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(57) Abstract: The present invention provides peptides partly derivable from FVIII which are capable of binding to an MHC class II molecule without further antigen processing and being recognised by a factor VIII specific T cell. In particular, the present invention provides peptides comprising the sequence DNIMVTFRNQASRPY or PRCLTRYSSFNME with modifications at the N- and C-termini. The present invention also relates to the use of such a peptide for the prevention or suppression of inhibitor antibody formation in haemophilia A and/or acquired haemophilia.



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PEPTIDES

FIELD OF THE INVENTION

5

The present invention relates to peptides, at least part of which is derivable from factor VIII (FVIII). The peptides can be used to reduce or prevent factor VIII inhibitor antibody formation, for example in haemophilia A treatment and acquired haemophilia.

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BACKGROUND TO THE INVENTION

HAEMOPHILIA

15 Haemophilia belongs to a group of inheritable blood disorders that includes haemophilia A, haemophilia B (Christmas disease) and Von Willebrand's disease.

In haemophilia, the blood's ability to clot is severely reduced because an essential clotting factor is partly or completely missing, resulting in increased bleeding time.

20

Haemophilia A is a deficiency of the clotting factor VIII, whereas Haemophilia B is a deficiency of clotting factor IX. In both diseases, the faulty gene is found on the X chromosome, so the conditions are X-linked. Haemophilia A is five times more common than haemophilia B.

25

Haemophilia is a lifelong inherited genetic condition, which affects females as carriers and males who inherit the condition. About a third of new diagnoses are where there is no previous family history. It appears world-wide and occurs in all racial groups. About 6,000 people are affected with haemophilia in the UK.

30

Haemophiliacs bleed for a prolonged period following injury. External injuries such as cuts and grazes do not usually pose serious problems: it is often possible to stop bleeding by applying a degree of pressure and covering the affected area (e.g with a plaster).

The main problem is internal bleeding into joints, muscles and soft tissues, which can occur spontaneously. Internal bleeding, such as haemorrhages into the brain, is very difficult to manage and can be fatal. Repeated bleeding in the joints causes acute pain and can cause arthritis and/or long-term joint damage leading to disability.

Treatment for haemophilia is usually by replacement of the missing clotting factor. In mild or moderate haemophilia injections may be given at the time a bleed occurs (on-demand therapy). However, in severe haemophilia regular prophylactic injections are given to help the blood to clot and minimise the likelihood of long term joint damage.

A potentially serious complication of coagulation factor replacement therapy for haemophilia A is the development of antibodies that neutralise the procoagulant function of factor VIII. Factor VIII inhibitors occur in approximately 25% of those with severe haemophilia A. Since patients with congenital haemophilia A can be genetically deficient in FVIII, the synthesis of inhibitors is an alloimmune response to the foreign protein administered to prevent or treat bleeding episodes.

CD4+ T cells play a central role in the immune response to FVIII. After being taken up by antigen-presenting cells (APCs), FVIII undergoes proteolytic degradation into peptide fragments (Reding *et al* (2006) Haemophilia 12(supp 6) 30-36). These peptides are then presented on the surface of the APC in association with MHC class II molecules. This complex is then recognised by the T cell receptor of a CD4+ cell specific for FVIII. In the presence of the appropriate costimulatory signals, this recognition ultimately causes the CD4+ cell to direct the synthesis of antibodies by B cells.

The incidence of inhibitor formation initially increases with the number of factor VIII treatments, but appears to plateau after 50-100 exposure days. Inhibitor formation is much more common in severe haemophilia than in moderate or mild disease and some molecular defects, most clearly large deletions and nonsense mutations in the factor VIII light chain, appear to predispose to inhibitor formation. Parameters such as the concentration, type (purified or recombinant) of

replacement factor, and treatment history may also affect the likelihood of antibody production.

The management of haemophilia patients with inhibitors is an ongoing challenge.

Immune tolerance induction (ITI) using a desensitization technique is successful in some patients with alloantibodies against factor VIII. This therapeutic approach requires ongoing exposure to factor replacement therapy, so is a long-term strategy.

Although ITI can be successful, a significant proportion (about 30%) of patients fails to respond to ITI. Patients with high inhibitor titres are much less likely to respond to treatment. Another significant contributing factor is age at the start of commencing ITI, with greatly decreased success rates when the patient is older than 20 (Hay *et al* (2005) *Seminars in Thrombosis and Hemostasis* 32:15-21)

When ITI therapy is unsuccessful, the inhibitor generally persists for life, and because such patients are usually high-responders, it is necessary to treat episodes of bleeding with FVIII bypassing products, such as activated prothombin complex concentrates (FEIBATM), and recombinant-activated FVII. However, the use of such agents is associated with adverse events such as disseminated intravascular coagulation, acute myocardial infarction, pulmonary embolus and thromboses (Acharya and DiMichele (2006) *Best Practice & Research Clinical Haematology* 19:51-66).

Immunosuppressive therapy is sometimes used for patients who fail to response to ITI. Treatment includes administration of immunosuppressive drugs such as cyclophosphamide, prednisone, azathioprine and cyclosporine which non-specifically target the immune system. These treatments can have side-effects associated with general immunosuppression.

There is renewed interest on selective B cell depletion using RituximabTM, a humanised monoclonal antibody to B cell CD20 antigen. However, infusion reactions, serum sickness and opportunistic infections have occurred in some children treated with this drug (DiMichele (2007) *J Thromb Haemost* 5:143-50).

ACQUIRED HAEMOPHILIA

Acquired haemophilia is a rare autoimmune condition which affects between 1 and 4 people in every million. In this condition, subjects who are not born with haemophilia develop antibodies against one of the clotting factors such as factor VIII. It is thought that pregnancy and autoimmune diseases such as rheumatoid arthritis and cancer may increase the risk of developing acquired haemophilia. Although there are differences in the underlying immune mechanisms leading to their production, the clinical manifestations of FVIII inhibitors produced in response to coagulation factor replacement therapy and those produced in acquired haemophilia are similar.

Acquired haemophiliac patients have a mortality rate that approaches 25%, partly because of the association of acquired inhibitors with severe bleeding complications. The therapy of acquired autoantibody inhibitors is based primarily on the need to control or prevent acute hemorrhagic complications, which frequently are life and limb threatening and secondarily to eradicate the autoantibody to restore normal coagulation.

Some bleeds associated with low titre autoantibody inhibitors (< 5 Bethesda Units) may be treated effectively with FVIII concentrates administered at high doses. Porcine FVIII concentrate was formerly considered a critical first-line therapy for acquired haemophilia-related bleeding since it was the only replacement therapy that provided an opportunity to actually measure post-infusion FVIII coagulation activity levels in the laboratory. The product was removed from the marketplace in 2004 because of contamination of the porcine plasma pools by porcine parvovirus. Now, "bypassing" agents are most commonly used, but potential risks of thrombogenicity exist and there is only about 80% efficacy for each product. Plasma exchange via plasmapheresis and extracorporeal immunoadsorption may be necessary to temporarily reduce the inhibitor titer enough for bypassing agents or FVIII replacement to provide adequate hemostasis.

Eradication of autoantibody inhibitors depends on immunosuppressive measures, such as: (1) administration of corticosteroids with 30%–50% efficacy in 3–6 weeks; (2) use of cytotoxic and myelosuppressive chemotherapeutic agents, e.g.,

cyclophosphamide, cyclosporine, 2-chlorodeoxyadenosine; (3) immunomodulation with intravenous immunoglobulin; and (4) selective B-lymphocyte depletion with rituximab. RituximabTM responders may require concurrent use of steroids and relapses may respond to retreatment.

Thus, all currently available methods for reducing alloantibody production associated with haemophilia A treatment, and autoantibody production in acquired haemophilia, have shortcomings. There is therefore a need for improved methods to address the issue of anti-FVIII antibodies in haemophilia A and acquired haemophilia.

The present inventors have found that it is possible to prevent FVIII inhibitor antibody formation by pre-tolerising the patient with FVIII-derived peptides and to treat conditions such as haemophilia by administration of FVIII-derived peptides in order to reduce FVIII inhibitor antibodies.

SUMMARY OF ASPECTS OF THE INVENTION

One aspect of the present invention, therefore, relates to a peptide, at least part of the sequence of which is derivable from FVIII, which is capable of inducing or restoring tolerance to FVIII.

In a first embodiment of the first aspect of the invention, the invention provides a peptide comprising the FVIII-derived sequence DNIMVTFRNQASRPY which peptide (a) has the formula

XXGDNIMVTFRNQASRPYGXX

wherein X is either Lysine or Glutamic acid; and

(b) has one of the following sequences:

KKGDNIMVTFRNQASRPYGKK (SEQ ID No. 17)

KKGDNIMVTFRNQASRPYGKE (SEQ ID No. 18)

KKGDNIMVTFRNQASRPYG EK (SEQ ID No. 19)

KEGDNIMVTFRNQASRPYGKK (SEQ ID No. 25)

KEGDNIMVTFRNQASRPYGKE (SEQ ID No. 26)

5 KEGDNIMVTFRNQASRPYGKEK (SEQ ID No. 27)

EKGDNIMVTFRNQASRPYGKK (SEQ ID No. 29)

10 EKGDNIMVTFRNQASRPYGKE (SEQ ID No. 30) and

EKGDNIMVTFRNQASRPYGKEK (SEQ ID No. 31).

In a second embodiment, the present invention provides a peptide comprising the FVIII-derived sequence PRCLTRYSSFVNME which peptide (a) has the formula

15

XXGPRCLTRYSSFVNMEGXX

wherein X is either Lysine or Glutamic acid; and
(b) has one of the following sequences:

20

KKGPRCLTRYSSFVNMEGKK (SEQ ID No. 1)

KKGPRCLTRYSSFVNMEGKE (SEQ ID No. 2)

25 KKGPRCLTRYSSFVNMEGEK (SEQ ID No. 3)

EEGPRCLTRYSSFVNMEGKK (SEQ ID No. 5)

EEGPRCLTRYSSFVNMEGEK (SEQ ID No. 7)

30

KEGPRCLTRYSSFVNMEGKK (SEQ ID No. 9)

KEGPRCLTRYSSFVNMEGKE (SEQ ID No. 10)

35 KEGPRCLTRYSSFVNMEGEK (SEQ ID No. 11) and

EKGPRCLTRYSSFVNMEGKK (SEQ ID No. 13).

In another aspect, the present invention provides a composition comprising a plurality of peptides, including one or more peptide(s) according to the first aspect the invention.

5 The composition may comprise at least one peptide according to the first embodiment and at least one peptide according to the second embodiment of the first aspect of the invention.

10 The composition may comprise a peptide having SEQ ID No. 1 and a peptide having SEQ ID No. 17.

The composition may be in the form of a kit, in which the plurality of peptides are provided separately for separate, subsequent, sequential or simultaneous administration.

15 The peptide or a composition of the invention may be for use in suppressing, reducing, or preventing the development of factor VIII inhibitor antibodies.

The present invention also provides the use of such a peptide or composition in the manufacture of a medicament to suppress, reduce or prevent the development of factor VIII inhibitor antibodies.

- 5 The present invention also provides a method for suppressing, preventing or reducing the development of Factor VIII inhibitor antibodies in a subject, which comprises the step of administration of such a peptide or composition to the subject.
- 10 The subject may be deficient in FVIII. In particular the subject may have haemophilia A, and may be, or be about to, undergo factor VIII replacement therapy.

Alternatively the subject may have, or be at risk from contracting, acquired
15 haemophilia.

Factor VIII inhibitors are found more frequently in individuals expressing HLA-DR2. The subject treated by the method of the invention may therefore be HLA-DR2 positive.
20

DESCRIPTION OF THE FIGURES

Figure 1: Solubility of modified FVIII peptides

- 25 A total of 32 peptides were tested: 16 of which were based on the FVIII peptide PRCLTRYSSFVNME ("PRCLT") (SEQ ID No. 1,2,3,4,5,6,7,8,9,10,11,12,13); and 16 of which were based on the FVIII peptide DNIMVTFRNQASRPY ("DNIMV") (SEQ ID No. 17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32). Their solubility was tested relative to an unrelated control peptide (4Y) which is known to be sufficiently
30 soluble for application as a tolerising antigen. In the diagram, green represents a peptide which is at least as soluble as 4Y, orange represents a peptide which is not quite as soluble as 4Y but is fairly close, and red represents a peptide which is not as soluble as 4Y. Peptides coded green were as soluble as control peptide 4Y, peptides coded red were poorly soluble, and one peptide coded amber had
35 intermediate solubility.

Figure 2: Solubility of tagged peptides in aqueous solution.

The concentration of peptide in solution following dissolution in DMSO (control) or PBS was determined spectrophotometrically and compared to the expected value of 4mg/mL. A greater disparity between the actual and expected concentration corresponds to lower relative solubility.

Figure 3: The tagged peptides act as apitopes

Antigen presentation assays with fresh and fixed antigen presenting cells were conducted to investigate whether the tagged peptides, like the parent peptides PRCLT and DNIMV, are capable of binding to an MHC molecule and being presented to a T cell without antigen processing. (a) PRCLT peptides; (b) DNIMV peptides.

Figure 4: Peptides having SEQ ID 1 and SEQ ID 2 regulate a T cell response to PRCLT

The figure shows recall responses from HLA-DR2 transgenic mice treated with either SEQ ID 1, SEQ ID 2 or control (PBS) and primed with PRCLT. T cell stimulation indices are shown across a range of peptide concentrations and against FVIII and the control PPD

Figure 5: Peptides having SEQ ID 17 and SEQ ID 18 regulate a T cell response to DNIMV.

The figure shows recall responses from HLA-DR2 transgenic mice treated with either SEQ ID 17, SEQ ID 18 or control (PBS) and primed with DNIMV. T cell stimulation indices are shown across a range of peptide concentrations and against FVIII and the control PPD.

