherein the nucleotide sequence encoding the signal peptide comprises SEQ ID NO: 57,
and corresponding to an AAV viral particle comprising the recombinant AAV construct, a composition comprising the Factor VIII polypeptide, AAV viral particle and a pharmaceutically acceptable excipient, for use in a method of treatment, corresponding use of the recombinant AAV construct for producing a population of AAV viral particles , method for producing a population of AAV viral particles ,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

method for increasing the vector genome yield during the production of a population of AAV viral particles, a method for decreasing the nucleic acid impurity level during the production of a population of AAV viral particles, and a population of AAV viral particles.

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3. claims: 1-9, 12-15, 38-60(all partially)

A recombinant AAV construct which is less than 4900 nucleotides in length and which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, a recombinant AAV construct which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, further comprising a nucleotide sequence encoding a signal peptide, wherein the signal peptide comprises SEQ ID NO: 58 or wherein the nucleotide sequence encoding the signal peptide comprises SEQ ID NO: 59,
and corresponding to an AAV viral particle comprising the recombinant AAV construct, a composition comprising the Factor VIII polypeptide, AAV viral particle and a pharmaceutically acceptable excipient, for use in a method of treatment, corresponding use of the recombinant AAV construct for producing a population of AAV viral particles , method for producing a population of AAV viral particles , method for increasing the vector genome yield during the production of a population of AAV viral particles, a method for decreasing the nucleic acid impurity level during the production of a population of AAV viral particles, and a population of AAV viral particles.

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4. claims: 1-9, 12-15, 38-60(all partially)

A recombinant AAV construct which is less than 4900 nucleotides in length and which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, a recombinant AAV construct which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, further comprising a nucleotide sequence encoding a signal peptide, wherein the signal peptide comprises SEQ ID NO: 60 or wherein the nucleotide sequence encoding the signal peptide comprises SEQ ID NO: 61,
and corresponding to an AAV viral particle comprising the recombinant AAV construct, a composition comprising the Factor VIII polypeptide, AAV viral particle and a pharmaceutically acceptable excipient, for use in a method

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

of treatment, corresponding use of the recombinant AAV construct for producing a population of AAV viral particles , method for producing a population of AAV viral particles , method for increasing the vector genome yield during the production of a population of AAV viral particles, a method for decreasing the nucleic acid impurity level during the production of a population of AAV viral particles, and a population of AAV viral particles.

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5. claims: 1-5, 7-9, 12-15, 38-60(all partially)

A recombinant AAV construct which is less than 4900 nucleotides in length and which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, a recombinant AAV construct which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, further comprising a nucleotide sequence encoding a signal peptide, the signal peptide as far as not covered in inventions 1-4,
and corresponding to an AAV viral particle comprising the recombinant AAV construct, a composition comprising the Factor VIII polypeptide, AAV viral particle and a pharmaceutically acceptable excipient, for use in a method of treatment, corresponding use of the recombinant AAV construct for producing a population of AAV viral particles , method for producing a population of AAV viral particles , method for increasing the vector genome yield during the production of a population of AAV viral particles, a method for decreasing the nucleic acid impurity level during the production of a population of AAV viral particles, and a population of AAV viral particles.

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6. claims: 37(completely); 38-60(partially)

A recombinant AAV construct which comprises a polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide comprising a Factor VIII amino acid sequence, corresponding AAV viral particle comprising the recombinant AAV construct, a composition comprising the Factor VIII polypeptide, AAV viral particle and a pharmaceutically acceptable excipient, for use in a method of treatment, corresponding use of the recombinant AAV construct for producing a population of AAV viral particles , method for producing a population of AAV viral particles , method for increasing the vector genome yield during the production of a population of AAV viral particles, a method for decreasing the nucleic acid impurity level during the production of a population of AAV viral particles, and a population of AAV

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viral particles, as far as not comprised in invention 1-5.

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7. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 9., the corresponding polynucleotide, and composition, for use in a method of treatment, in the manufacture of a medicament.

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8. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 10, the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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9. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 11, the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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10. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 12, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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11. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 14, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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12. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 13, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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13. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 15, and the corresponding polynucleotide.

