

MODIFIED COLLOIDAL PARTICLES FOR USE IN THE TREATMENT OF HAEMOPHILIA A

5 The present invention relates to the use of colloidal particles for the treatment of haemophilia A in patients with antibody inhibitors or with a history of developing antibody inhibitors to Factor VIII (FVIII). The colloidal particle comprises a first and second amphipathic lipid wherein the second amphipathic lipid may be a phospholipid moiety derivatised with a biocompatible hydrophilic polymer such as polyethylene glycol (PEG). The invention also relates to methods, kits and dosage forms comprising colloidal particles.

10 The coagulation cascade that leads to blood coagulation is a multi-step process, involving many different proteins and factors, coupled with regulatory feedback mechanisms that enable the safe formation of a blood clot in the event of an injury. In disorders of the blood such as haemophilia, one or more of these factors may be defective or absent, leading to defective or poor quality clots.

15 Blood clotting Factor VIII ('FVIII') is a blood protein that is involved in the amplification phase in the secondary haemostasis process of the coagulation cascade. The cascade's ultimate aim is to generate fibrin to form a clot to stop bleeding. On injury the platelets become everted – 'activated' – exposing a reaction surface to which the other factors bind where they undertake their specific action. The 'initiation' or 'extrinsic' phase, which leads to the activation of factor FX to FXa can be wholly
20 mediated on this reaction surface by TF-FVIIa, after the conversion of FVII by tissue factor (TF), leading to the production of thrombin from prothrombin and the conversion of fibrinogen to fibrin, which becomes cross-linked through the action of FXIII to form a clot. Following this initiation, a good quality clot normally forms quickly, due to the further 'amplification' or 'intrinsic' phase, which includes FVIII, among other factors: during this phase FVIII becomes activated (FVIIIa) and bound to the
25 platelet's reaction surface and binds FIXa to form the intrinsic 'tenase' complex which then converts FX to FXa. The formation of FIXa from FIX is also catalysed by the TF-FVIIa complex (except in the case of Haemophilia B, where FIX is missing). The thrombin produced during the initiation phase helps to catalyse the amplification phase, meaning that that the presence of TF, FVII, FIX and FVIII are required for optimal clot formation.

30 In the absence of FVIII, such as in congenital haemophilia A, the reaction proceeds much more slowly since it must rely on the initiation phase alone to catalyse the conversion of FX to FXa. It is this pathway (the 'extrinsic tenase' pathway) that is reinforced through the extraneous administration of FVIIa in haemophilia A patients who lack sufficient FVIII and in particular have developed
35 antibodies ('inhibitors') against either extraneous FVIII (in the case of congenital haemophiliacs who have been treated with FVIII) or to their own FVIII (in the case of acquired haemophilia – aHA).

The use of replacement FVIII from donated blood plasma or from recombinant sources to treat either congenital haemophilia A (cHA) or acquired haemophilia A (aHA) is very well established in clinical
40 practice. However, the efficacy of such products is limited due to the occurrence of inhibitory

antibodies ('inhibitors') being present in some patients that reduces the effectiveness of exogenously delivered Factor VIII. Inhibitors arise in 25-35% of cHA sufferers ('inhibitor patients') as a response to the exogenous FVIII they receive; in aHA the disease arises as the patient develops inhibitors to their own FVIII due to autoimmunity.

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The presence of inhibitors reduces the effectiveness of treating inhibitor patients with exogenous FVIII as the protein is bound by, neutralised and rapidly cleared from the circulation by the inhibitor antibody, making prophylactic treatment with replacement human FVIII very difficult or usually impossible. A sub-optimal quantity of FVIII means that even if a clot can be formed at all, it is formed slowly or once formed is of a poor quality that is rapidly broken down.

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The wild-type FVIII molecule comprises 2332 amino acids, organised in 6 domains: A1-A2-B-A3-C1-C2. Together the A1-A2-B domains comprise the 'Heavy Chain' (HC) and the A3-C1-C2 domains comprise the 'Light Chain' (LC) and these chains are linked non-covalently. In life, FVIII will normally associate with von Willebrand's Factor (VWF) in circulation. VWF facilitates the transport of FVIII and protects it from premature inactivation and clearance (Mannucci, P.M. et al. (2014) Novel investigations on the protective role of the FVIII/VWF complex in inhibitor development. *Haemophilia*. 20(suppl. 6), 2-16.). The association with VWF is also associated with reduced immunogenicity, efficacy in the presence of inhibitors and utility in immunotolerance treatment. The use of commercial concentrates of plasma-derived FVIII (pdFVIII) containing VWF were shown to be less immunogenic than recombinant concentrates of FVIII (rFVIII) in the SIPPET trial (Peyvandi, F. et al. (2016) A randomized trial of Factor VIII and neutralizing antibodies in Hemophilia A, *N Engl J Med*. 374, 2054-64). The reduced immunogenicity of the pdFVIII concentrates is associated with the VWF chaperone, which it is thought either masks critical epitopes on the FVIII molecule, and/or prevents its endocytosis by dendritic cells (Astermark, J. (2015) FVIII inhibitors: pathogenesis and avoidance. *Blood*. 125(13), 2045-51). Recombinant FVIII molecules also have the additional complication that most of these molecules are not humanised but are produced in non-human cell lines, resulting in the presence of non-human glycan epitopes potentially enhancing their immunogenicity.

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The development of inhibitors to these epitopes in FVIII remains the most frequent side effect of haemophilia treatment (Van den Berg et al. (2020). ITI treatment is not a first-choice in children with hemophilia A and low-responding inhibitors: Evidence from a PedNet study. *Coagulation and Fibrinolysis*. 120, 1166-1172). The risk is high in previously untreated patients (PUPs) with an overall incidence of up to 40% (ibid.), inactivating FVIII activity and requiring alternative and costly measures to protect these patients. In some patients, the use of immune tolerance induction therapy, a long and costly technique, can eradicate inhibitors but the technique does not work for all patients, preventing them from using replacement FVIII therapy. The risk of inhibitor development is highest during the first 20 exposure days (EDs) to replacement FVIII and persists up to 75 EDs (Liesner, R.J. et al. (2021) Simoctocog alfa (Nuwiq™) in previously untreated patients with severe haemophilia A:

final results of the NuProtect study. Thromb Haemost. online). Patients must be monitored for the development of inhibitors over this period.

There are currently two principal methods of dealing with the development of inhibitors: the use of inhibitor tolerance induction (ITI) or the use of bypass therapies. ITI utilises large, repeated doses of FVIII over several months to induce tolerance in the immune system to FVIII, with the aim of enabling the patient to return to a normal dosing regimen. The therapy is not always effective and the repeated high-dose injections of FVIII over several months are both unpleasant for the patient and extremely costly, representing a significant healthcare system cost. Accordingly, an 'inhibitor patient' is an individual who in their medical history has developed inhibitory antibodies ('inhibitor') in response to the application of a therapeutic blood factor (in the case of congenital haemophilia) or who has developed inhibitors to their own FVIII (in the case of acquired haemophilia). They may currently present with or without inhibitors but in the latter case will probably not have been tolerised via ITI and remain capable generating inhibitors if re-presented with FVIII.

Bypass therapies avoid the problem of inhibitors by 'bypassing' the amplification phase entirely. The most common treatments are FVIIa alone (e.g. NovoSeven) or in combination as a prothrombin complex concentrate (PCC) (e.g. FEIBA, an activated PCC), which help to support the initiation phase of the clotting cascade, as described above. These specialised treatments are also expensive, with on-demand use being roughly equivalent to ITI therapy. The prophylactic use becomes extremely expensive, meaning that these patients are unlikely to receive the peace of mind that a prophylactic treatment promises. More recent developments have attempted to replace the role of FVIIIa in bringing FIXa and FX together through the use of a bi-specific antibody.

Since the bypass method alone, in the continued absence of FVIII, does not enable the amplification phase, it is a suboptimal solution and other treatments have attempted to engage the intrinsic tenase complex through the use of simulacra and mimetics of human FVIII, such as porcine FVIII or other molecules, such as antibodies, that are able to perform a similar binding function to activated human FVIII (FVIIIa). While these may not be recognised by the normal FVIII inhibitor antibodies, there remain concerns that as they are protein products that are foreign to the body, these will in turn cause the generation of antibodies against themselves. Also, their mode of action does not fully replicate the regulatory function of native Factor VIII, which is highly adapted to prevent over production of thrombin in the general circulation. The long half-lives of some of the antibody-based products may also be concerning, since when a breakthrough bleed does occur, the administration of rescue therapy (usually FVIIa or an aPCC complex in inhibitor patients), in combination with a reserve of the mimetic may put the patient at danger of a thrombotic event.