Figure 6: Peptides having SEQ ID 1 and SEQ ID 17 regulate a T cell response to their parental peptides.

The figure shows recall responses from HLA-DR2 transgenic mice treated with either SEQ ID1 or SEQ ID 17 or control (PBS) and primed with PRCLT and DNIMV and recalled in vitro with PRCLT, DNIMV or FVIII. T cell stimulation indices

are shown across a range of peptide concentrations and against FVIII. There is a significant inhibition of T cell responses to the parental peptides.

Figure 7: A combination of a peptide having SEQ ID No. 1 and a peptide having SEQ ID No. 17 inhibits anti-FVIII antibody production *in vivo*.

The figure shows end point anti-FVIII antibody titres of mice treated with a combination of SEQ ID 1 and SEQ ID 17 (or PBS control) and immunised with 8 weekly FVIII immunisations

Figure 7a: results at day28

Figure 7b: results at day56

FIGURE 8: A combination of a peptide having SEQ ID No. 1 and a peptide having SEQ ID No. 17 prevents neutralising anti-FVIII antibody production *in vivo* upon FVIII exposure

The figure shows the levels of neutralising anti-FVIII antibodies in mice treated with a combination of SEQ ID 1 and SEQ ID 17 (or PBS control) prior to and during 8 weekly FVIII immunisations. At day56 there is a significant reduction of neutralising antibodies in peptide treated mice.

Figure 9: A combination of a peptide having SEQ ID No. 1 and a peptide having SEQ ID No. 17 inhibits T cell responses to FVIII.

The figure shows recall responses from HLA-DR2 transgenic mice treated with a combination of SEQ ID 1 and, SEQ ID 17 or control (PBS) and primed with DNIMV and PRCLT. T cell stimulation indices are shown across a range of FVIII concentrations.

FIGURE 10: A combination of a peptide having SEQ ID No. 1 and a peptide having SEQ ID No. 17 suppresses neutralising anti-FVIII antibody generation in a therapeutic animal model with an ongoing FVIII immune response

The figure shows the levels of neutralising anti-FVIII antibodies in mice treated with a combination of SEQ ID 1 and SEQ ID 17 or prostatic acid phosphatase (PAP) 133-152 control peptide (sequence as described in PCT/US2006/031961, SEQ ID No. 15) after a FVIII immune response and anti-FVIII antibody production was induced. Mice received peptide treatment three weeks after rFVIII immunisation and prior to a boost immunization with rFVIII. Two weeks after the FVIII boost

immunisation (week 7), there is a significant reduction of neutralizing anti-FVIII antibodies in peptide treated mice which is maintained during the course of the experiment for up to week 13.

5 DETAILED DESCRIPTION

PEPTIDE

The present invention relates to a peptide.

10

The term "peptide" is used in the normal sense to mean a series of residues, typically L-amino acids, connected one to the other typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids. The term includes modified peptides and synthetic peptide analogues.

15

The peptide of the present invention may be made using chemical methods (Peptide Chemistry, A practical Textbook. Mikos Bodansky, Springer-Verlag, Berlin.). For example, peptides can be synthesized by solid phase techniques (Roberge JY *et al* (1995) Science 269: 202-204), cleaved from the resin, and purified by preparative high performance liquid chromatography (e.g., Creighton (1983) Proteins Structures And Molecular Principles, WH Freeman and Co, New York NY). Automated synthesis may be achieved, for example, using the ABI 431 A Peptide Synthesizer (Perkin Elmer) in accordance with the instructions provided by the manufacturer.

25

The peptide may alternatively be made by recombinant means, or by cleavage of a peptide from factor VIII followed by modification of one or both ends. The composition of a peptide may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure).

30

For practical purposes, there are various other characteristics which the peptide may show. For example, it is important that the peptide is sufficiently stable *in vivo* to be therapeutically useful. The half-life of the peptide *in vivo* may be at least 10 minutes, 30 minutes, 4 hours, or 24 hours.

35

The peptide may also demonstrate good bioavailability *in vivo*. The peptide may maintain a conformation *in vivo* which enables it to bind to an MHC molecule at the cell surface without due hindrance.

5 APITOPES

In an adaptive immune response, T lymphocytes are capable of recognising internal epitopes of a protein antigen. Antigen presenting cells (APC) take up protein antigens and degrade them into short peptide fragments. A peptide may
10 bind to a major histocompatibility complex (MHC) class I or II molecule inside the cell and be carried to the cell surface. When presented at the cell surface in conjunction with an MHC molecule, the peptide may be recognised by a T cell (via the T cell receptor (TCR)), in which case the peptide is a T cell epitope.

15 An epitope is thus a peptide derivable from an antigen which is capable of binding to the peptide-binding groove of a MHC class I or II molecule and be recognised by a T cell.

The minimal epitope is the shortest fragment derivable from an epitope, which is
20 capable of binding to the peptide-binding groove of a MHC class I or II molecule and being recognised by a T cell. For a given immunogenic region, it is typically possible to generate a "nested set" of overlapping peptides which act as epitopes, all of which contain the minimal epitope but differ in their flanking regions.

25 By the same token, it is possible to identify the minimal epitope for a particular MHC molecule:T cell combination by measuring the response to truncated peptides. For example if a response is obtained to the peptide comprising residues 1-15 in the overlapping library, sets which are truncated at both ends (i.e. 1-14, 1-13, 1-12 etc. and 2-15, 3-15, 4-15 etc.) can be used to identify the minimal epitope.

30

The present inventors have previously determined that there is a link between the capacity of a peptide to bind to an MHC class I or II molecule and be presented to a T cell without further antigen processing, and the peptide's capacity to induce tolerance *in vivo* (WO 02/16410). If a peptide is too long to bind the peptide binding
35 groove of an MHC molecule without further processing (e.g. trimming), or binds in

an inappropriate conformation then it will not be tolerogenic *in vivo*. If, on the other hand, the peptide is of an appropriate size and conformation to bind directly to the MHC peptide binding groove and be presented to a T cell, then this peptide can be predicted to be useful for tolerance induction.

5

It is thus possible to investigate the tolerogenic capacity of a peptide by investigating whether it can bind to an MHC class I or II molecule and be presented to a T cell without further antigen processing *in vitro*.

10 The peptides of the present invention are apitopes (Antigen Processing-Independent epiTOPES) in that they are capable of binding to an MHC class II molecule and stimulating a response from factor VIII specific T cells without further antigen processing. Such apitopes can be predicted to cause tolerance to FVIII, following the rule-based method described in WO 02/16410.

15

A peptide of the present invention may be any length that is capable of binding to an MHC class I or II molecule without further processing. Typically, the peptide of the present invention is capable of binding MHC class II.

20 Peptides that bind to MHC class I molecules are typically 7 to 13, more usually 8 to 10 amino acids in length. The binding of the peptide is stabilised at its two ends by contacts between atoms in the main chain of the peptide and invariant sites in the peptide-binding groove of all MHC class I molecules. There are invariant sites at both ends of the groove which bind the amino and carboxy termini of the peptide.

25 Variations in peptide length are accommodated by a kinking in the peptide backbone, often at proline or glycine residues that allow the required flexibility.

Peptides which bind to MHC class II molecules are typically between 8 and 20 amino acids in length, more usually between 10 and 17 amino acids in length, and
30 can be longer (for example up to 40 amino acids). These peptides lie in an extended conformation along the MHC II peptide-binding groove which (unlike the MHC class I peptide-binding groove) is open at both ends. The peptide is held in place mainly by main-chain atom contacts with conserved residues that line the peptide-binding groove.

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PEPTIDE SEQUENCES

The first embodiment of the invention relates to a peptide comprising a FVIII-derived sequence. FVIII-derived sequences which were investigated in the Examples have the following sequences

SEQ ID No. 1: Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys

SEQ ID No. 2: Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu

SEQ ID No. 3: Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys

SEQ ID No. 4: Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu

SEQ ID No. 5: Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys

SEQ ID No. 6: Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu

SEQ ID No. 7: Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys

SEQ ID No. 8: Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu

SEQ ID No. 9: Lys-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys

SEQ ID No. 10: Lys-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu

SEQ ID No. 11: Lys-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys

5 SEQ ID No. 12: Lys-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu

SEQ ID No. 13: Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys

10

SEQ ID No. 14: Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu

SEQ ID No. 15: Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys

15

SEQ ID No. 16: Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu

20 SEQ ID No. 17: Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys

SEQ ID No. 18: Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Glu

25

SEQ ID No. 19: Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys

SEQ ID No. 20: Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu

30

SEQ ID No. 21: Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys

SEQ ID No. 22: Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Glu

5 SEQ ID No. 23: Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys

SEQ ID No. 24: Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu

10 SEQ ID No. 25: Lys-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys

SEQ ID No. 26: Lys-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Glu

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SEQ ID No. 27: Lys-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys

SEQ ID No. 28: Lys-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu

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SEQ ID No. 29: Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys

25 SEQ ID No. 30: Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Glu

SEQ ID No. 31: Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys.

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SEQ ID No. 32: Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu.

The term "has" or "having" is intended to mean that the peptide consists of the given amino acid sequence.

35

The present invention provides a peptide which comprises the sequence DNIMVTFRNQASRPY and has one of the following sequences: SEQ ID No. 17, 18, 19, 25, 26, 27, 29, 30 or 31.

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The peptide may have one of the following sequences: SEQ ID No. 17, 18, 25 or 26.

The peptide may have SEQ ID No. 17 or 18.

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The peptide may have SEQ ID No.17.

The present invention also provides a peptide which comprises the sequence PRCLTRYSSSFVNME and has one of the following sequences: SEQ ID No. 1, 2, 3, 5, 7, 9, 10, 11 or 13.

15

The peptide may have one of the following sequences: SEQ ID No. 1, 2, 9 or 10.

The peptide may have SEQ ID No. 1 or 2.

20

The peptide may have SEQ ID No.1.

FACTOR VIII

25 The sequences DNIMVTFRNQASRPY and PRCLTRYSSSFVNME which form the central portion of the peptides of the invention are both derivable from factor VIII.

Factor VIII participates in the intrinsic pathway of blood coagulation; factor VIII is a cofactor for factor IXa which, in the presence of Ca^{+2} and phospholipids, converts factor X to the activated form Xa.

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The factor VIII gene produces two alternatively spliced transcripts. Transcript variant 1 encodes a large glycoprotein, isoform a, which circulates in plasma and associates with von Willebrand factor in a noncovalent complex. This protein undergoes multiple cleavage events. Transcript variant 2 encodes a putative small

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protein, isoform b, which consists primarily of the phospholipid binding domain of factor VIIIc. This binding domain is essential for coagulant activity.

The complete 186,000 base-pair sequence of the human factor VIII gene was elucidated in the mid 1980s (Gitschier et al (1984) Nature 312 326-330). At the same time, DNA clones encoding the complete 2351 amino acid sequence were used to produce biologically active factor VIII in cultured mammalian cells (Wood *et al* (1984) Nature 312:330-337). The complete 2,351 amino acid sequence for human factor VIII is given in SEQ ID No. 33.

SOLUBILITY

The peptide of the first embodiment of the invention is a modified form of one of the following peptides:

DNIMVTFRNQASRPY
PRCLTRYYSFVNME

These peptides have already been shown to act as apitopes and be tolerogenic *in vivo* (See examples and International patent application No PCT/GB2008/003996).

It has since come to light that solubility is an important consideration in peptide-mediated tolerance induction.

The present inventors have found that solubility may be improved by incorporation of a Glycine spacer at both ends, followed by combinations of two additional amino acids which may be Lysine (K) and/or Glutamic acid (E) at both N and C termini.

The possible combination at a given terminus is therefore KK, KE, EK or EE.

The modified peptides of the present invention therefore have 6 additional amino acids (3 at each end) than the parent DNIVM and PRCLT peptides.

The peptides of the present invention have the general formula:

XXGDNIMVTFRNQASRPYGXX or
XXGPRCLTRYYSFVNMEGXX

wherein X is either Lysine or Glutamic acid.

The modified peptide may be more soluble than the parent (unmodified) peptide.

- 5 The modified peptide may have 2, 3, 4, or 5-fold greater solubility than the parent peptide. The peptide may be soluble at concentrations of up to 0.5 mg/ml, 1 mg/ml, or 5 mg/ml.

TOLERANCE

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T cell epitopes play a central role in the adaptive immune response to any antigen, whether self or foreign. The central role played by T cell epitopes in hypersensitivity diseases (which include allergy, autoimmune diseases and transplant rejection) has been demonstrated through the use of experimental models. It is possible to induce inflammatory or allergic diseases by injection of synthetic peptides (based on the structure of T cell epitopes) in combination with adjuvant.

15

By contrast, it has been shown to be possible to induce immunological tolerance towards particular antigens by administration of peptide epitopes in soluble form. Administration of soluble peptide antigens has been demonstrated as an effective means of inhibiting disease in experimental autoimmune encephalomyelitis (EAE – a model for multiple sclerosis (MS)) (Metzler and Wraith (1993) *Int. Immunol.* **5**:1159-1165; Liu and Wraith (1995) *Int. Immunol.* **7**:1255-1263; Anderton and Wraith (1998) *Eur. J. Immunol.* **28**:1251-1261); and experimental models of arthritis, diabetes, and uveoretinitis (reviewed in Anderton and Wraith (1998) as above). This has also been demonstrated as a means of treating an ongoing disease in EAE (Anderton and Wraith (1998) as above).