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14. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 16, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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15. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence

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comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 17, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament..

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16. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 18, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament..

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17. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 19, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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18. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 20, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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19. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between

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positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 21, the corresponding nucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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20. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 22 , and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament..

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21. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 23, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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22. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 24, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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23. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 25, and the corresponding polynucleotide and composition, for use in a

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method of treatment, in the manufacture of a medicament.

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24. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 26, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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25. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 27, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament..

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26. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 28, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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27. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 29., and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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28. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 30, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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29. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 31, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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30. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 32, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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31. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 33, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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32. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino

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acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 34, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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33. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 35, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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34. claims: 16-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises SEQ ID NOs: 36, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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35. claims: 16-22, 24, 25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises one or more substitution mutations at an interdomain interface selected from the group consisting of: the A1/A3 domain interface, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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36. claims: 16-22, 24, 25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence

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comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises one or more substitution mutations at an interdomain interface selected from the group consisting of: the A2/A3 domain interface, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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37. claims: 16-22, 24, 25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein the Factor VIII amino acid sequence comprises one or more substitution mutations at an interdomain interface selected from the group consisting of: the A1/C2 domain interface, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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38. claims: 20-25, 35, 36, 44-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein: the Factor VIII amino acid sequence comprises a one or more substitution mutations selected from the group consisting of a substitution of an amino acid corresponding to M662 of SEQ ID NO: 1, and the corresponding polynucleotide and composition, for use in a method of treatment, in the manufacture of a medicament.

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39. claims: 20-25, 35, 36, 40-50(all partially)

A Factor VIII polypeptide comprising a Factor VIII amino acid sequence wherein the Factor VIII amino acid sequence comprises a modified beta domain related (BDR) region which is modified relative to wild-type BDR region, which wild-type BDR region corresponds to the region between positions 713 and 1697 of SEQ ID NO: 1, wherein: the Factor VIII amino acid sequence comprises a one or more substitution mutations selected from the group consisting of a substitution of an amino acid corresponding to H693 of SEQ ID NO: 1, and the corresponding polynucleotide and composition, for use in a method of treatment, in the

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manufacture of a medicament, as far as not comprised in invention 38.

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40. claims: 26-36, 44-50(all partially)

A polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide and wherein at least a portion of the Factor VIII nucleotide sequence is not wild-type, wherein the Factor VIII polypeptide encoded by the Factor VIII nucleotide sequence is expressed in human liver cells at at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100% of the level expressed by a polypeptide encoded by the polynucleotide of:
SEQ ID NO: 3, corresponding polypeptide, composition, for use in a method of treatment, in the manufacture of a medicament.

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41. claims: 26-36, 44-50(all partially)

A polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide and wherein at least a portion of the Factor VIII nucleotide sequence is not wild-type, wherein the Factor VIII polypeptide encoded by the Factor VIII nucleotide sequence is expressed in human liver cells at at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100% of the level expressed by a polypeptide encoded by the polynucleotide of:
SEQ ID NO: 4, corresponding polypeptide, composition, for use in a method of treatment, in the manufacture of a medicament.

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42. claims: 26-36, 44-50(all partially)

A polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide and wherein at least a portion of the Factor VIII nucleotide sequence is not wild-type, wherein the Factor VIII polypeptide encoded by the Factor VIII nucleotide sequence is expressed in human liver cells at at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100% of the level expressed by a polypeptide encoded by the polynucleotide of:
SEQ ID NO: 5,corresponding polypeptide, composition, for use in a method of treatment, in the manufacture of a

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medicament.

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43. claims: 26-36, 44-50(all partially)

A polynucleotide comprising a Factor VIII nucleotide sequence, wherein the Factor VIII nucleotide sequence encodes a Factor VIII polypeptide and wherein at least a portion of the Factor VIII nucleotide sequence is not wild-type, wherein the Factor VIII polypeptide encoded by the Factor VIII nucleotide sequence is expressed in human liver cells at at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or at least 100% of the level expressed by a polypeptide encoded by the polynucleotide of:
SEQ ID NO: 6, corresponding polypeptide, composition, for use in a method of treatment, in the manufacture of a medicament.