Another class of novel agents, "rebalancing agents", are focusing on downregulating the feedback loops that would ordinarily prevent unwanted thrombotic events by slowing down the coagulation cascade once coagulation has been achieved. Three current approaches involve attacking Tissue

Factor Pathway Inhibitor (TFPI), anti-thrombin or activated protein C. At date of writing not have reached market and the more advanced programmes have encountered difficulties with thrombotic events during trials.

- 5 For cHA patients, there remains the longer term hope of gene therapy to enable these patients to generate sufficient amounts of their own FVIII for the first time in their lives: this is not an option for aHA patients who generate sufficient FVIII but have developed inhibitors against their own FVIII. This therapy normally uses a viral vector to deliver genetic code to liver cells to stimulate FVIII expression from the liver. Whether antibodies will develop against the delivery vectors remains to be seen and
10 is yet a concern.

- An alternative approach taken by a few groups has been to target expression in megakaryocytes (bone marrow) so that the resultant platelet cells have FVIII stored in their α -granules. This approach first proposed by Shi et al. (2006) (*J. Clin. Invest.* **116**, 1974-1982) has the dual advantage of
15 concealing the FVIII from any inhibitors and other clearance mechanisms and of placing the molecule at the right location for the coagulation cascade. A more recent piece of work by the same group (Baumgartner et al (2015) (*J Thromb. Haemost.* **13**, 2210-2219) compared the relative efficacy of platelet-derived FVIII ('2bF8') and plasma FVIII (rhFVIII – Xyntha, Pfizer). This work looked at thrombin generation over a range of doses and found that while both generated similar levels of
20 thrombin, the platelet-associated FVIII not only significantly accelerated thrombin generation, but only one tenth of the dose was required to stimulate the onset and peak of thrombin generation. The group concluded that the higher therapeutic efficacy of 2bF8 compared with factor replacement therapy seemed to be due to acceleration of thrombin generation.

- 25 WO 2009/140598 describes the gene therapy platelet-derived work of the Shi group as an exemplar of the approach of associating FVIII with platelets. The stated objectives of that filing are to reduce immunogenicity, reduce side effects and obtain further advantages by releasing the therapeutic protein in the immediate vicinity of its site of action *in vivo*. The factors that are claimed are FVIII and FIX, as the two core commercially available exogenously administered factors. The method of
30 targeting is relatively specific and involves engineering a domain into the structure of the factor that specifically binds to a membrane protein on a blood cell. This approach involves altering the normal structure of the protein, which carries the danger of it being recognised as foreign and stimulating the generation of antibodies.

- 35 The present invention seeks to re-enable the amplification phase by fusing and or otherwise associating with platelets and other cellular bodies and thereby associating an exogenously applied FVIII (in the case of haemophilia A) to blood platelets and/or or enabling it to be phagocytosed into blood platelets, thus both placing it at the site where it will be needed and protecting it from recognition and damage by inhibitory antibodies or memory B or T cells. No further modification of

the FVIII should therefore be necessary to enable the association with the invention as described herein.

5 The present invention allows for the additional association of the patient's own FVII (and FVIIa) to blood platelets and/or enable it to be phagocytosed into blood platelets, thus also placing it at the site where it is most needed to provide a thrombin burst to stimulate the amplification phase. If FVIIa were to be exogenously administered as a bypassing agent, use of the invention may also be used to target the FVIIa to become associated with the platelets and the TF-bearing pro-coagulatory microparticles that arise following platelet activation.

10 The present invention allows for the additional association of the patient's own FVIII (where this is not deficient to the point of absence) to blood platelets and/or enable it to be phagocytosed into blood platelets, thus also placing it at the site where it is most needed to provide a thrombin burst to stimulate the amplification phase.

15 The present invention allows for the disruption of the platelets and other cellular bodies that it fuses with, creating long-lived, TF-bearing pro-coagulant microparticles (TFBPM) that catalyse the conversion of FVII to TF-FVIIa, which also catalyses the conversion of FIX to FIXa and FX to FXa, thereby enhancing the potency of the extrinsic pathway for an extended period, resulting in a greater
20 thrombin burst and more sustained, FVIII independent thrombin production.

By concentrating and fixing both FVIII on and/or within the blood platelets (and thereafter their activated forms, following activation) and through the bypassing action production of TF-bearing pro-coagulant microparticles (TFBPM), the ultimate object of the invention is to enable and maintain an
25 unusually rapid burst of thrombin generation at the onset of injury in order to accelerate the formation of a clot which can be quickly stabilised into a firm, good quality clot that is resistant to degradation.

By concentrating and amplifying the effect of a limited amount of a FVIII that is at a lower than normal level in the disease to be treated, it is anticipated that the invention will be FVIII-sparing, enabling
30 either lower doses to be administered and/or reducing the number of injections that are normally required to achieve haemostasis in haemophilia patients and in particular in inhibitor patients who cannot normally be administered FVIII as their inhibitory antibodies will destroy the protein and leave them unprotected.

35 The invention may act in multiple ways to improve the conversion of FX to FXa in the presence of both a limited amount of FVIII and inhibitors to FVIII. Firstly by protecting, enhancing and maximising the potential of a limited amount of FVIII to be able to form the tenase complex with FIXa to catalyse the conversion of FX to FXa; secondly, by mimicking FVIIa and binding FIXa to provide a substitute tenase complex to catalyse the conversion of FX and FXa; thirdly by upregulating the extrinsic
40 pathway both through the production of TFBPM and by concentrating FVII/FVIIa to stimulate the

conversion of FX to FXa through the extrinsic tenase complex; and finally, enhancing, via the upregulated extrinsic pathway, the conversion of FIX to FIXa to feed the formation of the intrinsic tenase complex. Through these actions the invention has multiple modes of action, by protecting, preserving and maximising the activity of FVIII in the tenase complex of the intrinsic pathway, while
5 mimicking the functionality of FVIIIa in the tenase complex and also simultaneously bypassing that pathway through up-regulation of the extrinsic pathway.

By enabling the use of standard plasma-derived or recombinantly produced forms of target blood factor (for example FVIII in the case of haemophilia A) it is an ultimate objective that the invention
10 will enable a cost-effective solution to enabling prophylactic treatment with FVIII in haemophilia A patients with inhibitors.

The invention allows for the co-administration or separate administration of the invention (a multi-specific lipidic vesicle) with the target blood factor, enabling it to be used alongside the standard of
15 care exogenously applied factor (being FVIII in the case of haemophilia A). The separate administration of the invention may also be of use in patients where a small but suboptimal amount of target blood factor exists endogenously, for example in moderate haemophilia patients or patients who have recovered a limited ability to generate their own target blood factor following gene therapy.

20 SUMMARY OF THE INVENTION

The present invention provides compositions, methods, kits and dosage forms comprising a colloidal particle for treating haemophiliac patients with antibody inhibitors or with a history of developing antibody inhibitors to Factor VIII (FVIII), for example those with acquired haemophilia (aHA) or congenital haemophilia (cHA) with inhibitors to FVIII.

25 In a first aspect of the invention there is provided a composition comprising a colloidal particle comprising (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI), wherein
30 said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer, for use in the treatment of haemophilia A in a subject. The subject has previously generated antibody inhibitors to Factor VIII (FVIII).

The biocompatible hydrophilic polymer may be selected from the group consisting of polyalkylethers, poly
35 lactic acids and polyglycolic acids, preferably, the biocompatible hydrophilic polymer is polyethylene glycol (PEG). The polyethylene glycol may have a molecular weight of between about 500 to about 5000 Daltons, preferably about 2000 Daltons or about 5000 Daltons.

The second amphipathic lipid may be N-(Carbonyl-methoxypolyethyleneglycol)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG) such as N-(Carbonyl-methoxypolyethyleneglycol-
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2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000) or N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000).

- 5 The phosphatidyl choline (PC) may be 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

The first amphipathic lipid and the second amphipathic lipid may be in a molar ratio of from 90 to 110:10 to 1 or 90 to 99:10 to 1, such as 100:3 or 97:3.

- 10 The colloidal particle may further comprise (iii) a non-ionic surfactant. The non-ionic surfactant may be selected from the group consisting of a polyoxyethylene sorbitan, a polyhydroxyethylene stearate and a polyhydroxyethylene laurylether. The non-ionic surfactant may be polyoxyethylene (20) sorbitan monooleate. The colloidal particle may comprise the first amphipathic lipid and the second amphipathic lipid to the non-ionic surfactant in a ratio of from 30:1 to 2:1 w/w ({first amphipathic lipid + second amphipathic lipid}:{non-ionic surfactant}).

The first amphipathic lipid to the second amphipathic lipid to the non-ionic surfactant may be in a ratio of from 10 to 40:1:0 to 4 w/w ({first amphipathic lipid}:{second amphipathic lipid}:{non-ionic surfactant}).