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25

Tolerance is the failure to respond to an antigen. Tolerance to self-antigens is an essential feature of the immune system, when this is lost, autoimmune disease can result. The adaptive immune system must maintain the capacity to respond to an enormous variety of infectious agents while avoiding autoimmune attack of the self-antigens contained within its own tissues. This is controlled to a large extent by the sensitivity of immature T lymphocytes to apoptotic cell death in the thymus

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(central tolerance). However, not all self-antigens are detected in the thymus, so death of self-reactive thymocytes remains incomplete. There are thus also mechanisms by which tolerance may be acquired by mature self-reactive T lymphocytes in the peripheral tissues (peripheral tolerance). A review of the mechanisms of central and peripheral tolerance is given in Anderton *et al* (1999) (*Immunological Reviews* **169**:123-137).

In haemophilia A, patients have a defect in the factor VIII gene. This means that factor VIII is not recognised as a "self" antigen by the immune system. When factor VIII is administered during coagulation factor replacement therapy, therefore, an alloimmune response is generated to the foreign protein, leading to the production of FVIII inhibitor antibodies.

The peptides of the present invention are capable of inducing tolerance to factor VIII such that when FVIII is administered prophylactically, it does not induce an immune response and FVIII inhibitors do not develop; and when it is administered therapeutically, ongoing FVIII inhibitor production is reduced or inhibited.

Acquired haemophilia is an autoimmune disease in which tolerance to factor VIII breaks down. In this case, peptides of the present invention may be administered to reinstate tolerance to this self-protein and curtail the pathogenic immune response.

Tolerance may result from or be characterised by the induction of anergy in at least a portion of CD4⁺ T cells. In order to activate a T cell, a peptide must associate with a "professional" APC capable of delivering two signals to T cells. The first signal (signal 1) is delivered by the MHC-peptide complex on the cell surface of the APC and is received by the T cell via the TCR. The second signal (signal 2) is delivered by costimulatory molecules on the surface of the APC, such as CD80 and CD86, and received by CD28 on the surface of the T cell. It is thought that when a T cell receives signal 1 in the absence of signal 2, it is not activated and, in fact, becomes anergic. Anergic T cells are refractory to subsequent antigenic challenge, and may be capable of suppressing other immune responses. Anergic T cells are thought to be involved in mediating T cell tolerance.

Without wishing to be bound by theory, the present inventors predict that peptides which require processing before they can be presented in conjunction with MHC molecules do not induce tolerance because they have to be handled by mature antigen presenting cells. Mature antigen presenting cells (such as macrophages, B cells and dendritic cells) are capable of antigen processing, but also of delivering both signals 1 and 2 to a T cell, leading to T cell activation. Apitopes, on the other hand, will be able to bind class II MHC on immature APC. Thus they will be presented to T cells without costimulation, leading to T cell anergy and tolerance.

Of course, apitopes are also capable of binding to MHC molecules at the cell surface of mature APC. However, the immune system contains a greater abundance of immature than mature APC (it has been suggested that less than 10% of dendritic cells are activated, Summers et al. (2001) Am. J. Pathol. **159**: 285-295). The default position to an apitope will therefore be anergy/tolerance, rather than activation.

The induction of tolerance to FVIII can be monitored *in vivo* by looking for a reduction in the level of:

- (i) FVIII inhibitory antibodies;
 - (ii) CD4+ T cells specific for FVIII
 - (iii) B cells capable of secreting FVIII inhibitory antibodies
- by techniques known in the art.

The induction of tolerance can therefore also be monitored by various techniques including:

- (a) the induction of anergy in CD4+ T cells (which can be detected by subsequent challenge with FVIII *in vitro*);
- (b) changes in the CD4+ T cell population, including
 - (i) reduction in proliferation;
 - (ii) down-regulation in the production of IL-2, IFN- γ and IL-4; and
 - (iii) increase in the production of IL-10.

As used herein, the term "tolerogenic" means capable of inducing tolerance.

COMPOSITION

The present invention also relates to a composition, such as a pharmaceutical composition comprising one or more peptide(s) according to the invention.

The composition may comprise a modified form of the peptide DNIMVTFRNQASRPY and a modified form of the peptide PRCLTRYSSFVNME.

The peptide may comprise a plurality of peptides, for example, two, three, four, five or six peptides.

The composition of the present invention may be for prophylactic or therapeutic use.

When administered for prophylactic use, the composition may reduce or prevent the generation of an immune response to FVIII. The level of immune response is less than would have been obtained in the patient had not been treated with the composition. The term "reduce" indicates that a partial reduction in immune response is observed, such as a 50%, 70%, 80% or 90% reduction in the response that would have been observed in the patient had not been treated with the composition (or in the response observed in an untreated patient over the same time-frame). The term "prevent" indicates that no appreciable immune response to FVIII is observed.

When administered for therapeutic use, the composition may suppress an already ongoing immune response to FVIII. The term "suppress" indicates a reduction in the level of an on-going immune response, compared to the level before peptide treatment, or the level which would have been observed at the same time point had the treatment not been given.

Treatment with the composition of the present invention may cause a reduction in levels of any or all of the following:

(i) FVIII inhibitory antibodies:

(ii) CD4+ T cells specific for FVIII

(iii) B cells secreting FVIII inhibitory antibodies.

Detection of all these factors can be carried out by techniques known in the art, such as ELISA, Flow cytometry, chromogenic clotting assay etc.

5

Treatment with the composition of the present invention may also or alternatively cause anergy in CD4+ T cells specific for FVIII. Anergy can be detected by for example subsequent challenge with FVIII *in vitro*.

10 It is important to bear in mind that not all immune responses to FVIII are pathogenic. Non-inhibitory anti-FVIII antibodies may be found in haemophilia patients without inhibitors (Moreau *et al* (2000) Blood 95:3435-41) and approximately 15% of healthy blood donors (Algiman *et al* (1992) 89:3795-9).

15 FVIII inhibitors may be detected by the Nijmegen modification of the clotting Bethesda assay, in which the ability of the patient's plasma to inactivate FVIII in normal plasma is tested. A Bethesda unit is defined as the amount of antibody that neutralizes 50% of plasma FVIII activity, and titres of 0.6BU or greater suggest the presence of antibody.

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Inhibitors are generally classified as low titre if the level is <5 BU and high titre if $\geq$ 5 BU.

25 The level of circulating FVIII inhibitory antibodies may be reduced to 90%, 75%, 50%, 20%, 10% 5% of the level of antibodies which would have been observed had the patient not received treatment.

The level of circulating FVIII inhibitory antibodies may be reduced to 5, 4, 3, 2, 1 or 0.5 BU.

30

The peptides and composition of the invention may increase the amount or proportion of therapeutically administered FVIII which is available to aid clotting in a patient. This is due to the reduction in FVIII inhibitors which may effectively remove a proportion of FVIII from exerting its therapeutic function. The peptide or

composition of the invention may increase the amount of available FVIII by, for example, 10%, 25%, 50% 75% or 100%.

The peptides and composition of the invention may thus reduce the amount of FVIII which needs to be administered to aid clotting in a patient.

FORMULATION

The composition may be prepared as an injectable, either as liquid solution or suspension; solid form suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the peptides encapsulated in liposomes. The active ingredients may be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline (for example, phosphate-buffered saline), dextrose, glycerol, ethanol, or the like and combinations thereof.

In addition, if desired, the composition may contain minor amounts of auxiliary substances such as wetting or emulsifying agents and/or pH buffering agents. Buffering salts include phosphate, citrate, acetate, hydrochloric acid and/or sodium hydroxide may be used for pH adjustment. For stabilisation, disaccharides may be used such as sucrose or trehalose.

If the composition comprises a plurality of peptides, the relative ratio of the peptides may be approximately equal. Alternatively the relative ratios of each peptide may be altered, for example, to focus the tolerogenic response on a particular sub-set of autoreactive T-cells or if it is found that one peptide works better than the others in particular HLA types.

After formulation, the composition may be incorporated into a sterile container which is then sealed and stored at a low temperature, for example 4°C, or it may be freeze-dried.

Conveniently the composition is prepared as a lyophilized (freeze dried) powder. Lyophilisation permits long-term storage in a stabilised form. Lyophilisation

procedures are well known in the art, see for example <http://www.devicelink.com/ivdt/archive/97/01/006.html>. Bulking agents are commonly used prior to freeze-drying, such as mannitol, dextran or glycine.

- 5 The composition may be administered in a convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, sublingual, intranasal, intradermal or suppository routes or implanting (e.g. using slow release molecules).
- 10 The composition may advantageously be administered via intranasal, subcutaneous or intradermal routes.

The peptide and composition of the invention may be used to treat a human subject. The subject may have haemophilia A, in particular severe haemophilia A.

- 15 The subject may be genetically deficient in FVIII. The subject may have acquired haemophilia. The subject may have inhibitory anti-FVIII antibodies.

The subject may be undergoing or about to undergo coagulant replacement therapy with FVIII.

20

The subject may be undergoing or about to undergo therapy with by-passing agents [with or without coagulant replacement therapy with FVIII].

- The subject may be undergoing or about to undergo gene therapy with the FVIII gene.
- 25

- The subject may be an HLA-haplotype which is associated with a predisposition to develop inhibitory anti-FVIII alloantibodies or autoantibodies. The subject may express HLA-DR2. Methods for determining the HLA haplotype of an individual are
- 30 known in the art.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient.

35

In a preferred embodiment a “dose escalation” protocol may be followed, where a plurality of doses is given to the patient in ascending concentrations. Such an approach has been used, for example, for phospholipase A2 peptides in immunotherapeutic applications against bee venom allergy (Müller *et al* (1998) J. Allergy Clin Immunol. 101:747-754 and Akdis *et al* (1998) J. Clin. Invest. 102:98-106).

KITS

Conveniently, if the composition comprises a plurality of peptides, they may be administered together, in the form of a mixed composition or cocktail. However, there may be circumstances in which it is preferable to provide the peptides separately in the form of a kit, for simultaneous, separate, sequential or combined administration.

The kit may also comprise mixing and/or administration means (for example a vapouriser for intranasal administration; or a syringe and needle for subcutaneous/intradermal dosing). The kit may also comprise instructions for use.

The pharmaceutical composition or kit of the invention may be used to treat and/or prevent a disease.

In particular, the composition/kit may be used to treat and/or prevent anti-FVIII inhibitor formation in haemophilia A or acquired haemophilia patients. The composition/kit may be used to treat haemophilia impaired by the presence of neutralising FVIII antibodies.

HAEMOPHILIA A

Haemophilia A (classic haemophilia), is caused by the deficiency of Factor VIII.

Haemophilia A has an estimated incidence of 1 in 10,000 males, while haemophilia B is estimated to occur in one in 40,000 males. Approximately 1 woman in 5,000 is a carrier for haemophilia A, and 1 in 20,000 is a carrier of haemophilia B.

Haemophilia is typically divided into three classes: severe, moderate and mild, based on the level of clotting factor in the blood. In severe haemophilia, there is less than 1 percent of normal clotting factor. The degree of severity tends to be consistent from generation to generation.

5

Contrary to popular belief, minor cuts and wounds do not usually present a threat to haemophiliacs. Rather, the greatest danger comes from spontaneous bleeding that may occur in joints and muscles. This is most prone to occur during years of rapid growth, typically between the ages of 5 and 15 years.

10

Repeated spontaneous bleeding in joints may cause arthritis, and adjacent muscles become weakened. Pressure on nerves caused by the accumulation of blood may result in pain, numbness, and temporary inability to move the affected area.

15

Haemophilia A is usually diagnosed with a blood test to determine the effectiveness of clotting and to investigate whether the levels of clotting factors are abnormal.

20

The development of purified clotting factors in the 1970s, isolated from donated blood, significantly improved the long-term outlook for haemophiliacs. Mild to moderate haemophiliacs can use treatment with FVIII on an *ad hoc* basis, whereas severe haemophiliacs may require regular, indefinite treatment.

25

Previously, patients were given factor VIII concentrates pooled from thousands of plasma donations. This leads to significant problems of contamination with viral pathogens, particularly the human immunodeficiency virus and the hepatitis viruses. Monoclonal antibody purification techniques, heat inactivation, and virucidal detergent treatments have rendered plasma-derived concentrates relatively safe.

30

Recombinant DNA technology has now provided a series of synthetic products, such as Recombinate™ and Kogenate™. Kogenate is made using baby hamster kidney cells expressing human factor VIII. The resulting factor is highly purified, eliminating any possibility of transmission of virus from plasma.

35

The peptide or composition of the present invention may be administered before and/or during factor VIII replacement therapy.

5 Haemophilia A is an ideal disease target for gene therapy since i) it is caused by a mutations in a single identified gene, ii) a slight increase in clotting factor levels *in vivo* can convert severe haemophilia into milder disease, and iii) current replacement therapies are considered suboptimal. Also, there is a wide range of safety if there is an "overshoot" of desired level of coagulation activity.

10 Unfortunately, to date the promise of gene therapy as a cure for haemophilia has not been realized, primarily because of difficulties in finding a gene delivery system which is sufficiently non-immunogenic to allow for long term expression of the clotting factor.

15 The peptides of the present invention is also suitable for tolerising a subject prior to gene therapy with factor VIII and/or managing FVIII inhibitor formation in a patient following gene therapy.

20 ACQUIRED HAEMOPHILIA

Acquired haemophilia is characterised by the presence of autoantibody inhibitors against FVIII in individuals with previously normal coagulation. It is a rare condition, with an estimated incidence of 1–3 per million population per year. The mortality
25 rate associated with acquired autoantibody inhibitors approaches 25% versus the substantially lower risk of death in those with alloantibodies.

Compared to alloantibody inhibitor patients, acquired haemophilia is characterized by: (1) a more severe bleeding pattern; (2) higher incidence in older population; (3)
30 occurrence in conjunction with identifiable underlying autoimmune diseases, lymphoproliferative or solid tumor malignancies, pregnancy, and use of certain antibiotics such as penicillin and sulfonamides in approximately 50% of cases; and (4) *in vitro* inhibitor activity that follow a type II pharmacokinetic pattern with incomplete neutralization of the targeted clotting factor activity by the autoantibody,

typically resulting in residual factor VIII levels ranging between 2%–18% in patient plasma.

The peptide or composition of the present invention may be administered to a patient with acquired haemophilia, or to a patient believed to be at risk of developing acquired haemophilia due to, for example:

- i) imminent treatment with, for example penicillin or a sulfonamide
- ii) progression of a tumour or other malignancy
- iii) imminent or early pregnancy.

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

EXAMPLES

Example 1 - Investigating the solubility of FVIII-derived peptides.

A total of 32 peptides were tested: 16 of which were based on the FVIII peptide PRCLTRYSSFVNME ("PRCLT"); and 16 of which were based on the FVIII peptide DNIMVTFRNQASRPY ("DNIMV"). The peptides are summarised in Tables 1 and 2 below.

Table 1 - PRCLT-derived peptides

Peptide No	Sequence	SEQ ID No.
1	Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys	1
2	Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu	2
3	Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys	3
4	Lys-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu	4

5	Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys	5
6	Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu	6
7	Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys	7
8	Glu-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu	8
9	Lys-Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys	9
10	Lys- Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu	10
11	Lys- Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys	11
12	Lys- Glu-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu	12
13	Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Lys	13
14	Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Lys-Glu	14
15	Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Lys	15
16	Glu-Lys-Gly-Pro-Arg-Cys-Leu-Thr-Arg-Tyr-Tyr-Ser-Ser-Phe-Val-Asn-Met-Glu-Gly-Glu-Glu	16

Table 2 - DNIMV-derived peptides

5

Peptide No	Sequence	SEQ ID No.
17	Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys	17
18	Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-	18

	Ser-Arg-Pro-Tyr-Gly-Lys-Glu	
19	Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys	19
20	Lys-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu	20
21	Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys	21
22	Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Glu	22
23	Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys	23
24	Glu-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu	24
25	Lys-Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys	25
26	Lys- Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Glu	26
27	Lys- Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys	27
28	Lys- Glu-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu	28
29	Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Lys	29
30	Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Lys-Glu	30
31	Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Lys	31
32	Glu-Lys-Gly-Asp-Asn-Ile-Met-Val-Thr-Phe-Arg-Asn-Gln-Ala-Ser-Arg-Pro-Tyr-Gly-Glu-Glu	32

A stock solution of each test peptide of 20mg/mL was prepared in DMSO. A stock solution of the control peptide, an MBP-derived peptide known as 4Y was also prepared in DMSO. Peptide 4Y has the sequence Ac-ASQYRPSQR. It is

structurally dissimilar to the test peptides but is known to be sufficiently soluble for application as a tolerising antigen.

Sequential dilutions of a 40µL volume of peptide solution were made by adding water in a 96-well plate to give concentrations of 10, 5, 2.5 and 1.25 mg/mL

The plates were left at room temperature for 1 hour to allow any precipitate to form and then centrifuged at 14,800rpm for 10 minutes to separate precipitated/colloidal peptide from truly dissolved solute.

A 2µL sample from the very top of each tube was removed for analysis. Absorbance was measured at a wavelength of 280nm and concentration (mg/mL) was calculated.

The results are shown in Figure 1. Some tagged peptides demonstrated a solubility equivalent to 4Y, even at high concentration. Peptides coded green were as soluble as control peptide 4Y, peptides coded red were poorly soluble, and one peptide coded amber had intermediate solubility.

Example 2 - Direct dissolution of tagged peptides in aqueous solvent at clinically relevant concentration

For each test peptide and the parent peptides (PRCLT and DNIMV) two tubes were prepared at 4mg/mL in either DMSO (control) or PBS.

Each tube was spun at full speed in a mini-centrifuge for 5 minutes. A 10µL sample was removed from the top of each solution and the molecular weight and coefficient of absorption was recorded for each peptide.

The absorbance of the supernatant was measured using NanoDrop and the concentration calculated. The actual concentration was compared to the expected value of 4mg/mL and the results are shown in Figure 2. In this Figure, greater disparity between the actual and expected concentration corresponds to lower relative solubility.

As shown in Figure 2, the tagged peptides SEQ ID No. 1, 2, 9, 10, 17, 18, 25 and 26 all had improved solubility in PBS compared to the parent peptides.

Example 3 - The tagged peptides act as apitopes

5 HLA-DR2 transgenic mice were primed with the parent peptides PRCLT or DNIMV. Spleen and lymph nodes were then harvested, and after CD4 purification, cells were restimulated with 1ug/ml human recombinant FVIII. After fusion with BW5147 cells, the resulting clones were expanded and 'screened' for specificity to
 10 DNIMV or PRCLT. This is performed by incubating hybridoma cells with 5×10^4 DR2-positive antigen presenting cells (MGAR cell line) and 10ug/ml PRCLT/DNIMV or media for 48 hours. The supernatants were then analysed for IL-2 production by ELISA.

15 Clones which produced IL-2 specifically when incubated with peptide were expanded and screened again with FVIII (at 1ug/ml).

Clones which produced IL-2 in response to both FVIII and peptide were then used to assess whether the modified DNIMV and PRCLT peptides are apitopes i.e.
 20 whether they are capable of binding to an MHC molecule and being presented to a T cell by both fixed and fresh APC.

5×10^4 hybridoma cells were incubated with 5×10^4 fresh or fixed MGAR cells, and 10ug/ml of peptide, 1ug/ml FVIII or media for 48 hours. Supernatants were then
 25 analysed for IL-2 using ELISA. The results are shown for PRCLT in Figure 3a and for DNIMV in Figure 3b.

All of the tagged peptides were capable of being presented by fixed antigen presenting cells, indicating that they are all apitopes like the parent peptides
 30 PRCLT and DNIMV.

Example 4: Ex vivo T cell tolerance

To determine if modified apitopes were capable of regulating T cell responses to
 35 parent peptides, male HLA-DR2 transgenic mice were treated with $3 \times 100 \mu\text{g}$

peptide (peptides SEQ ID No. 1, 2, 17 or 18) or PBS as a control, and then immunised with 100µg of each of the parent peptides (PRCLT and DNIMV) in complete Freund's Adjuvant. Ten days later, draining lymph nodes and spleens were collected and stimulated *in vitro* with either PRCLT or DNIMV. The results are shown in Figures 4 (PRCLT) and 5 (DNIMV). Treatment with modified peptides SEQ ID No. 1 or SEQ ID No. 2 reduces T cell responses to PRCLT and treatment with modified peptides SEQ ID No. 17 or SEQ ID No. 18 reduces T cell responses to DNIMV. Thus the modified peptides are able to reduce an immune response from mice immunised with the parent peptide.

Example 5: Ex vivo T cell tolerance

In order to determine if the effect of modified epitopes on the regulation of T cell responses to parental peptides extended to regulating responses to FVIII, female HLA-DR2 transgenic mice were treated with 3x100µg peptide (peptides SEQ ID No. 1 or SEQ ID No. 17), and then primed with 100µg of each of the parent peptides (PRCLT and DNIMV) in complete Freund's Adjuvant. Ten days later, draining lymph nodes were collected and stimulated *in vitro* with either PRCLT, DNIMV or recombinant FVIII. The results are shown in Figure 6. Peptide SEQ ID no 1 significantly ($p<0.01$) reduced the T cell response to the PRCLT peptide. Peptide SEQ ID no 17 significantly ($p<0.02$) reduced the T cell response to DNIMV.

Peptide SEQ ID No.1 regulated responses to PRCLT and peptide SEQ ID No.17 regulated responses to DNIMV. There was also some reduction, which was non-significant, in response to FVIII with individual peptides. To enhance efficacy of the peptides against FVIII immune responses a combination of modified DNIMV and modified PRCLT were used in further experiments.

Example 6: Antibody generation prevention with a dose escalation of a combination of PRCLT and DNIMV modified peptides

Male HLA-DR2 transgenic mice were treated with the combination of peptide SEQ ID No. 1 and peptide SEQ ID No. 17 following a dose escalation protocol. Six

injections of the combination were given at the following dose: 0.1; 1.0; 10 and 3x100 µg (for each peptide in the combination).

Animals were then primed weekly with 1µg recombinant human FVIII and a concurrent treatment (3 days after rFVIII immunisation) with a combination of peptides SEQ ID No.1/SEQ ID No.17 (100 µg each) and the generation of anti-FVIII antibodies analysed from blood samples taken after 4 and 8 FVIII immunisations (day 28, Figure 7a; day 56, Figure 7b, respectively). Sera were titrated and analysed by direct ELISA. The antibody titre for unimmunised mice was negative in all cases, whereas anti-FVIII antibody was considered present in the sera of treated mice where binding ratio (using negative sera to calculate the ratio) was greater than 1.9. Results are shown in Figure 7 a and b for day 28 and day 56 of the experiment, respectively.

In addition, neutralizing FVIII antibodies were measured on day 56. FVIII neutralizing antibodies were determined using a chromogenic method. Briefly, samples were mixed with 1 IU/ml FVIII and coagulation was initiated by addition of FIX, FX, Trombin, CaCl₂ and phospholipids. After incubation the amount of generated FXa was determined by addition of the chromogenic substrate S-2760 and the OD signal was measured. The OD signal is proportional to the FVIII activity in the samples and is compared to samples containing a known amount of FVIII and no neutralizing antibodies. The % remaining activity of the test sample was calculated compared to the reference samples and expressed in Bethesda like units and shown in Figure 8. Mice treated with peptides SEQ ID1 and SEQ ID17 had a significantly lower level of neutralizing anti-FVIII antibodies than mice treated with PBS.

Example 7: Ex vivo T cell tolerance using a dose escalation of a combination of PRCLT and DNIMV modified peptides

Male/female DR2 mice were treated with the combination of peptides SEQ ID No1/SEQ ID No17 following a dose escalation protocol as described in Example 6.

Animals were primed with 100µg PRCLT and 100µg DNIMV in CFA. Ten days later draining lymph nodes cells and splenocytes were isolated and restimulated *in vitro* with recombinant human FVIII.

5 T cell responses were assessed by proliferation assays (Figure 9). A significant reduction of T cell responses to FVIII were seen across all concentrations of FVIII tested when the mice were treated with the combination of peptides SEQ ID No1 and SEQ ID No17.

10 **Example 8: Suppression of neutralising antibody generation with a dose escalation of a combination of PRCLT and DNIMV modified peptides in a therapeutic animal model**

HLA-DR2 transgenic mice were primed with 3µg rFVIII in CFA and three weeks
15 after the immunisation treated with the combination of peptide SEQ ID No. 1 and peptide SEQ ID No. 17 following a dose escalation protocol. Six injections of the combination were given at the following dose: 0.1; 1.0; 10 and 3x100 µg (for each peptide in the combination). Control animals were treated with a non-related FVIII DR2-binding prostatic acid phosphatase (PAP) 133-152 peptide (sequence as
20 described in patent PCT/US2006/031961, SEQ ID No. 15) using the same dose-escalation protocol. All animals received a boost immunization with 3µg rhFVIII in IFA five weeks after rFVIII immunisation and 4 days after the last round of peptide treatment. Generation of neutralising antibodies was analysed from plasma samples taken 3 weeks after the first FVIII immunisation and prior to any peptide
25 treatment, at week 5 (prior to the FVIII boost immunisation and after the peptide treatment) and during a follow-up period at week 7, 9 and 13. Neutralising FVIII antibody levels in the plasma samples were determined as described in Example 6.

30 Results in figure 10 show that treatment with peptides SEQ ID No. 1 and SEQ ID No. 17 three weeks after immunisation with FVIII (Week 3) suppress the formation of neutralizing anti-FVIII antibodies significantly after the boost immunization (Week 5) compared to treatment with control peptide.

5 All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular immunology or related fields are intended to be within the scope of the following claims.