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The composition may further comprise a Factor VIII (FVIII) molecule. The colloidal particle and the Factor VIII (FVIII) molecule may be in a stoichiometric ratio of from 1 to 90:1 such as 10 to 20:1 or 5 to 10:1.

- 25 The haemophilia A may be congenital haemophilia A (cHA) or acquired haemophilia A (aHA).

The composition may further comprise a therapeutically active compound. The composition may also further comprise an excipient, diluent and/or adjuvant.

- 30 The subject may be a paediatric patient.

- 35 The compositions of the invention may be formulated in an aqueous suspension ready for use, or the compositions may be prepared as a lyophilised formulation. Lyophilised formulations of the invention may be supplied as separate dosage forms along with a suitable diluent, adjuvant or excipient provided also, e.g. a physiologically acceptable buffer. As described herein, such compositions may additionally comprise Factor VIII as a separate dosage form, or formulated with the colloidal particles as described herein.

- 40 In a second aspect of the invention there is provided a method of treating haemophilia A in a subject comprising the step of administering a composition comprising a colloidal particle. The colloidal

particle comprises (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI). The second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer. The subject has previously generated antibody inhibitors to Factor VIII (FVIII).

The biocompatible hydrophilic polymer may be selected from the group consisting of polyalkylethers, polylactic acids and polyglycolic acids, preferably, the biocompatible hydrophilic polymer is polyethylene glycol (PEG). The polyethylene glycol may have a molecular weight of between about 500 to about 5000 Daltons, preferably about 2000 Daltons or about 5000 Daltons.

The phosphatidyl choline (PC) may be 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

The second amphipathic lipid may be N-(Carbonyl-methoxypolyethyleneglycol)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG) such as N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000) or N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000).

The colloidal particle may further comprise (iii) a non-ionic surfactant.

The composition may further comprise Factor VIII (FVIII). Alternatively, the method may comprise a further step of separately or subsequently administering a composition comprising Factor VIII (FVIII).

The haemophilia may be congenital haemophilia A (cHA); or acquired haemophilia A (aHA).

The subject may be a paediatric patient.

In a third aspect of the invention there is provided a kit comprising (i) a composition comprising a colloidal particle and (ii) a composition comprising a Factor VIII (FVIII) molecule for use in the treatment of haemophilia A in a subject. The subject has previously generated antibody inhibitors to Factor VIII (FVIII). The colloidal particle is composed (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI). The second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.

The colloidal particle may further comprise (iii) a non-ionic surfactant.

In a fourth aspect of the invention there is provided a kit comprising (i) a composition comprising a colloidal particle and (ii) a composition comprising a Factor VIII (FVIII) molecule for separate, simultaneous or subsequent use in the treatment of haemophilia A in a subject. The subject has previously generated antibody inhibitors to Factor VIII (FVIII). The colloidal particle is composed (i) 5 a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI). The second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.

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The colloidal particle may further comprise (iii) a non-ionic surfactant.

In a fifth aspect of the invention there is provided a dosage form of a pharmaceutical composition comprising a colloidal particle comprising (i) a first amphipathic lipid comprising a 15 phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI), wherein said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer for use in the treatment of haemophilia A in a subject. The subject has previously generated antibody inhibitors to Factor VIII 20 (FVIII).

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The colloidal particle may further comprise (iii) a non-ionic surfactant.

In an embodiment, the colloidal particle is capable of non-covalently, reversibly FVIII, as well as 25 binding and fusing with blood platelets and other cellular elements of the blood, such that a limited amount of exogenously applied Factor VIII (FVIII) will be concentrated with other endogenous blood factors (for example FVII/FVIIa) at the surface and/or within the platelet and that this association will both protect the Factor VIII (FVIII) (that is present in lower than normal concentration) from degradation by inhibitors and the normal clearance mechanisms as well as enable the rapid onset 30 and acceleration of the clotting cascade on injury due to the parallel production of tissue factor (TF) bearing pro-coagulant microparticles (TFBPM). This results in the faster formation of a better quality clot in haemophilia patients and especially in those with inhibitors where an unprotected target blood factor (i.e. without the PEGLiP, e.g. FVIII in the case of haemophilia A) would be rapidly cleared, providing a much safer treatment option for these patients.

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The present invention is based on the surprising and unexpected finding that in a patient that is both deficient in a clotting factor and who is an inhibitor patient, such that the use of a replacement factor would ordinarily be ineffective, phospholipids derivatised with a bio-compatible polymer have two beneficial effects on the coagulation cascade when they are administered either co-formulated with 40 or as a separate injection to an administration of the missing factor in a patient that is deficient in a

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blood factor and with a history of producing antibodies to that blood factor. First, they can extend the apparent half-life of the exogenously applied target blood factor in the presence of inhibitors to that target blood factor (for example FVIII in the case of haemophilia A), providing extended haemostatic cover. Secondly, they enhance the coagulation cascade in a FVIII-independent manner, for example

5 reducing the onset of coagulation ("Clotting Time") and clot quality ("Maximum Clot Firmness"). Without wishing to be bound by theory the latter effects may be due to a variety of causes, for example: the binding and thus concentration of pre-existing endogenous blood factors, for example FVII, and FIX (in the case of haemophilia A) at and within blood platelets through having a reversible

10 non-covalent binding affinity with multiple entities within the coagulation cascade; the upregulation of the extrinsic coagulation pathway through the production of pro-coagulatory microparticles bearing tissue factor, stimulated by the fusion of the invention with cellular components of the blood, which microparticles serve as both an expanded reaction surface for the components of the clotting cascade, as well as providing TF to boost the extrinsic pathway in a manner similar to other

15 bypassing agents; working with FIXa to form a substitute tenase complex for the catalysis of FX to FXa; enhancing the production of FIXa to feed the tenase complex, through the upregulation of the extrinsic pathway.

In an embodiment there is provided an idealised formulation of the PEGLiP particles with respect to the amount of PEGLiP being injected, as well as the characteristics of the particular target blood

20 factor (for example FVIII in the case of haemophilia A), especially its specific activity (IU/mg) and molecular mass (g/mol or kDa), which should be an amount appropriate for the patient's severity of haemophilia. If co-formulating prior to injection, a number of the PEGLiP particles will reversibly bind these target blood factor molecules. It is a critical aspect of the invention that sufficient PEGLiP remains free and unbound to the target blood factor at the time of injection to enable the target blood

25 factor-independent actions, for example in the case of haemophilia A, the reversible capture of enough of the patient's FVII and FIX from the blood stream, to enhance the extrinsic pathway or form a substitute tenase complex; or the adequate interaction with the platelets and other cellular components to produce sufficient TFBPM to enhance the extrinsic pathway, in order to maximise the benefit of the invention.

30 In an embodiment there is therefore provided a composition comprising a suspension PEGLiP (colloidal particle) admixed with a Factor VIII to be administered to a patient with inhibitors to a target blood factor, for example FVIII in the case of haemophilia A, in a ratio of PEGLiP particles to FVIII molecules prior to administration that leaves sufficient un-associated PEGLiP to effectively enable

35 its FVIII-independent actions to enhance and extend the haemostatic cover beyond that expected of the target blood factor alone.

If the target blood factor (for example FVIII in the case of haemophilia A) is to be separately administered, or if it is intended to administer the invention alone to a patient with a suboptimal

40 endogenous concentration of target blood factor, the optimal ratio of PEGLiP to target blood factor in

blood may be calculated prior to injection, based on a) the amount of target blood factor to be administered or an assessment of the patient's existing, though sub-optimal concentration of target blood factor; b) the specific activity of the particular protein used; and c) the molecular mass of the particular protein used.

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DESCRIPTION OF THE INVENTION

The invention provides compositions, methods, kits and dosage forms comprising a colloidal particle for use in the treatment of haemophilia A in a subject wherein the subject has previously generated antibody inhibitors to a critical intrinsic blood factor. The colloidal particle is able to both bind blood clotting factors and bind and fuse with blood platelets to accelerate and improve the formation of blood clots in the treatment of haemophilia A.

Inhibitors or antibody inhibitors to FVIII refer to antibodies, also interchangeably known antibody inhibitors or neutralising antibodies, to FVIII. The antibodies may be auto-antibodies to endogenous FVIII or antibodies to exogenous FVIII.

In accordance with the first aspect described above, the colloidal particle comprises (i) a first amphipathic lipid and (ii) a second amphipathic lipid. The first amphipathic lipid may be a phosphatidylcholine (PC) moiety. A suitable example of a phosphatidyl choline (PC) moiety may be 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC). The second amphipathic lipid is a phospholipid moiety selected from the group consisting of phosphatidylethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol (PI). A suitable example of phosphatidyl ethanolamine (PE) may be 1,2-distearoyl-sn-glycero-3-phosphoethanol-amine (DSPE). Aminopropanediol distearoyl (DS) lipid is a carbamate-linked uncharged lipopolymer which is also an amphipathic lipid. Other examples of phosphatidyl ethanolamine (PE) include DPPE, DMPE and DOPE.

The colloidal particles of the invention are typically in the form of lipid vesicles or liposomes and are well known in the art. References to colloidal particles in the present specification include liposomes and lipid vesicles unless the context specifies otherwise.

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The second amphipathic lipid is a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.

The purpose of the biocompatible hydrophilic polymer is to sterically stabilize the colloidal particle, thus preventing fusion of the colloidal particle *in vitro*, and allowing the colloidal particle to escape adsorption by the reticuloendothelial system *in vivo*. The biocompatible hydrophilic polymer may be selected from the group consisting of polyalkylethers, polylactic acids and polyglycolic acids. The biocompatible hydrophilic polymer may be polyethylene glycol (PEG). The polyethylene glycol may be branched or unbranched. The biocompatible polymer may have a molecular weight of between about 100 to about 10,000 Da, suitably of from about 2000 to about 5000 Da, with preferred values of about 100 Da, 250 Da, 350 Da, 550 Da, 750 Da, 1000 Da, 1500 Da, 2000 Da, 2500 Da, 3000 Da, 3500 Da, 4000 Da, 4500 Da, 5000 Da, 5500 Da, 6000 Da, 6500 Da, 7000 Da, 7500 Da, 8000 Da,

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8500 Da, 9500 Da and 10,000 Da. A suitable example of a phospholipid derivatised with a biocompatible hydrophilic polymer may be N-(Carbonyl-methoxypolyethyleneglycol)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG) such as N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG(2000)) and N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000).

The first amphipathic lipid and the second amphipathic lipid may be provided in a molar ratio of from 90 to 110:10 to 1, 90 to 100:10 to 1, 90 to 99:10 to 1, 93 to 99:7 to 1, 95 to 99:5 to 1 suitably 100:3, 100:3, 99:3, 98:3, 97:3, 96:3 or 95:3. The molar ratio of 97:3 may also be expressed as a molar ratio of 32.4:1, likewise the molar ratio of 100:3 may be expressed as a molar ratio of 33.2:1. The ratio of the first amphipathic lipid and the second amphipathic lipid may also be expressed as a weight/weight ratio for example, 1:1 to 20:1 w/w, suitably 2:1 to 12:1 w/w or 4:1 to 9:1 w/w, for example 4:1, 5:1, 6:1, 9:1 or 12:1 w/w. Suitably, the composition may comprise a colloidal particle composed of a mixture of palmitoyl-oleoyl phosphatidyl choline (POPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) in a molar ratio (POPC:DSPE) of from 90 to 99:10 to 1, 93 to 99:7 to 1, 95 to 99:5 to 1 suitably 97:3. Expressed as a weight/weight ratio this may be, for example, 1:1 to 20:1 w/w, suitably 2:1 to 12:1 w/w, for example 4:1, 5:1, 6:1, 9:1 or 12:1 w/w.

In one instance, the colloidal particle may be composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG(2000)) in a 97:3 molar ratio or 9:1 w/w ratio.

The colloidal particle may have a mean particle size (average particle size) ranging from 0.05 to 0.3 μm diameter, suitably around 0.1, 0.15, 0.2 or 0.25 microns (μm). The average particle size (mean particle size) may be from 100 to 130 nanometres (nm), suitably 110 to 120 nm, 112 to 118 nm, 150 to 170 nm, 155 to 165 nm, more suitably 110, 112, 114, 116, 118, 120, 160, 162, 164, 166, 168 or 170 nm.

Mean particle size may be measured using a Malvern Zetasizer Ultra ZSU 5700. This instrument determines particle size by light scattering, whereby the back-scatter from laser light shone into the sample and hitting particles is detected at an angle of 173° (173° being almost back on itself & hence the term back-scatter). Brownian motion of particles causes the light to be scattered at different intensities. Because the velocity of Brownian motion relates to particle size, particle size can be inferred via the Stokes-Einstein relationship.

Mean particle size corresponds to the mean diameter of the colloidal particle. The polydispersity index (PDI) quoted in relation to particle size measurements corresponds to the measure of distribution around the mean diameter of the colloidal particle. For example, in the present invention, the polydispersity index may be a maximum of 0.2, suitably 0.15, 0.12, 0.1 or 0.5.

The PDI is calculated as the square of the standard deviation/mean, i.e. $\text{PDI} = (\text{s/m})^2$.

From the mean particle size and the polydispersity index, the standard deviation can be calculated. Twice the standard deviation facilitates the calculation of the 95% confidence intervals around the particle size, i.e. the range in which 95% of the colloidal particles in the sample lie.

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Suitably, the 95% mean particle size may be 50 to 500 nm, suitably 50 to 290 nm, 50 to 285 nm, 65 to 265 nm, 65 to 260 nm, 65 to 180 nm, 65 to 175 nm, 65 to 170 nm, 65 to 165 nm, 65 to 160 nm, more suitably 65 to 173 nm, 64 to 161 nm, 65 to 263 nm or 54 to 282 nm.

- 10 The colloidal particle may be stored as a suspension of 9% (w/v) total lipids in an aqueous citrate buffer, suitably the particles may be stored as a suspension of 7%, 6%, 5%, 4% (w/v) total lipids.

- 15 The colloidal particle may further comprise (iii) a surfactant such as a non-ionic surfactant. The non-ionic surfactant may be selected from the group consisting of a polyoxyethylene sorbitan, a polyhydroxyethylene stearate and a polyhydroxyethylene lauryl ether. The non-ionic surfactant may be polyoxyethylene (20) sorbitan monooleate (also known as polysorbate 80 or Tween 80). The first amphipathic lipid and the second amphipathic lipid to the non-ionic surfactant may be provided in a ratio of from 30:1 to 2:1 w/w, suitably 25:1, 20:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, or 5:1 w/w ({first amphipathic lipid + second amphipathic lipid}:{non-ionic surfactant}). Expressed as a molar ratio, this may be for example 10 to 20:1, 12 to 18:1, 14 to 16:1, suitably 14:1, 15:1 or 16:1.
- 20 The surfactant concentration may be from 0.25% to 5% by weight, for example 1% to 3%, 1 to 2%, some exemplary values may be 0.47%, 0.85%, or 3.5%.

- 25 The non-ionic surfactant may also be PEGylated. A PEGylated non-ionic surfactant may be polyoxyethylene (20) sorbitan monooleate (also known as polysorbate 80 or Tween 80). The polyethylene glycol may be branched or unbranched. The biocompatible polymer may have a molecular weight of between about 100 to about 10,000 Da, suitably of from about 2000 to about 5000 Da, with preferred values of about 100 Da, 250 Da, 350 Da, 550 Da, 750 Da, 1000 Da, 1500 Da, 2000 Da, 2500 Da, 3000 Da, 3500 Da, 4000 Da, 4500 Da, 5000 Da, 5500 Da, 6000 Da, 6500 Da, 7000 Da, 7500 Da, 8000 Da, 8500 Da, 9500 Da and 10,000 Da.
- 30

- The non-ionic surfactant may be associated with the colloidal particle, incorporated into the lipid bilayer membrane of the colloidal particle, incorporated into the outer layer of the lipid bilayer membrane of the colloidal particle or incorporated into the inner layer lipid bilayer membrane of the colloidal particle.
- 35

- Accordingly, the first amphipathic lipid to the second amphipathic lipid to the non-ionic surfactant may be provided in a ratio of from 2 to 10:1:0 to 2, 3 to 9:1:0.5 to 1.5, 4 to 9:1:0.5 to 1 w/w, suitably 9:1:0, 9:1:1, 4:1:0 or 4:1:0.5 w/w ({first amphipathic lipid}:{second amphipathic lipid}:{non-ionic surfactant}).
- 40 Expressed as a molar ratio this may be, for example, 30 to 40:1:0 to 5, 30 to 35:1:0 to 2.5, 30 to 35:1:0 to 2.5, suitably 33:1:0, 33:1:2 or 32:1:3.