10 The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

20 Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

SEQ ID No.33

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 61 tsvvykktlf veftdhlfni akprppwmgl lgptiqaevy dtvvtlknm ashpvsilhav
 121 gvsywkaseg aeyddqtsqr ekeddkvfpg gshtyvwqvl kengpmasdp lcltysylsh
 5 181 vdlvkdlnsg ligallvcre gslakektqt lhkfillfav fdegkswhse tknslnmqdrd
 241 aasarawpkm htvngyvnrs lpgligchrk svywhvigmg tpevhsifl eghtflvrnh
 301 rqaaleispi tfltaqtllm dlqqlflfch issqhhdgme ayvkvdscpe epqlrmknne
 361 eaedydddlf dsemdivrfd ddnspsfqi rsvakkhpkf wvhyiaaeae dwdyaplvla
 421 pddrsyksqy lnnqpqrigr kykkvrfmay tdetfktrea iqhesgilgp llygevgdtl
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 601 nrswylteni qrlfnpagv qledpefqas nimhsingyv fdlqlsvcl hevaywyils
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 1261 gayapvlqdf rslndstnr kkhtahfskk geeenlegl nqtqiveky acttrispt
 1321 sqqnfvtrs kralkqrlp leetelekri ivddtstqws knmkhltpst ltqidyneke
 25 1381 kgaitqspls dcltrshsp qanrslpia kvssfpsirp yltrvlfqd nsshlpaaasy
 1441 rkkdsgvqes shflqgakkn nlsailtle mtgdqrevgs lgsatnsvt ykkventvlp
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 30 1681 idyddtisve mkkedfdiyd edenqsprsf qkktrhyfia averlwdygm sssphvlrnr
 1741 aqsgsvpqfk kvvfqeftdg sftqplyrge lnehlgllgp yiraevedni mvtrfnqasr
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 1921 pcniqmedpt fkenyrfhai ngymdtlpg lvmaqdqir wyllsmgsne nihsihfsg
 35 1981 vftvrkkeey kmalynlypg vftvempls kagiwrvecl ighlhagms tflvysnkc

2041 qtplgmasgh irdfqitasg qygqwapkla rhysgsina wstkepfswi kvdlapmii
2101 hgiktqgarq kfsslyisqf iimysldgkk wqtyrgnstg tlmvffgnvd ssgikhnifn
2161 ppiaryirl hpthysirst lrmewmgcdl nscsmplgme skaisdaqit assyftnmfa
2221 twspskarlh lqgrsnawrp qvnnpkewlq vdfqktmkvt gvtqgvksl ltsmyvkefl
5 2281 isssqdghqw tlffqngkvk vfqgnqdsft pvnslppl ltrylrihpq swvhqialrm
2341 evlgceaql y

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A peptide comprising the FVIII-derived sequence DNIMVTFRNQASRPY which peptide (a) has the formula

XXGDNIMVTFRNQASRPYGXX

wherein X is either Lysine or Glutamic acid; and

(b) has one of the following sequences:

KKGDNIMVTFRNQASRPYGKK (SEQ ID No. 17)

KKGDNIMVTFRNQASRPYGKE (SEQ ID No. 18)

KKGDNIMVTFRNQASRPYGKEK (SEQ ID No. 19)

KEGDNIMVTFRNQASRPYGKK (SEQ ID No. 25)

KEGDNIMVTFRNQASRPYGKE (SEQ ID No. 26)

KEGDNIMVTFRNQASRPYGKEK (SEQ ID No. 27)

EKGDNIMVTFRNQASRPYGKK (SEQ ID No. 29)

EKGDNIMVTFRNQASRPYGKE (SEQ ID No. 30) and

EKGDNIMVTFRNQASRPYGKEK (SEQ ID No. 31).

2. A peptide comprising the FVIII-derived sequence PRCLTRYSSFVNME which peptide (a) has the formula

XXGPRCLTRYSSFVNMEGXX

wherein X is either Lysine or Glutamic acid; and

(b) has one of the following sequences:

KKGPRCLTRYSSSFVNMEGKK (SEQ ID No. 1)

KKGPRCLTRYSSSFVNMEGKE (SEQ ID No. 2)

KKGPRCLTRYSSSFVNMEGEK (SEQ ID No. 3)

EEGPRCLTRYSSSFVNMEGKK (SEQ ID No. 5)

EEGPRCLTRYSSSFVNMEGEK (SEQ ID No. 7)

KEGPRCLTRYSSSFVNMEGKK (SEQ ID No. 9)

KEGPRCLTRYSSSFVNMEGKE (SEQ ID No. 10)

KEGPRCLTRYSSSFVNMEGEK (SEQ ID No. 11) and

EKGPRCLTRYSSSFVNMEGKK (SEQ ID No. 13).

3. A composition comprising a plurality of peptides, including one or more peptide(s) according to claim 1 or 2.

4. A composition according to claim 3, which comprises at least one peptide according to claim 1 and at least one peptide according to claim 2.

5. A composition according to claim 4, which comprises a peptide having SEQ ID No. 1 and a peptide having SEQ ID No. 17

6. A peptide according to claim 1 or 2, or a composition according to any of claims 3 to 5, for use in suppressing or preventing the production of factor VIII inhibitor antibodies *in vivo*.

7. The use of a peptide according to claim 1 or 2, or a composition according to any of claims 3 to 5, in the manufacture of a medicament to suppress or prevent the production of factor VIII inhibitor antibodies *in vivo*.

8. A method for suppressing or preventing the production of factor VIII inhibitor antibodies in a subject, which comprises the step of administration of a peptide according to claim 1 or 2, or a composition according to any of claims 3 to 5, to the subject.

9. A method for treating haemophilia in a subject which comprises the step of administration of a peptide according to claim 1 or 2, or a composition according to any of claims 3 to 5, to the subject.

10. A method according to claim 8 or 9, wherein the subject has haemophilia A, and is undergoing, or is about to undergo, factor VIII replacement therapy and/or FVIII by-passing therapy.

11. A method according to claim 8 or 9, wherein the subject has, or is at risk from contracting, acquired haemophilia.

12. A method according to any of claims 8 to 11, wherein the subject is HLA-DR2.

Figure 1

	PRCLT	DNIMV	
KK-KK	1	17	Further Investigation
KK-KE	2	18	
KK-EK	3	19	
KK-EE	4	20	
EE-KK	5	21	Further Investigation
EE-KE	6	22	
EE-EK	7	23	
EE-EE	8	24	
KE-KK	9	25	Further Investigation
KE-KE	10	26	
KE-EK	11	27	
KE-EE	12	28	
EK-KK	13	29	
EK-KE	14	30	
EK-EK	15	31	
EK-EE	16	32	