The composition may further comprise a Factor VIII (FVIII) molecule or a fragment thereof. Where the composition comprises a fragment of Factor VIII, the Factor VIII fragment may suitably be an active fragment in which the fragment retains the biological activity, or substantially the same biological activity as the native Factor VIII molecule. For example, one such active fragment is the

5 B-domain truncated Factor VIII. It is further possible that the composition may comprise both the native blood factor and a fragment thereof. The colloidal particle and the Factor VIII (FVIII) molecule may be provided in a stoichiometric ratio of from 1 to 90:1, suitably 2 to 90:1, 5 to 85:1, 6 to 10:1, 7 to 8:1, 7.5 to 20:1, 10 to 80:1, 10 to 15:1, 10 to 16:1, 10 to 20:1, 13 to 19:1, 15 to 16:1, 15 to 75:1, 20 to 70:1, 25 to 65:1, 30 to 60:1, 35 to 55:1, 40 to 50:1, such as 10 to 20:1 and 5 to 10:1. Alternatively

10 expressed, the colloidal particle and the Factor VIII (FVIII) molecule may be provided in a stoichiometric ratio of 1:1, 2:1, 5:1, 7.5:1, 10:1, 15:1, 16:1, 17:1, 18:1, 19:1, 20:1, 22:1, 25:1, 26:1, 27:1, 28:1, 29:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 86:1, 90:1 such as 15.5:1, 13:1, 8:1, 7.7:1, 7:1. More specifically for full-length FVIII molecules the stoichiometric ratio may be 10:1 to 19:1 and optimally 10 to 15:1 or 5 to 10:1 and optimally 7.5:1. For beta-domain

15 deleted or beta-domain truncated FVIII molecules the ranges may be 13 to 19:1 and optimally 10 to 16:1 or 6 to 10:1 and optimally 8:1.

Without wishing to be bound by theory, there is a presumption that the excess colloidal particles present in the composition are in an amount sufficient to allow free colloidal particles to reversibly

20 bind other core blood factors (for example FVII and FIX in the case of haemophilia A) which with an amount of particle-associated Factor VIII (FVIII) may be captured and reversibly bound to the platelets following administration, to concentrate factors at the platelet and boost the extrinsic blood coagulation pathway.

25 Factor VIII may be from any suitable source and may be a recombinant protein produced by recombinant DNA technology using molecular biological techniques or synthesised chemically or produced transgenically in the milk of a mammal, or the Factor VIII may be isolated from natural sources (e.g. purified from blood plasma). Suitably the Factor VIII is a mammalian Factor VIII, such as a human Factor VIII.

30 Blood factors, such as Factor VIII, are characterised by the property of surface adhesion. This is a necessary feature of the coagulation cascade which requires that enzymes and cofactors adhere to other participants in the cascade, to the surface of platelets and to tissue at the site of injury. It is particularly important that a blood clot remains at the site of injury and does not drift to cause a

35 dangerous thrombosis. This property presents a challenge in the formulation of drug products, since blood factors such as Factor VIII will adhere excessively to any glass and plastic surfaces. In practical terms this is mitigated by the extensive use of a non-ionic surfactant such as polyoxyethylene (20) sorbitan monooleate (Tween® 80).

40 To determine the stoichiometric ratio of the colloidal particle to the Factor VIII (FVIII) molecule, the following calculation should be performed. First the molecules of FVIII per IU of FVIII should be determined. Note, the mass of FVIII varies depending on the variant of FVIII (for example full length

versus β -domain deleted). Second, the number of particles per gram of colloidal particle should be determined. Finally, a stoichiometric ratio can be determined accordingly. Example calculations with 35 IU/kg FVIII (both β -domain deleted and full-length) and 22 mg/kg colloidal (PEGLip) are as follows:

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Calculation example 1, a β -domain deleted rFVIII

FVIII, e.g. Nuwiq			
Specific activity	9,500	IU/mg	A
Mass of 1 IU of FVIII	0.105	mg	$B = 1/A \times 1,000$
=	1.05×10^{-7}	g	$C = B/1,000,000$
Molecular mass of FVIII	170,000	g/mol	D
Moles in 1 IU	6.19×10^{-13}	Moles	$E = C/D$
N(A)	6.02×10^{23}	Molecules/mole	F
Molecules of FVIII per IU	3.73×10^{11}	Molecules/IU	$G = E \times F$
PEGLip			
	POPC	DSPE	
Mass of 1g PEGLip solids	0.9	0.1	g
Molecular mass	760.1	2748	g/mol
Moles / g PEGLip solids	1.19×10^{-3}	3.64×10^{-5}	Moles
	J1	J2	
Total moles lipid	1.22×10^{-3}		Moles
N(A)	6.02×10^{23}		Molecules/mole
N(tot)	80,047		Lipid/surface
N(Lipo)	9.18×10^{15}		Liposomes/g PEGLip solids
Ratio Calculation			
35IU/kg BDDFVIII	1.30×10^{13}		Molecules
22mg/kg PEGLip	2.02×10^{14}		Liposomes
Ratio	15:1		Liposomes:FVIII (rounded)
			$Q = P / O$

Calculation example 2, a full-length rFVIII

FVIII, e.g. Kogenate			
Specific activity	4,000	IU/mg	A
Mass of 1 IU of FVIII	0.250	mg	$B = 1/A \times 1,000$
=	2.50×10^{-7}	g	$C = B/1,000,000$
Molecular mass of FVIII	265,000	g/mol	D
Moles in 1 IU	9.43×10^{-13}	Moles	$E = C/D$
N(A)	6.02×10^{23}	Molecules/mole	F
Molecules of FVIII per IU	5.68×10^{11}	Molecules/IU	$G = E \times F$
PEGLip			
	POPC	DSPE	
Mass of 1g PEGLip solids	0.9	0.1	g
Molecular mass	760.1	2748	g/mol
Moles / g PEGLip solids	1.19×10^{-3}	3.64×10^{-5}	Moles
	J1	J2	
Total moles lipid	1.22×10^{-3}		Moles
N(A)	6.02×10^{23}		Molecules/mole
N(tot)	80,047		Lipid/surface
N(Lipo)	9.18×10^{15}		Liposomes/g PEGLip solids
Ratio Calculation			
35IU/kg BDDFVIII	1.99×10^{13}		Molecules
22mg/kg PEGLip	2.02×10^{14}		Liposomes
Ratio	10:1		Liposomes:FVIII (rounded)
			$Q = P / O$

- 10 The composition may comprise a further therapeutically active compound or molecule, e.g. an anti-inflammatory drug, analgesic or antibiotic, or other pharmaceutically active agent which may promote or enhance the activity of Factor VIII.

The compositions of the invention may be formulated as pharmaceutical compositions suitable for administration as described herein according to standard practice. For example, the composition may further comprise any suitable excipient, diluent and/or adjuvant. Suitable diluents, such as buffers may be formulated with a water-soluble salt of an alkali metal or an alkaline earth metal and a suitable acid. Suitable buffer solutions may include, but are not limited to amino acids (for example histidine), salts of inorganic acids (for example an acid selected from the group consisting of citric acid, lactic acid, succinic acid, citric acid and phosphoric acid) and alkali metals or alkaline earth metals, (for example sodium salts, magnesium salts, potassium salts, lithium salts or calcium salts – exemplified as sodium chloride, sodium phosphate or sodium citrate). Examples of such excipient, buffer and/or adjuvants, include phosphate buffered saline (PBS), potassium phosphate, sodium phosphate and/or sodium citrate. Other biological buffers can include PIPES, MOPS etc.

A suitable aqueous citrate buffer may be a sodium citrate buffer or a potassium citrate buffer, for example a 50mM sodium citrate buffer. A suitable phosphate buffer may be a sodium phosphate buffer, for example a 25mM sodium phosphate buffer.

Suitable pH values for the composition include any generally acceptable pH values for administration in vivo, such as for example pH 5.0 to pH 9.0, suitably from pH 6.7 to pH 7.4, or pH6.8, pH 6.9, pH 7.0, pH 7.2. The pH may be adjusted accordingly with a suitable acid or alkali, for example hydrochloric acid.

The compositions of the invention may be formulated in an aqueous suspension ready for use, or the compositions may be prepared as a lyophilised formulation. Lyophilised formulations of the invention may be supplied as separate dosage forms along with a suitable diluent, adjuvant or excipient provided also, e.g. a physiologically acceptable buffer. As described herein, such compositions may additionally comprise Factor VIII as a separate dosage form, or formulated with the colloidal particles as described herein. Typically, a vial of lyophilised Factor VIII (FVIII) and a separate vial of colloidal particle (PEGLip) solution, for reconstitution will be provided.

The colloidal particle may be stored as a suspension of 9% (w/v) total lipids in an aqueous citrate buffer, suitably the particles may be stored as a suspension of 7%, 6%, 5%, 4% (w/v) total lipids. Once the required concentration of exogenous Factor VIII (FVIII) is known for the patient, the bulk solution may be diluted if necessary with 50mM sodium citrate solution to adjust the concentration of the colloidal particles so that when the Factor VIII (FVIII) is added the desired ratio of colloidal particles to Factor VIII (FVIII) molecules is obtained.

The Factor VIII may be entirely exogenous and formulated with the invention prior to injection, for example in the case of a severe haemophiliac with inhibitors, for which use it may be either derived from plasma concentrates or recombinantly produced. Alternatively, if the patient retains some ability

to self-manufacture Factor VIII (for example mild or moderate haemophiliacs, or patients with acquired haemophilia), a lesser amount or no exogenous Factor VIII will be administered.

Without wishing to be bound by theory, there is a presumption that the excess colloidal particles present in the composition in an amount sufficient to allow free colloidal particles to reversibly bind other core blood factors (for example FVII and FIX in the case of haemophilia A) which with an amount of particle-associated Factor VIII (FVIII) may be captured and reversibly bound to the platelets following administration, to concentrate factors at the platelet and boost the extrinsic blood coagulation pathway.

Upon injection the colloidal particle will reversibly bind to the surface of blood platelets and fuse with the membrane of others. Where the colloidal particle particles are already bound with an exogenous Factor VIII, this will concentrate the Factor VIII at the surface of the platelet with some maybe phagocytosed into the platelets or associated with or within the TF-bearing pro-coagulant microparticles that are produced, protecting the protein from inhibitors and also the normal clearance mechanisms, e.g. LRP-1, conferring a longer half-life on the protein. In patients with moderate or mild haemophilia, colloidal particles will also capture any circulating Factor VIII, concentrating it at or within the platelet or within the arising TF-bearing pro-coagulant microparticles. Colloidal particles that are not associated with Factor VIII on injection will begin to capture and concentrate FVII as well as other endogenous blood factors (e.g. FIX) at the surface of the platelets and to associate these with any TF-bearing pro-coagulant microparticles produced; it is also feasible that particles with no attached factors will also bind and fuse to the surface of the platelets, both forming TF-bearing pro-coagulant microparticles and acting as opportunistic traps to capture and concentrate further factors, including the activated forms, FVIIa and FIXa, at the platelet reaction surface during the maelstrom of the clotting cascade.

Ordinarily, on injury to the endothelium, tissue factor converts FVII to FVIIa and combines with it. The TF-FVIIa complex then migrates towards and binds onto the surface of the activated platelets and starts to convert FX to FXa to cleave prothrombin to generate thrombin, a process which becomes optimised when FXa complexes with FVIIa (released from the activated platelets) to form the prothrombinase complex, which is also assembled on the exposed membrane surfaces of the activated platelets and TF-bearing, pro-coagulatory microparticles derived from them. The invention places FVII in close proximity to this reaction surface, which may have shattered into many TF-bearing pro-coagulatory microparticles. Thus, the conversion to FVIIa (which remains bound to colloidal particle, and thus the platelet and microparticles) and the formation of the TF-FVIIa complex occurs on the reaction surface of the platelets and their microparticles with two important and immediate effects:

- 1) The localised conversion of FX to FXa, which combines with FV (the 'prothrombinase complex'), also on these platelet and TF-bearing procoagulant microparticle surfaces, to

produce a highly localised thrombin burst, which will both initiate the production of fibrin catalyse the amplification phase; and simultaneously

- 2) The localised conversion of FIX to FIXa which can then associate with the FVIII, which have been co-localised via the colloidal particle, to form the tenase complex on the same membrane surfaces, thus feeding and optimising the amplification phase that has been catalysed by the thrombin from the now augmented initiation phase in (1), above. Alternatively, and additionally the colloidal particle membrane may form a substitute tenase complex, attracting and converting FX to FXa

- Once the clotting cascade is initiated and fibrin is produced, platelets ordinarily coagulate to infill the fibrin mesh. The ability of the colloidal particle to bind and fuse with platelets has a final role to play here in reinforcing adherence of the platelets together in the mesh to stabilise the clot.

- The invention may act in multiple ways to improve the conversion of FX to FXa in the presence of both a limited amount of FVIII and inhibitors to FVIII. Firstly by protecting, enhancing and maximising the potential of a limited amount of FVIII to be able to form the tenase complex with FIXa to catalyse the conversion of FX to FXa; secondly, by mimicking the action of FVIIIa and binding FIXa to provide a substitute tenase complex to catalyse the conversion of FX to FXa; thirdly by upregulating the extrinsic pathway both through the production of TF-bearing pro-coagulant microparticles and by concentrating FVII/FVIIa to stimulate the conversion of FX to FXa through the extrinsic tenase complex; and finally, enhancing, via the upregulated extrinsic pathway, the conversion of FIX to FIXa to feed the formation of the intrinsic tenase complex. Through these actions the invention has multiple modes of action, by protecting, preserving and maximising the activity of FVIII in the tenase complex of the intrinsic pathway, while mimicking the functionality of FVIIIa in the tenase complex and also simultaneously bypassing that pathway through up-regulation of the extrinsic pathway.

- The colloidal particle has a dual action, both as a bypassing agent to enhance FX to FXa conversion via the extrinsic pathway, as well as amplifying the intrinsic pathway, through both the protection of FVIII and by concentrating FIX/FIXa accelerating the formation of the tenase complex or forming a FVIII-independent tenase complex with FIXa. Together the enhanced initiation (extrinsic) and amplification (intrinsic) phases enable both the more rapid onset of clotting and the faster generation of fibrin that can be bound into a firmer clot than would normally be possible with such a reduced amount of Factor VIII – especially in the presence of inhibitory antibodies - leading to the faster resolution of a bleed for the patient.

- The invention thus relies on the ability of the colloidal particle, and in particular its specific formulation ratio of colloidal particle to Factor VIII, both to concentrate correct amounts of both endogenous and exogenous Factor VIII at the platelet surface and inside the platelets, as well as stimulating the production of TF-bearing pro-coagulant microparticles, so that both the TF-FVIIa-centric initiation phase and the amplification phase of the clotting cascade are optimised together with the synergistic

effect of accelerating the onset of thrombin generation with a limited amount of Factor VIII in the presence of inhibitors to Factor VIII in the case of haemophilia A).

5 Since the injected exogenous Factor VIII is protected from degradation by both inhibitors and normal clearance mechanisms, and since a better quality clot is formed faster both through concentrating the factors at the platelet, and the accelerating effect of the TF-bearing pro-coagulant microparticles, the invention will be Factor VIII sparing over other methods of supplying Factor VIII, as found by production of ectopic FVIII in platelets via gene therapy. This benefit will manifest in smaller or less frequent injections for patients, increasing compliance with prescribed treatment and decreasing the
10 likelihood of an accidental and possibly fatal bleed.

While the invention concentrates exogenous Factor VIII and endogenous factors (FVII/FVIIa and FIX/FIXa) at and within the platelets, unlike the successful attempts to product FVIII ectopically in platelets via gene therapy it does not require the long-development programmes, regulatory burden
15 or the irreversible nature of a virally-mediated transgene therapy.

By concentrating and amplifying the effect of a limited amount of Factor VIII that is at a lower than normal level in the disease to be treated (for example FVIII in the case of haemophilia A (HA)), it is anticipated that the invention will be Factor VIII-sparing, enabling either lower doses to be
20 administered and/or reducing the number of injections that are normally required to achieve haemostasis in haemophilia patients and in particular in inhibitor patients who cannot normally be administered Factor VIII as their inhibitory antibodies will destroy the protein and leave them unprotected.

25 Unlike approaches to create novel engineered Factor VIII molecules or mimetics of these molecules or their activated forms, the invention can be used with any current plasma-derived or recombinant Factor VIII, without the need to engineer foreign sequences into the molecule, for example recombinant human FVIII (rhFVIII). This reduces the danger of an immunomodulatory response arising to a novel, unrecognised protein.

30 Unlike both of these approaches, i.e. the production of FVIII in platelets or the use of mimetics, the invention has the novel and very necessary dual action of not only concentrating an exogenously applied component of the extrinsic, acceleratory pathway, but in also both concentrating endogenous factors and stimulating the production of TF-bearing pro-coagulant microparticles to amplify the
35 intrinsic pathway to a rapid thrombin burst and the local generation of the other major component (FIXa) of the extrinsic pathway, which may continue to drive the common pathway to thrombin production as Factor VIII levels fall again.

Use of the invention is Factor VIII-sparing over free Factor VIII. This means more convenience for
40 patients (smaller injections), better compliance (fewer missed prophylactic injections) and better

healthcare economics (less cost of Factor VIII, fewer emergency infusions when haemophiliacs have not been compliant and had bleeds).

5 By enabling the use of standard plasma-derived or recombinantly produced forms of Factor VIII it is an ultimate objective that the invention will enable a cost-effective solution to enabling prophylactic treatment with FVIII in haemophilia A patients with inhibitors.

10 Use of the invention is sparing over the use of exogenous FVIIa as a bypass agent in haemophilia patients with inhibitors. The invention not only uses the patient's own FVII but also both concentrates this at the platelets and stimulates the production of TF-bearing pro-coagulant microparticles to maximise the effectiveness of FVII, thus avoiding the cost of exogenous FVIIa and any concerns of thrombotic reactions due to overdosing with the protein.

15 The composition may be administered by injection or infusion, preferably intravenous, subcutaneous, intradermal or intramuscular. Injection comprises the administration of a single dose of the composition. Infusion comprises the administration of a composition over an extended period of time.