Figure 2

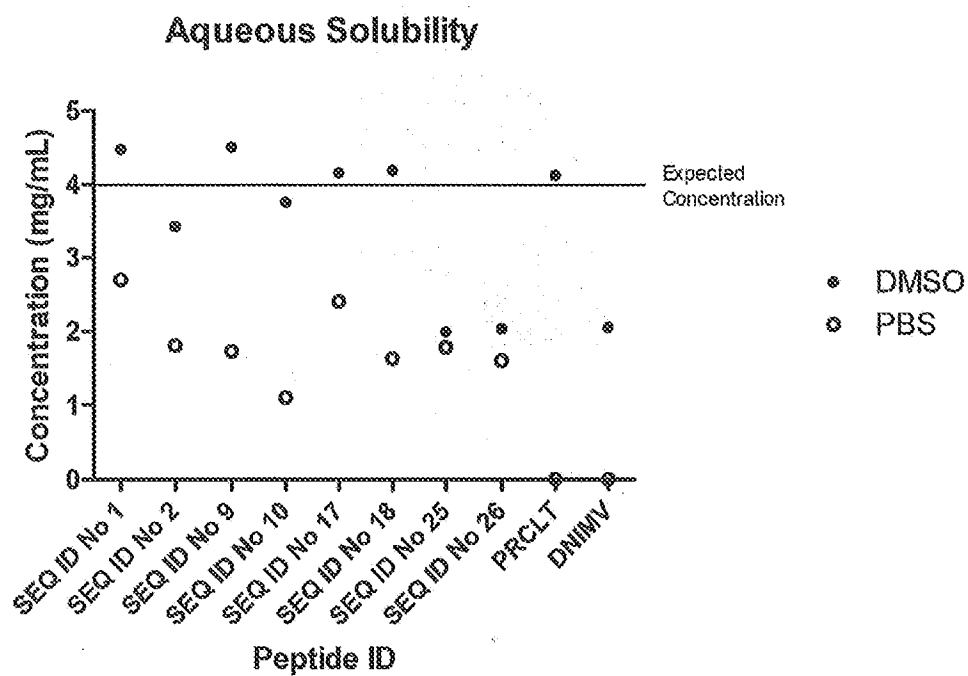


Figure 3a

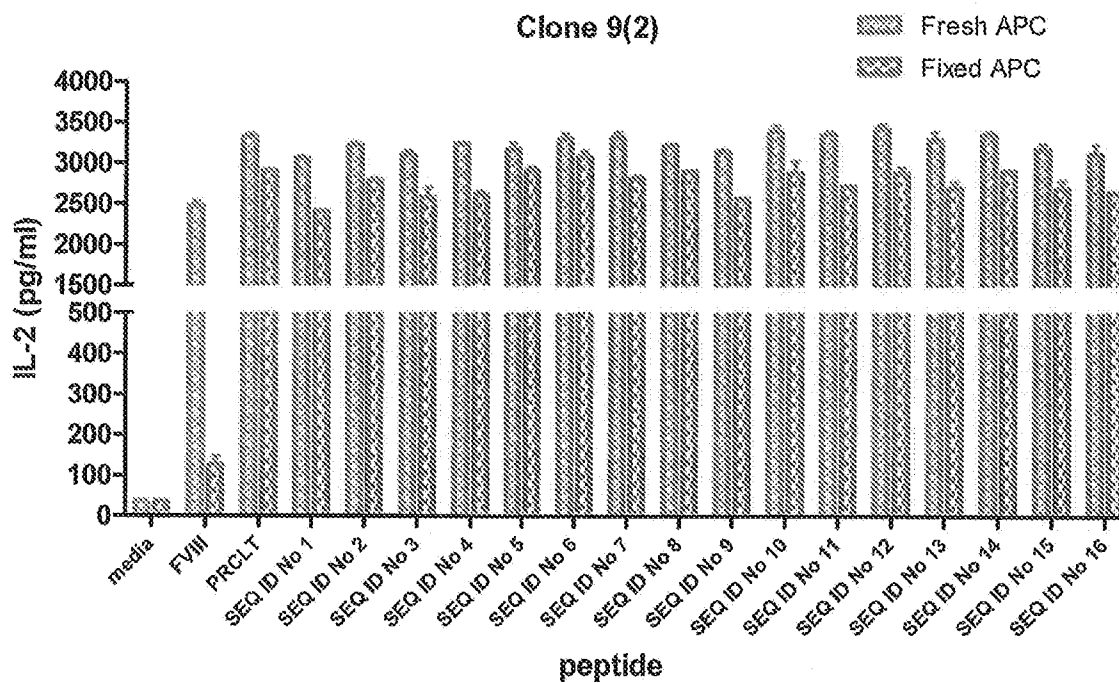


Figure 3b

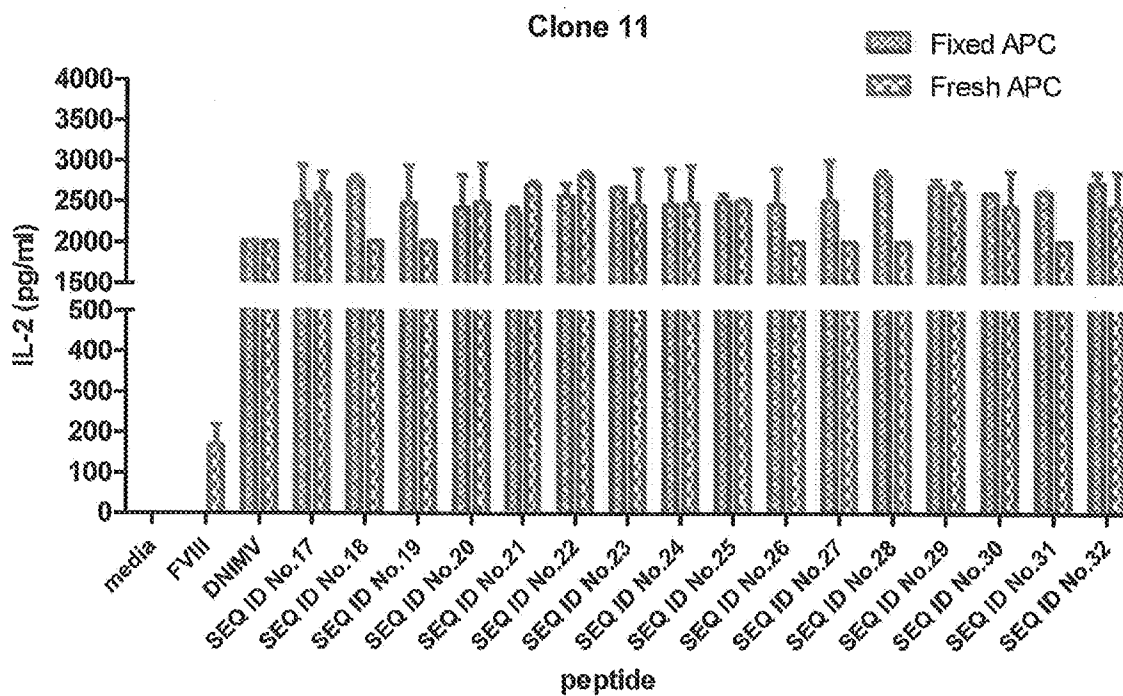


Figure 4

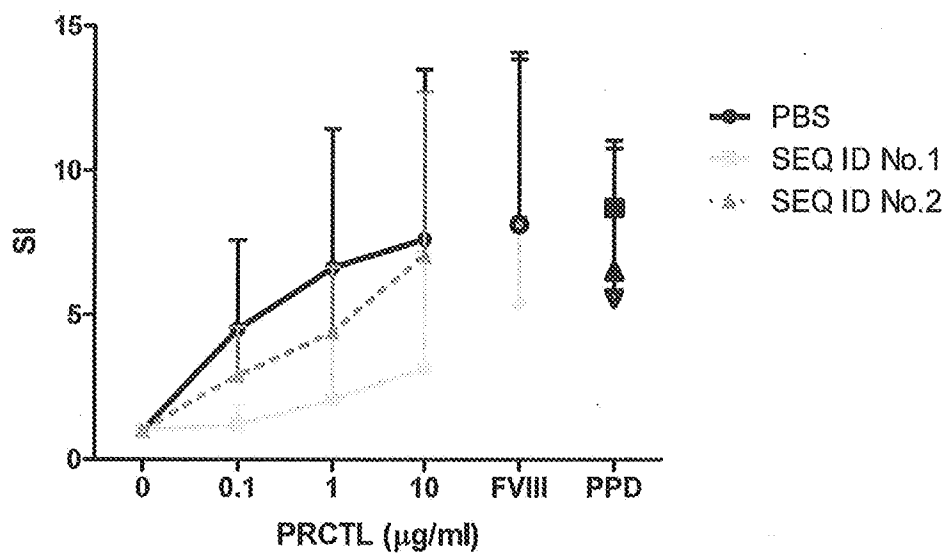


Figure 5

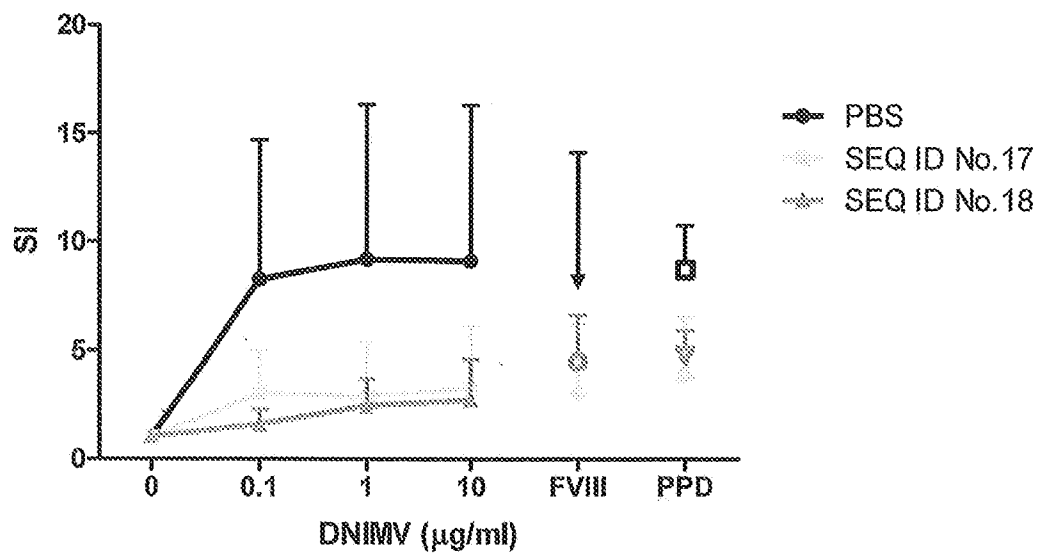


Figure 6

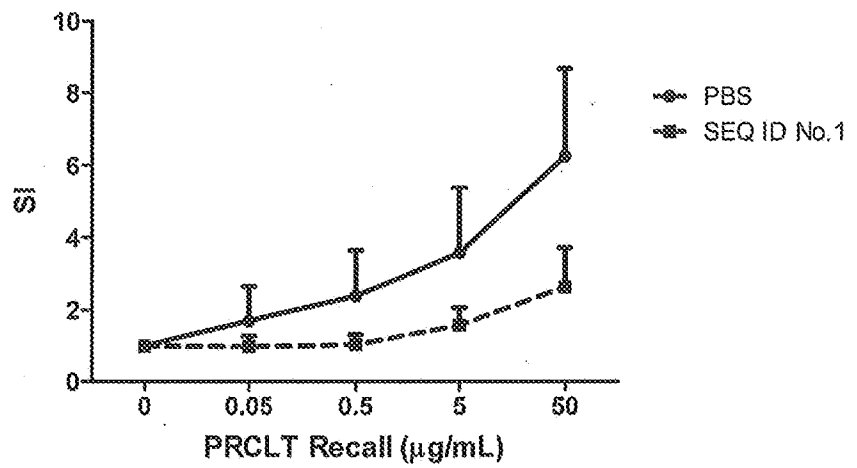
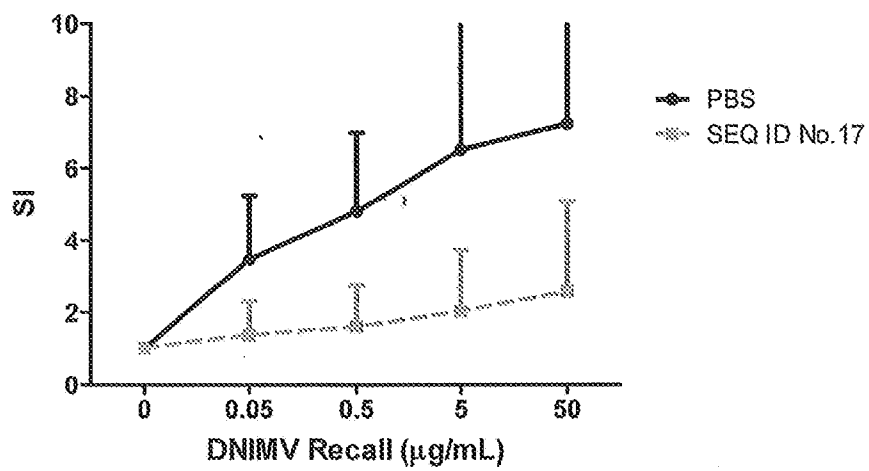
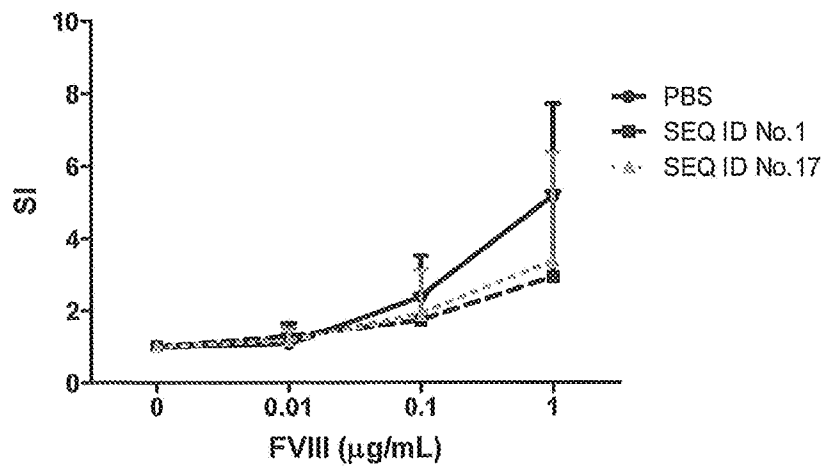


Figure 7

Figure 7a: anti-FVIII antibody titres at day28:

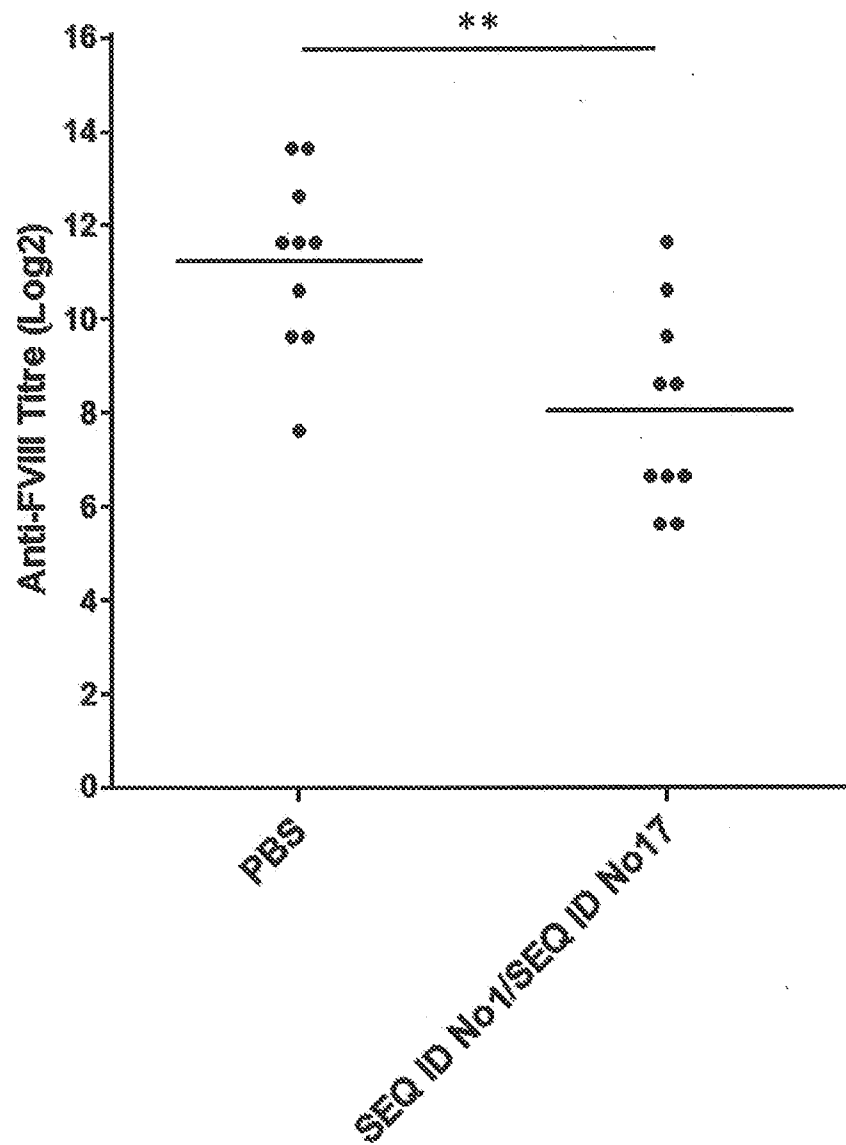


Figure 7b: anti-FVIII antibody titres at day56:

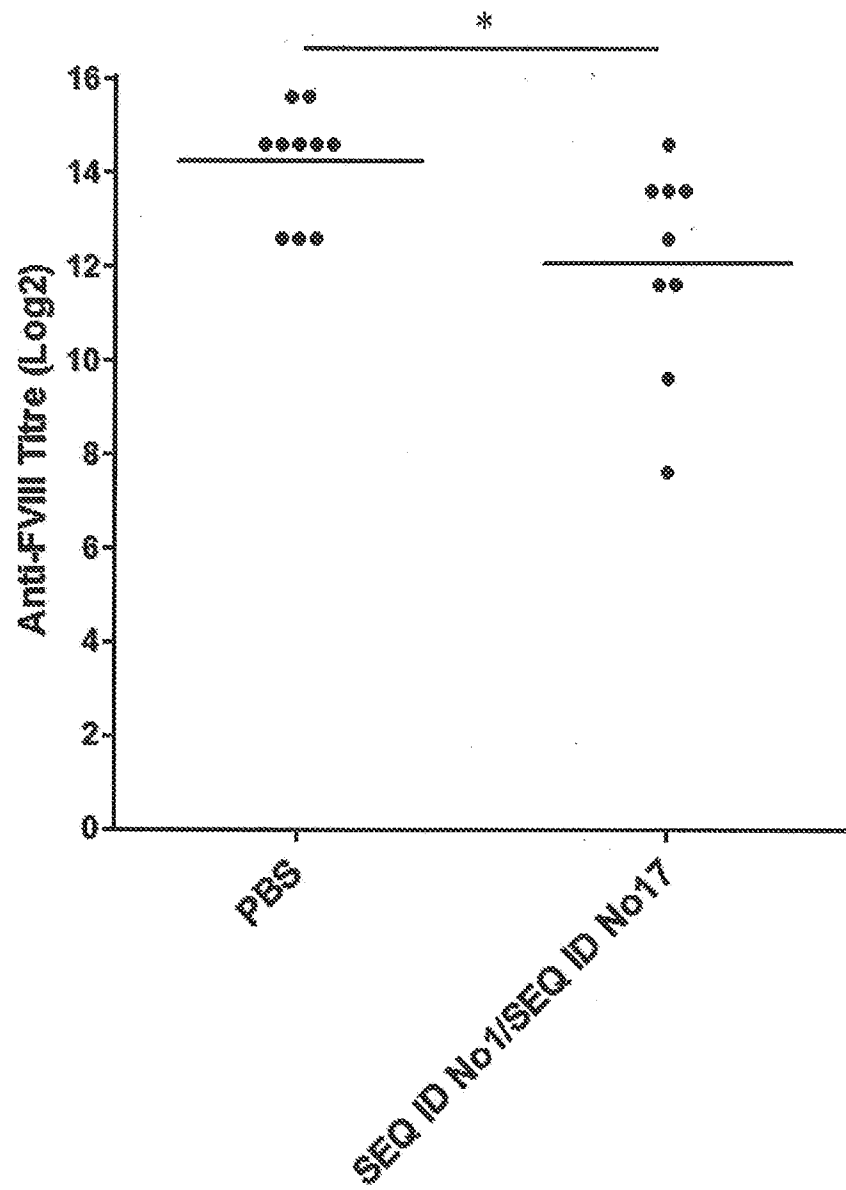


Figure 8

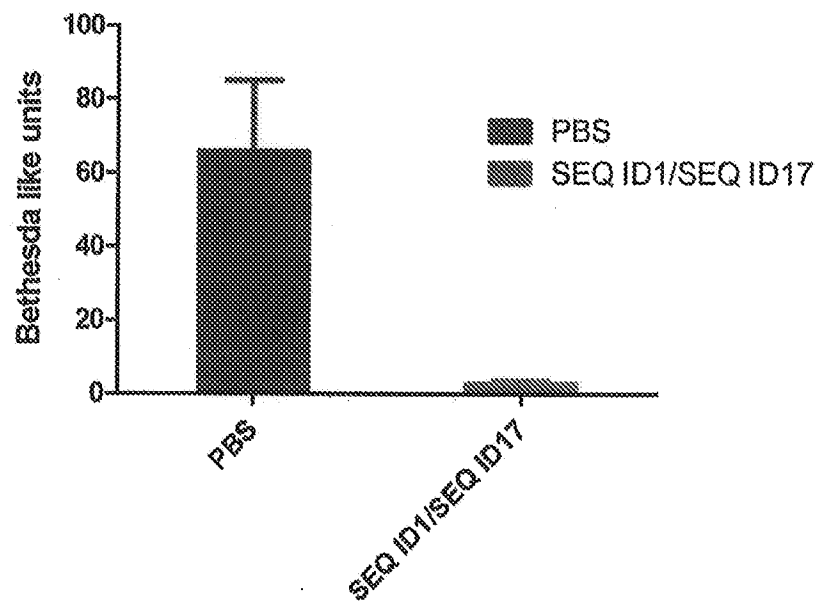
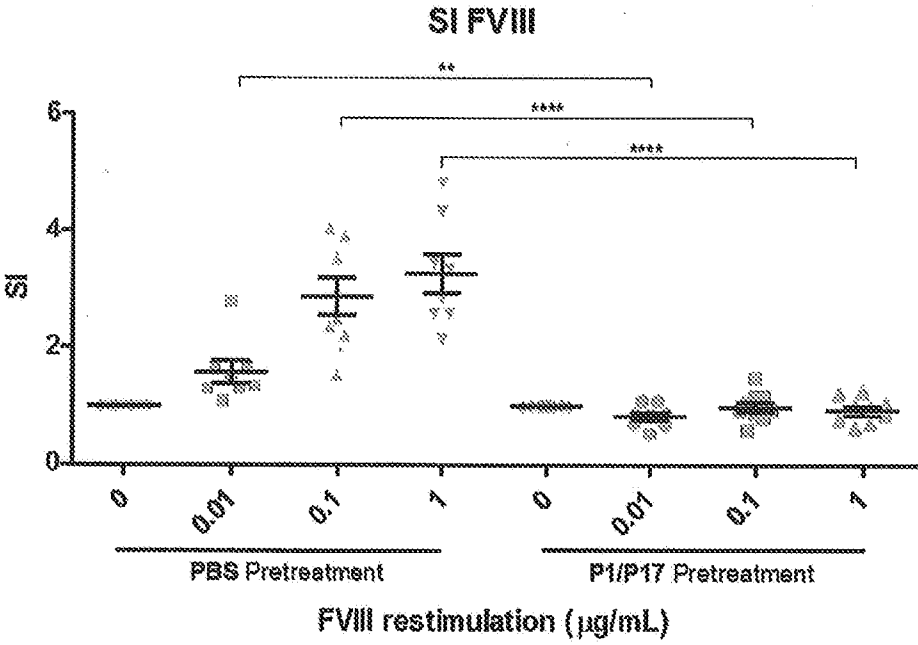


Figure 9



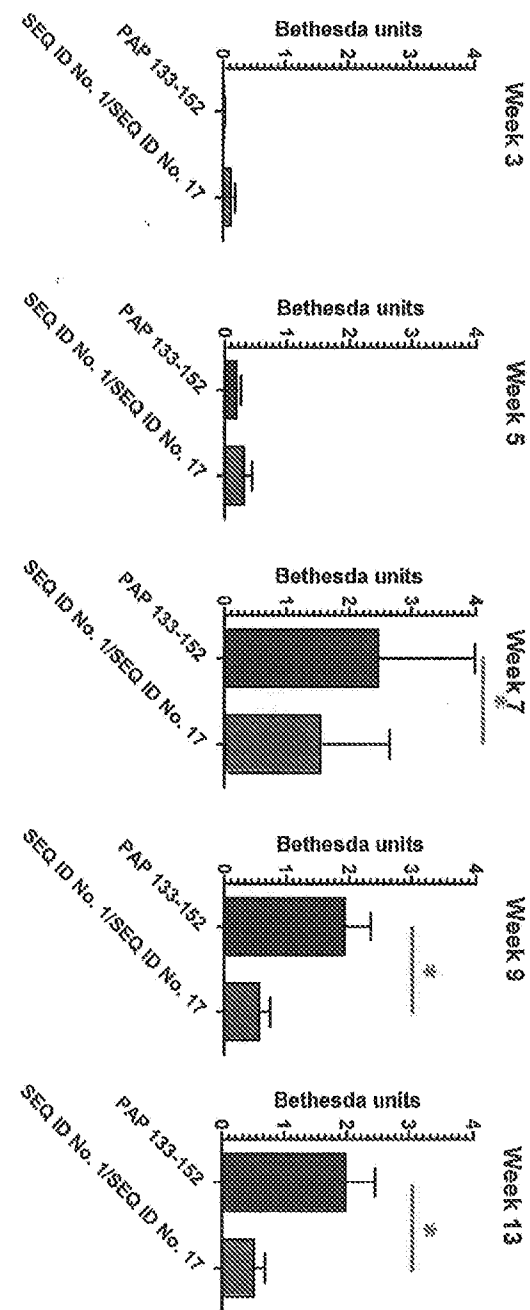


Figure 10

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<170> PatentIn version 3.5

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<213> Artificial Sequence

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<223> Modified FVIII-derived peptide sequence

<400> 22

Glu Glu Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

Pro Tyr Gly Lys Glu
20

<210> 23

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified FVIII-derived peptide sequence

<400> 23

Glu Glu Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

Pro Tyr Gly Glu Lys
20

<210> 24

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified FVIII-derived peptide sequence

<400> 24

Glu Glu Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

P046272PCT sequence listing.txt

Pro Tyr Gly Glu Glu
20

<210> 25
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified FVIII-derived peptide sequence

<400> 25

Lys	Glu	Gly	Asp	Asn	Ile	Met	Val	Thr	Phe	Arg	Asn	Gln	Ala	Ser	Arg
1				5					10					15	

Pro Tyr Gly Lys Lys
20

<210> 26
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified FVIII-derived peptide sequence

<400> 26

Lys	Glu	Gly	Asp	Asn	Ile	Met	Val	Thr	Phe	Arg	Asn	Gln	Ala	Ser	Arg
1				5					10					15	

Pro Tyr Gly Lys Glu
20

<210> 27
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> Modified FVIII-derived peptide sequence

<400> 27

Lys	Glu	Gly	Asp	Asn	Ile	Met	Val	Thr	Phe	Arg	Asn	Gln	Ala	Ser	Arg
1				5					10					15	

Pro Tyr Gly Glu Lys
20

<210> 28
<211> 21
<212> PRT
<213> Artificial Sequence

P046272PCT sequence listing.txt

<220>

<223> Modified FVIII-derived peptide sequence

<400> 28

Lys Glu Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

Pro Tyr Gly Glu Glu
20

<210> 29

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified FVIII-derived peptide sequence

<400> 29

Glu Lys Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

Pro Tyr Gly Lys Lys
20

<210> 30

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified FVIII-derived peptide sequence

<400> 30

Glu Lys Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

Pro Tyr Gly Lys Glu
20

<210> 31

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified FVIII-derived peptide sequence

<400> 31

Glu Lys Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

Pro Tyr Gly Glu Lys

20

<210> 32
 <211> 21
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modified FVIII-derived peptide sequence

<400> 32

Glu Lys Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
 1 5 10 15

Pro Tyr Gly Glu Glu
 20

<210> 33
 <211> 2351
 <212> PRT
 <213> Homo sapiens

<400> 33

Met Gln Ile Glu Leu Ser Thr Cys Phe Phe Leu Cys Leu Leu Arg Phe
 1 5 10 15

Cys Phe Ser Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser
 20 25 30

Trp Asp Tyr Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg
 35 40 45

Phe Pro Pro Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val
 50 55 60

Tyr Lys Lys Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile
 65 70 75 80

Ala Lys Pro Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln
 85 90 95

Ala Glu Val Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser
 100 105 110

His Pro Val Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser
 115 120 125

Glu Gly Ala Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp
 130 135 140

P046272PCT sequence listing.txt

Asp	Lys	Val	Phe	Pro	Gly	Gly	Ser	His	Thr	Tyr	Val	Trp	Gln	Val	Leu	145	150	155	160
Lys	Glu	Asn	Gly	Pro	Met	Ala	Ser	Asp	Pro	Leu	Cys	Leu	Thr	Tyr	Ser	165	170	175	
Tyr	Leu	Ser	His	Val	Asp	Leu	Val	Lys	Asp	Leu	Asn	Ser	Gly	Leu	Ile	180	185	190	
Gly	Ala	Leu	Leu	Val	Cys	Arg	Glu	Gly	Ser	Leu	Ala	Lys	Glu	Lys	Thr	195	200	205	
Gln	Thr	Leu	His	Lys	Phe	Ile	Leu	Leu	Phe	Ala	Val	Phe	Asp	Glu	Gly	210	215	220	
Lys	Ser	Trp	His	Ser	Glu	Thr	Lys	Asn	Ser	Leu	Met	Gln	Asp	Arg	Asp	225	230	235	240
Ala	Ala	Ser	Ala	Arg	Ala	Trp	Pro	Lys	Met	His	Thr	Val	Asn	Gly	Tyr	245	250	255	
Val	Asn	Arg	Ser	Leu	Pro	Gly	Leu	Ile	Gly	Cys	His	Arg	Lys	Ser	Val	260	265	270	
Tyr	Trp	His	Val	Ile	Gly	Met	Gly	Thr	Thr	Pro	Glu	Val	His	Ser	Ile	275	280	285	
Phe	Leu	Glu	Gly	His	Thr	Phe	Leu	Val	Arg	Asn	His	Arg	Gln	Ala	Ser	290	295	300	
Leu	Glu	Ile	Ser	Pro	Ile	Thr	Phe	Leu	Thr	Ala	Gln	Thr	Leu	Leu	Met	305	310	315	320
Asp	Leu	Gly	Gln	Phe	Leu	Leu	Phe	Cys	His	Ile	Ser	Ser	His	Gln	His	325	330	335	
Asp	Gly	Met	Glu	Ala	Tyr	Val	Lys	Val	Asp	Ser	Cys	Pro	Glu	Glu	Pro	340	345	350	
Gln	Leu	Arg	Met	Lys	Asn	Asn	Glu	Glu	Ala	Glu	Asp	Tyr	Asp	Asp	Asp	355	360	365	
Leu	Thr	Asp	Ser	Glu	Met	Asp	Val	Val	Arg	Phe	Asp	Asp	Asp	Asn	Ser	370	375	380	
Pro	Ser	Phe	Ile	Gln	Ile	Arg	Ser	Val	Ala	Lys	Lys	His	Pro	Lys	Thr	385	390	395	400

P046272PCT sequence listing.txt

Trp Val His Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro
405 410 415

Leu Val Leu Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn
420 425 430

Asn Gly Pro Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met
435 440 445

Ala Tyr Thr Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu
450 455 460

Ser Gly Ile Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu
465 470 475 480

Leu Ile Ile Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro
485 490 495

His Gly Ile Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys
500 505 510

Gly Val Lys His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe
515 520 525

Lys Tyr Lys Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp
530 535 540

Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg
545 550 555 560

Asp Leu Ala Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu
565 570 575

Ser Val Asp Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val
580 585 590

Ile Leu Phe Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu
595 600 605

Asn Ile Gln Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp
610 615 620

Pro Glu Phe Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val
625 630 635 640

Phe Asp Ser Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp
645 650 655

P046272PCT sequence listing.txt

Tyr Ile Leu Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe
 660 665 670
 Ser Gly Tyr Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr
 675 680 685
 Leu Phe Pro Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro
 690 695 700
 Gly Leu Trp Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly
 705 710 715 720
 Met Thr Ala Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp
 725 730 735
 Tyr Tyr Glu Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys
 740 745 750
 Asn Asn Ala Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Pro
 755 760 765
 Ser Thr Arg Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp
 770 775 780
 Ile Glu Lys Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys
 785 790 795 800
 Ile Gln Asn Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser
 805 810 815
 Pro Thr Pro His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr
 820 825 830
 Glu Thr Phe Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn
 835 840 845
 Ser Leu Ser Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly
 850 855 860
 Asp Met Val Phe Thr Pro Glu Ser Gly Leu Gln Leu Arg Leu Asn Glu
 865 870 875 880
 Lys Leu Gly Thr Thr Ala Ala Thr Glu Leu Lys Lys Leu Asp Phe Lys
 885 890 895
 Val Ser Ser Thr Ser Asn Asn Leu Ile Ser Thr Ile Pro Ser Asp Asn
 900 905 910