20 The compositions of the invention may be for administration at least once per day, at least twice per day, about once per week, about twice per week, about once per two weeks, or about once per month. The composition may also be administered and/or re-dosed at intervals to allow the blood concentration of FVIII to be maintained at a consistent level, providing a sustained, constant and predictable therapeutic effect without the need to wait to re-dose until the concentration of FVIII in the blood of the patient reaches sub-therapeutic or therapeutically irrelevant levels. In traditional practice, subsequent doses of FVIII are not normally given to the patient while "healthy levels", or
25 therapeutically effective/relevant levels, of FVIII are still present in the bloodstream. Thus, the invention provides for a more consistent therapeutic level of FVIII in the bloodstream that is more ideally suited to prophylaxis.

30 Sub-therapeutic or therapeutically irrelevant levels of FVIII in the blood of a patient may be characterised as being when a patient is not able to maintain a whole blood clotting time of 20 minutes, or less, 15 minutes, or less, or 12 minutes or less.

The invention provides a composition wherein a patient is able to maintain a whole blood clotting time of no more than 20 minutes, no more than 15 minutes or not more than 12 minutes.

35 It has been surprisingly found that formulations of blood factors in association with colloidal particles (liposomes) derivatized with a biocompatible polymer can be successfully administered subcutaneously and achieve a therapeutically effective dose of blood factor to a subject suffering from haemophilia.

40

In the examples of the present invention, the PEG is incorporated into the colloidal particle during vesicle formation, before association with the blood factor. It is believed that specific amino acid sequences on the blood factor may bind non-covalently to carbamate functions of the PEG molecules on the outside of the liposomes.

5

The colloidal particle does not encapsulate the blood factor. The blood factor interacts non-covalently with the polymer chains on the external surface of the liposomes, and no chemical reaction is carried out to activate the polymer chains. The nature of the interaction between the blood factor and the liposome derivatized with a biocompatible hydrophilic polymer may be by any non-covalent mechanism, such as ionic interactions, hydrophobic interactions, hydrogen bonds and Van der Waals attractions (Arakawa, T. and Timasheff, S. N., *Biochemistry* 24: 6756- 6762 (1985); Lee, J. C. and Lee, L. L. Y., *J. Biol. Chem.* 226: 625-631 (1981)). An example of such a polymer is polyethylene glycol (PEG).

10

15 A variety of known coupling reactions may be used for preparing vesicle forming lipids derivatized with hydrophilic polymers. For example, a polymer (such as PEG) may be derivatized to a lipid such as phosphatidylethanolamine (PE) through a cyanuric chloride group. Alternatively, a capped PEG may be activated with a carbonyl diimidazole coupling reagent, to form an activated imidazole compound. A carbamate-linked compound may be prepared by reacting the terminal hydroxyl of
20 MPEG (methoxyPEG) with p-nitrophenyl chloroformate to yield a p-nitrophenyl carbonate. This product is then reacted with 1-amino-2,3-propanediol to yield the intermediate carbamate. The hydroxyl groups of the diol are acylated to yield the final product. A similar synthesis, using glycerol in place of 1-amino-2, 3-propanediol, can be used to produce a carbonate-linked product, as described in WO 01/05873. Other reactions are well known and are described, e.g. in US 5,013,556.

25

Colloidal particles (liposomes) can be classified according to various parameters. For example, when the size and number of lamellae (structural parameters) are used as the parameters then three major types of liposomes can be described: Multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and large unilamellar vesicles (LW).

30

MLV are the species which form spontaneously on hydration of dried phospholipids above their gel to liquid crystalline phase transition temperature (T_m). The size of the MLVs is heterogeneous and their structure resembles an onion skin of alternating, concentric aqueous and lipid layers.

35 SUV are formed from MLV by sonication or other methods such as extrusion, high pressure homogenisation or high shear mixing and are single layered. They are the smallest species with a high surface-to-volume ratio and hence have the lowest capture volume of aqueous space to weight of lipid.

The third type of liposome LUV has a large aqueous compartment and a single (unilamellar) or only a few (oligolamellar) lipid layers. Further details are disclosed in D. Lichtenberg and Y. Barenholz, in "Liposomes: Preparation, Characterization, and Preservation, in Methods of Biochemical Analysis", Vol. 33, pp. 337 – 462 (1988).

5

As used herein the term "loading" means any kind of interaction of the biopolymeric substances to be loaded, for example, an interaction such as encapsulation, adhesion (to the inner or outer wall of the vesicle) or embedding in the wall with or without extrusion of the biopolymeric substances.

- 10 As used herein and indicated above, the term "liposome" refers to colloidal particles and is intended to include all spheres or vesicles of any amphipathic compounds which may spontaneously or non-spontaneously vesiculate, for example phospholipids where at least one acyl group replaced by a complex phosphoric acid ester. The liposomes may be present in any physical state from the glassy state to liquid crystal. Most triacylglycerides are suitable and the most common phospholipids
- 15 suitable for use in the present invention are the lecithins (also referred to as phosphatidylcholines (PC)), which are mixtures of the diglycerides of stearic, palmitic, and oleic acids linked to the choline ester of phosphoric acid. The lecithins are found in all animals and plants such as eggs, soybeans, and animal tissues (brain, heart, and the like) and can also be produced synthetically. The source of the phospholipid or its method of synthesis are not critical, any naturally occurring or synthetic
- 20 phosphatide can be used.

Examples of specific phosphatides are L- α -(distearoyl) lecithin, L- α -(dipalmitoyl) lecithin, L- α -phosphatide acid, L- α -(dilauroyl)-phosphatidic acid, L- α -(dimyristoyl) phosphatidic acid, L- α -(dioleoyl)phosphatidic acid, DL- α (di- palmitoyl) phosphatidic acid, L- α (distearoyl) phosphatidic

25 acid, and the various types of L- α -phosphatidylcholines prepared from brain, liver, egg yolk, heart, soybean and the like, or synthetically, and salts thereof. Other suitable modifications include the controlled peroxidation of the fatty acyl residue cross-linkers in the phosphatidylcholines (PC) and the zwitterionic amphipathates which form micelles by themselves or when mixed with the PCs such as alkyl analogues of PC.

30

The phospholipids can vary in purity and can also be hydrogenated either fully or partially. Hydrogenation reduces the level of unwanted peroxidation, and modifies and controls the gel to liquid/crystalline phase transition temperature (T_m) which effects packing and leakage.

- 35 The liposomes can be "tailored" to the requirements of any specific reservoir including various biological fluids, maintains their stability without aggregation or chromatographic separation, and remains well dispersed and suspended in the injected fluid. The fluidity in situ changes due to the composition, temperature, salinity, bivalent ions and presence of proteins. The liposome can be used with or without any other solvent or surfactant.

40

Generally suitable lipids may have an acyl chain composition which is characteristic, at least with respect to transition temperature (T_m) of the acyl chain components in egg or soybean PC, i.e., one chain saturated and one unsaturated or both being unsaturated. However, the possibility of using two saturated chains is not excluded.

5

The liposomes may contain other lipid components, as long as these do not induce instability and/or aggregation and/or chromatographic separation. This can be determined by routine experimentation.

10 The PEGylated phospholipid may be physically attached to the surface of the colloidal particle or inserted into the membrane of the colloidal particle. The polymer may therefore be covalently bound to the colloidal particle.

A variety of methods for producing the modified colloidal particle which are unilamellar or multilamellar are known and available (see Lichtenberg and Barenholz, (1988)):

15

1. A thin film of the phospholipid is hydrated with an aqueous medium followed by mechanical shaking and/or ultrasonic irradiation and/or extrusion through a suitable filter;
2. Dissolution of the phospholipid in a suitable organic solvent, mixing with an aqueous medium followed by removal of the solvent;
- 20 3. Use of gas above its critical point (i.e., freons and other gases such as CO₂ or mixtures of CO₂ and other gaseous hydrocarbons) or
4. Preparing lipid detergent mixed micelles then lowering the concentration of the detergents to a level below its critical concentration at which liposomes are formed.

25 In general, such methods produce colloidal particles with heterogeneous sizes from about 0.02 to 10 μm or greater. Since colloidal particles which are relatively small and well defined in size are preferred for use in the present invention, a second processing step defined as "colloidal particle down-sizing" can be used for reducing the size and size heterogeneity of colloidal particle suspensions.

30 The colloidal particle suspension may be sized to achieve a selective size distribution of vesicles in a size range less than about 5 μm , for example $< 0.4 \mu\text{m}$. In one embodiment of the invention, the colloidal particles have an average particle size diameter of from about 0.03 to 0.4 microns (μm), suitably around 0.1 microns (μm).

35 Colloidal particles in this range can readily be sterilized by filtration through a suitable filter. Smaller vesicles also show less of a tendency to aggregate on storage, thus reducing potentially serious blockage or plugging problems when the liposome is injected intravenously or subcutaneously. Finally, liposomes which have been sized down to the submicron range show more uniform distribution.