P046272PCT sequence listing.txt

Leu Ala Ala Gly Thr Asp Asn Thr Ser Ser Leu Gly Pro Pro Ser Met
915 920 925

Pro Val His Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys
930 935 940

Ser Ser Pro Leu Thr Glu Ser Gly Gly Pro Leu Ser Leu Ser Glu Glu
945 950 955 960

Asn Asn Asp Ser Lys Leu Leu Glu Ser Gly Leu Met Asn Ser Gln Glu
965 970 975

Ser Ser Trp Gly Lys Asn Val Ser Ser Thr Glu Ser Gly Arg Leu Phe
980 985 990

Lys Gly Lys Arg Ala His Gly Pro Ala Leu Leu Thr Lys Asp Asn Ala
995 1000 1005

Leu Phe Lys Val Ser Ile Ser Leu Leu Lys Thr Asn Lys Thr Ser
1010 1015 1020

Asn Asn Ser Ala Thr Asn Arg Lys Thr His Ile Asp Gly Pro Ser
1025 1030 1035

Leu Leu Ile Glu Asn Ser Pro Ser Val Trp Gln Asn Ile Leu Glu
1040 1045 1050

Ser Asp Thr Glu Phe Lys Lys Val Thr Pro Leu Ile His Asp Arg
1055 1060 1065

Met Leu Met Asp Lys Asn Ala Thr Ala Leu Arg Leu Asn His Met
1070 1075 1080

Ser Asn Lys Thr Thr Ser Ser Lys Asn Met Glu Met Val Gln Gln
1085 1090 1095

Lys Lys Glu Gly Pro Ile Pro Pro Asp Ala Gln Asn Pro Asp Met
1100 1105 1110

Ser Phe Phe Lys Met Leu Phe Leu Pro Glu Ser Ala Arg Trp Ile
1115 1120 1125

Gln Arg Thr His Gly Lys Asn Ser Leu Asn Ser Gly Gln Gly Pro
1130 1135 1140

Ser Pro Lys Gln Leu Val Ser Leu Gly Pro Glu Lys Ser Val Glu
1145 1150 1155

P046272PCT sequence listing.txt

Gly Gln	Asn Phe Leu Ser	Glu	Lys Asn Lys Val	Val	Val Gly Lys
1160		1165		1170	
Gly Glu	Phe Thr Lys Asp	Val	Gly Leu Lys Glu	Met	Val Phe Pro
1175		1180		1185	
Ser Ser	Arg Asn Leu Phe	Leu	Thr Asn Leu Asp	Asn	Leu His Glu
1190		1195		1200	
Asn Asn	Thr His Asn Gln	Glu	Lys Lys Ile Gln	Glu	Glu Ile Glu
1205		1210		1215	
Lys Lys	Glu Thr Leu Ile	Gln	Glu Asn Val Val	Leu	Pro Gln Ile
1220		1225		1230	
His Thr	Val Thr Gly Thr	Lys	Asn Phe Met Lys	Asn	Leu Phe Leu
1235		1240		1245	
Leu Ser	Thr Arg Gln Asn	Val	Glu Gly Ser Tyr	Asp	Gly Ala Tyr
1250		1255		1260	
Ala Pro	Val Leu Gln Asp	Phe	Arg Ser Leu Asn	Asp	Ser Thr Asn
1265		1270		1275	
Arg Thr	Lys Lys His Thr	Ala	His Phe Ser Lys	Lys	Gly Glu Glu
1280		1285		1290	
Glu Asn	Leu Glu Gly Leu	Gly	Asn Gln Thr Lys	Gln	Ile Val Glu
1295		1300		1305	
Lys Tyr	Ala Cys Thr Thr	Arg	Ile Ser Pro Asn	Thr	Ser Gln Gln
1310		1315		1320	
Asn Phe	Val Thr Gln Arg	Ser	Lys Arg Ala Leu	Lys	Gln Phe Arg
1325		1330		1335	
Leu Pro	Leu Glu Glu Thr	Glu	Leu Glu Lys Arg	Ile	Ile Val Asp
1340		1345		1350	
Asp Thr	Ser Thr Gln Trp	Ser	Lys Asn Met Lys	His	Leu Thr Pro
1355		1360		1365	
Ser Thr	Leu Thr Gln Ile	Asp	Tyr Asn Glu Lys	Glu	Lys Gly Ala
1370		1375		1380	
Ile Thr	Gln Ser Pro Leu	Ser	Asp Cys Leu Thr	Arg	Ser His Ser
1385		1390		1395	

P046272PCT sequence listing.txt

Ile	Pro	Gln	Ala	Asn	Arg	Ser	Pro	Leu	Pro	Ile	Ala	Lys	Val	Ser
1400						1405						1410		
Ser	Phe	Pro	Ser	Ile	Arg	Pro	Ile	Tyr	Leu	Thr	Arg	Val	Leu	Phe
1415						1420					1425			
Gln	Asp	Asn	Ser	Ser	His	Leu	Pro	Ala	Ala	Ser	Tyr	Arg	Lys	Lys
1430						1435					1440			
Asp	Ser	Gly	Val	Gln	Glu	Ser	Ser	His	Phe	Leu	Gln	Gly	Ala	Lys
1445						1450					1455			
Lys	Asn	Asn	Leu	Ser	Leu	Ala	Ile	Leu	Thr	Leu	Glu	Met	Thr	Gly
1460						1465					1470			
Asp	Gln	Arg	Glu	Val	Gly	Ser	Leu	Gly	Thr	Ser	Ala	Thr	Asn	Ser
1475						1480					1485			
Val	Thr	Tyr	Lys	Lys	Val	Glu	Asn	Thr	Val	Leu	Pro	Lys	Pro	Asp
1490						1495					1500			
Leu	Pro	Lys	Thr	Ser	Gly	Lys	Val	Glu	Leu	Leu	Pro	Lys	Val	His
1505						1510					1515			
Ile	Tyr	Gln	Lys	Asp	Leu	Phe	Pro	Thr	Glu	Thr	Ser	Asn	Gly	Ser
1520						1525					1530			
Pro	Gly	His	Leu	Asp	Leu	Val	Glu	Gly	Ser	Leu	Leu	Gln	Gly	Thr
1535						1540					1545			
Glu	Gly	Ala	Ile	Lys	Trp	Asn	Glu	Ala	Asn	Arg	Pro	Gly	Lys	Val
1550						1555					1560			
Pro	Phe	Leu	Arg	Val	Ala	Thr	Glu	Ser	Ser	Ala	Lys	Thr	Pro	Ser
1565						1570					1575			
Lys	Leu	Leu	Asp	Pro	Leu	Ala	Trp	Asp	Asn	His	Tyr	Gly	Thr	Gln
1580						1585					1590			
Ile	Pro	Lys	Glu	Glu	Trp	Lys	Ser	Gln	Glu	Lys	Ser	Pro	Glu	Lys
1595						1600					1605			
Thr	Ala	Phe	Lys	Lys	Lys	Asp	Thr	Ile	Leu	Ser	Leu	Asn	Ala	Cys
1610						1615					1620			
Glu	Ser	Asn	His	Ala	Ile	Ala	Ala	Ile	Asn	Glu	Gly	Gln	Asn	Lys
1625						1630					1635			

P046272PCT sequence listing.txt

Pro Glu	Ile Glu Val Thr	Trp	Ala Lys Gln Gly Arg	Thr Glu Arg
1640		1645		1650
Leu Cys	Ser Gln Asn Pro	Pro	Val Leu Lys Arg His	Gln Arg Glu
1655		1660		1665
Ile Thr	Arg Thr Thr Leu Gln	Ser Asp Gln Glu Glu	Ile Asp Tyr	
1670		1675		1680
Asp Asp	Thr Ile Ser Val Glu	Met Lys Lys Glu Asp	Phe Asp Ile	
1685		1690		1695
Tyr Asp	Glu Asp Glu Asn Gln	Ser Pro Arg Ser Phe	Gln Lys Lys	
1700		1705		1710
Thr Arg	His Tyr Phe Ile Ala	Ala Val Glu Arg Leu	Trp Asp Tyr	
1715		1720		1725
Gly Met	Ser Ser Ser Pro His	Val Leu Arg Asn Arg	Ala Gln Ser	
1730		1735		1740
Gly Ser	Val Pro Gln Phe Lys	Lys Val Val Phe Gln	Glu Phe Thr	
1745		1750		1755
Asp Gly	Ser Phe Thr Gln Pro	Leu Tyr Arg Gly Glu	Leu Asn Glu	
1760		1765		1770
His Leu	Gly Leu Leu Gly Pro	Tyr Ile Arg Ala Glu	Val Glu Asp	
1775		1780		1785
Asn Ile	Met Val Thr Phe Arg	Asn Gln Ala Ser Arg	Pro Tyr Ser	
1790		1795		1800
Phe Tyr	Ser Ser Leu Ile Ser	Tyr Glu Glu Asp Gln	Arg Gln Gly	
1805		1810		1815
Ala Glu	Pro Arg Lys Asn Phe	Val Lys Pro Asn Glu	Thr Lys Thr	
1820		1825		1830
Tyr Phe	Trp Lys Val Gln His	His Met Ala Pro Thr	Lys Asp Glu	
1835		1840		1845
Phe Asp	Cys Lys Ala Trp Ala	Tyr Phe Ser Asp Val	Asp Leu Glu	
1850		1855		1860
Lys Asp	Val His Ser Gly Leu	Ile Gly Pro Leu Leu	Val Cys His	
1865		1870		1875

P046272PCT sequence listing.txt

Thr Asn	Thr Leu Asn Pro	Ala His Gly Arg Gln Val	Thr Val Gln
1880		1885	1890
Glu Phe	Ala Leu Phe Phe Thr	Ile Phe Asp Glu Thr	Lys Ser Trp
1895		1900	1905
Tyr Phe	Thr Glu Asn Met Glu	Arg Asn Cys Arg Ala	Pro Cys Asn
1910		1915	1920
Ile Gln	Met Glu Asp Pro Thr	Phe Lys Glu Asn Tyr	Arg Phe His
1925		1930	1935
Ala Ile	Asn Gly Tyr Ile Met	Asp Thr Leu Pro Gly	Leu Val Met
1940		1945	1950
Ala Gln	Asp Gln Arg Ile Arg	Trp Tyr Leu Leu Ser	Met Gly Ser
1955		1960	1965
Asn Glu	Asn Ile His Ser Ile	His Phe Ser Gly His	Val Phe Thr
1970		1975	1980
Val Arg	Lys Lys Glu Glu Tyr	Lys Met Ala Leu Tyr	Asn Leu Tyr
1985		1990	1995
Pro Gly	Val Phe Glu Thr Val	Glu Met Leu Pro Ser	Lys Ala Gly
2000		2005	2010
Ile Trp	Arg Val Glu Cys Leu	Ile Gly Glu His Leu	His Ala Gly
2015		2020	2025
Met Ser	Thr Leu Phe Leu Val	Tyr Ser Asn Lys Cys	Gln Thr Pro
2030		2035	2040
Leu Gly	Met Ala Ser Gly His	Ile Arg Asp Phe Gln	Ile Thr Ala
2045		2050	2055
Ser Gly	Gln Tyr Gly Gln Trp	Ala Pro Lys Leu Ala	Arg Leu His
2060		2065	2070
Tyr Ser	Gly Ser Ile Asn Ala	Trp Ser Thr Lys Glu	Pro Phe Ser
2075		2080	2085
Trp Ile	Lys Val Asp Leu Leu	Ala Pro Met Ile Ile	His Gly Ile
2090		2095	2100
Lys Thr	Gln Gly Ala Arg Gln	Lys Phe Ser Ser Leu	Tyr Ile Ser
2105		2110	2115

P046272PCT sequence listing.txt

Gln Phe	Ile Ile Met Tyr	Ser	Leu Asp Gly Lys Lys	Trp Gln Thr
2120		2125		2130
Tyr Arg	Gly Asn Ser Thr	Gly	Thr Leu Met Val	Phe Phe Gly Asn
2135		2140		2145
Val Asp	Ser Ser Gly Ile	Lys	His Asn Ile Phe	Asn Pro Pro Ile
2150		2155		2160
Ile Ala	Arg Tyr Ile Arg	Leu	His Pro Thr His	Tyr Ser Ile Arg
2165		2170		2175
Ser Thr	Leu Arg Met Glu	Trp	Met Gly Cys Asp	Leu Asn Ser Cys
2180		2185		2190
Ser Met	Pro Leu Gly Met	Glu	Ser Lys Ala Ile	Ser Asp Ala Gln
2195		2200		2205
Ile Thr	Ala Ser Ser Tyr	Phe	Thr Asn Met Phe	Ala Thr Trp Ser
2210		2215		2220
Pro Ser	Lys Ala Arg Leu	His	Leu Gln Gly Arg	Ser Asn Ala Trp
2225		2230		2235
Arg Pro	Gln Val Asn Asn	Pro	Lys Glu Trp Leu	Gln Val Asp Phe
2240		2245		2250
Gln Lys	Thr Met Lys Val	Thr	Gly Val Thr Thr	Gln Gly Val Lys
2255		2260		2265
Ser Leu	Leu Thr Ser Met	Tyr	Val Lys Glu Phe	Leu Ile Ser Ser
2270		2275		2280
Ser Gln	Asp Gly His Gln	Trp	Thr Leu Phe Phe	Gln Asn Gly Lys
2285		2290		2295
Val Lys	Val Phe Gln Gly	Asn	Gln Asp Ser Phe	Thr Pro Val Val
2300		2305		2310
Asn Ser	Leu Asp Pro Pro	Leu	Leu Thr Arg Tyr	Leu Arg Ile His
2315		2320		2325
Pro Gln	Ser Trp Val His	Gln	Ile Ala Leu Arg	Met Glu Val Leu
2330		2335		2340
Gly Cys	Glu Ala Gln Asp	Leu	Tyr	
2345		2350		

P046272PCT sequence listing.txt

<210> 34
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 34

Asp	Asn	Ile	Met	Val	Thr	Phe	Arg	Asn	Gln	Ala	Ser	Arg	Pro	Tyr
1				5				10						15

<210> 35
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 35

Pro	Arg	Cys	Leu	Thr	Arg	Tyr	Tyr	Ser	Ser	Phe	Val	Asn	Met	Glu
1				5				10						15

<210> 36
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide

<400> 36

Pro	Arg	Cys	Leu	Thr
1				5

<210> 37
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Peptide

<400> 37

Asp	Asn	Ile	Met	Val
1				5

<210> 38
 <211> 21
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Modified FVIII-derived peptide general formula

<220>
 <221> MISC_FEATURE
 <222> (1)..(2)
 <223> Xaa may be Lys or Glu

P046272PCT sequence listing.txt

<220>

<221> MISC FEATURE

<222> (20)..(21)

<223> Xaa may be Lys or Glu

<400> 38

Xaa Xaa Gly Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg
1 5 10 15

Pro Tyr Gly Xaa Xaa
20

<210> 39

<211> 21

<212> PRT

<213> Artificial Sequence

<220>

<223> Modified FVIII-derived peptide general formula

<220>

<221> MISC FEATURE

<222> (1)..(2)

<223> Xaa may be Lys or Glu

<220>

<221> MISC FEATURE

<222> (20)..(21)

<223> Xaa may be Lys or Glu

<400> 39

Xaa Xaa Gly Pro Arg Cys Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn
1 5 10 15

Met Glu Gly Xaa Xaa
20

<210> 40

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Control peptide 4Y

<220>

<221> MISC FEATURE

<222> (1)..(1)

<223> N-terminal acetylation

<400> 40

Ala Ser Gln Tyr Arg Pro Ser Gln Arg
1 5