40

Several techniques are available for reducing the sizes and size heterogeneity of colloidal particles, in a manner suitable for the present invention. Ultrasonic irradiation of a colloidal particle suspension either by standard bath or probe sonication produces a progressive size reduction down to small unilamellar vesicles (SUVs) between 0.02 and 0.08 μm in size.

5

Homogenization is another method which relies on shearing energy to fragment large colloidal particles into smaller ones. In a typical homogenization procedure, the colloidal particle suspension is recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 μm are observed. In both methods, the particle size distribution can be

10

Extrusion of colloidal particles through a small-pore polycarbonate filter or equivalent membrane is also an effective method for reducing colloidal particle sizes down to a relatively well-defined size distribution whose average is in the range between about 0.02 and 5 μm , depending on the pore size

15

Typically, the suspension is cycled through one or two stacked membranes several times until the desired colloidal particle size distribution is achieved. The colloidal particle may be extruded through successively smaller pore membranes to achieve a gradual reduction in liposome size.

20

Centrifugation and molecular sieve chromatography are other methods which are available for producing a liposome suspension with particle sizes below a selected threshold less than 1 μm . These two respective methods involve preferential removal of large liposomes, rather than conversion of large particles to smaller ones. Colloidal particle yields are correspondingly reduced.

25

The size-processed colloidal particle suspension may be readily sterilized by passage through a sterilizing membrane having a particle discrimination size of about 0.4 μm , such as a conventional 0.45 μm depth membrane filter. The liposomes are stable in lyophilized form and can be reconstituted shortly before use by taking up in water.

30

Suitable lipids for forming colloidal particles are described above. Suitable examples include but are not limited to phospholipids such as dimyristoylphosphatidylcholine (DMPC) and/or dimyristoyl - phosphatidylglycerol (DMPG), egg and soybean derived phospholipids as obtained after partial or complete purification, directly or followed by partial or complete hydrogenation.

35

The following four methods are described in WO 95/04524 and are generally suitable for the preparation of the colloidal particles (liposomes) used in accordance with the present invention.

40

Method A

- a) mixing amphipathic substances, such as lipids suitable for forming vesicles in water-immiscible organic solvents
- b) removing of the solvent in presence of a solid support, alternatively, dried amphipathic substances or mixtures thereof can be used in any form (powder, granular, etc.) directly,
- c) taking up the product of step b) into a solution of the biopolymeric substances in a physiologically compatible solution
- d) adding an organic solvent having solubilizing or dispersing properties, as well as
- e) drying the fraction obtained in step d) under conditions retaining the function of the biopolymeric substances.

According to step a) of Method A amphipathic substances suitable for forming vesicles as mentioned above are mixed in a water-immiscible organic solvent. The water-immiscible organic solvent may be a polar-protic solvent such as fluorinated hydrocarbons, chlorinated hydrocarbons and the like.

In step b) of the method of the invention the solvent is removed in presence of a solid support. The solid support may be an inert organic or inorganic material having a bead-like structure. The material of the inorganic support material may be glass and the organic material can be Teflon™ or other similar polymers.

The step c) of Method A of the invention is for taking up the product of step b) into a solution of the substances to be encapsulated in a physiologically compatible solution.

The physiological compatible solution may be equivalent to a sodium chloride solution up to about 1.5 by weight. It is also possible to use other salts as long as they are physiologically compatible e.g. as a cryoprotectant e.g., sugars and/or amino acids. For example, lactose, sucrose or trehalose may be used as a cryoprotectant.

Optionally, between step a) and b) a step of virus inactivation, sterilizing, depyrogenating, filtering the fraction or the like of step a) can be provided. This might be advantageous in order to have a pharmaceutically acceptable solution at an early stage of the preparation.

The step d) of the Method A is adding an organic solvent having solubilizing or dispersing properties.

The organic solvent may be an organic polar-protic solvent miscible with water. Lower aliphatic alcohols having 1 to 5 carbon atoms in the alkyl chain can also be used, such as tertiary butanol (tert-butanol). The amount of organic polar-protic solvent miscible with water is strongly dependent on its interference with the substance to be loaded to the liposomes. For example, if a protein is to be loaded the upper limit is set by the amount of solvent by which the activity of the protein becomes affected. This may strongly vary with the nature of the substance to be loaded. For example, if the

blood clotting factor comprises Factor IX then the amount of about of tert-butanol is around 30%, whereas, for Factor VIII an amount of less than 10% of tert-butanol is suitable (Factor VIII is much more sensitive to the impact of tert-butanol). The percentage of tert-butanol in these examples is based on percent by volume calculated for final concentration.

5

Optionally, subsequent to step d), virus inactivation sterilizing and/or portioning of the fraction yielded after step d) can be carried out.

The step e) of the present invention is drying the fraction obtained in step d) under conditions retaining the function of the substance to be loaded. One method for drying the mixture is lyophilization. The lyophilization may be carried out in presence of a cryoprotectant, for example, lactose or other saccharides or amino acids. Alternatively, evaporation or spray-drying can be used.

10

The dried residue can then be taken up in an aqueous medium prior to use. After taking up of the solid it forms a dispersion of the respective liposomes. The aqueous medium may contain a saline solution and the dispersion formed can optionally be passed through a suitable filter in order to down size the liposomes if necessary. Suitably, the liposomes may have a size of 0.02 to 5 μm , for example in the range of $< 0.4 \mu\text{m}$.

15

The liposomes obtainable by the Method A show high loading of the blood factors.

20

The compositions of the invention can also be an intermediate product obtainable by isolation of either fraction of step c) or d) of the method A. Accordingly, the formulation of the invention also comprises an aqueous dispersion obtainable after taking up the product of step e) of method A in water in form of a dispersion (liposomes in aqueous medium).

25

Alternatively, the pharmaceutical compositions of the invention are also obtainable by the following methods which are referred to as Methods B, C, D and E.

30 Method B

This method comprises also the steps a), b) and c) of the Method A. However, step d) and e) of Method A are omitted.

Method C

In Method C step d) of method A is replaced by a freeze and thaw cycle which has to be repeated at least two times. This step is well-known in prior art to produce liposomes.

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Method D

Method D excludes the use of any osmotic component. In method D the steps of preparation of vesicles, admixing and substantially salt free solution of the substances to be loaded and co-drying of the fractions thus obtained is involved.

5

Method E

Method E is simpler than methods A - D described above. It requires dissolving the compounds used for liposome preparation (lipids antioxidants, etc.) in a polar-protic water miscible solvent such as tert.-butanol. This solution is then mixed with an aqueous solution or dispersion containing the blood
10 factor. The mixing is performed at the optimum volume ratio required to maintain the biological and pharmacological activity of the agent.

The mixture is then lyophilized in the presence or absence of cryoprotectant. Rehydration is required before the use of the liposomal formulation. These liposomes are multilamellar, their downsizing can
15 be achieved by one of the methods described in WO 95/04524.

The composition for use in the treatment of haemophilia A in a subject of the first aspect of the invention may be used for a subject that has previously generated antibody inhibitors to Factor VIII (FVIII). The composition for use in the treatment of haemophilia A in a subject of the first aspect of
20 the invention may be used for a subject that initiates or generates an immune response to exogenously administered Factor VIII (FVIII). The composition for use in the treatment of haemophilia A in a subject of the first aspect of the invention may be used for a subject that is resistant to treatment with exogenous Factor VIII (FVIII), i.e. Factor VIII therapy. Expressed another way, the composition for use in the treatment of haemophilia A in a subject of the first aspect of the invention may be used
25 for a subject that has tested positive for an inhibitor (an antibody) to FVIII. In keeping with the present invention, the patient may therefore be tested for the presence of an FVIII inhibitor (an antibody to FVIII) prior to treatment according to the present invention. Optionally, therefore a method of treatment according to the present invention may include a step of testing the subject for the generation of antibodies to Factor VIII prior to the step of treating the subject with a composition as
30 defined herein.

Suitably, the subject may be tested in an immune challenge assay to determine if the subject initiates an immune response to exogenously administered Factor VIII (FVIII). Increasing amounts of exogenous Factor VIII (FVIII) may be administered to the subject over a defined number of exposures
35 to determine if the subject initiates an immune response to exogenously administered Factor VIII (FVIII), for example the number of exposures to exogenous Factor VIII (FVIII) may be 50 exposures, or less. The increasing amounts of exogenous Factor VIII (FVIII) may be calculated as part of a titration curve, i.e. the amount of Factor VIII (FVIII) administered over 50 exposures.

The composition for use in the treatment of haemophilia A in a subject of the first aspect of the invention may be used for a paediatric subject. A paediatric patient is defined in the European Union (EU) as that part of the population aged between birth and 18 years. The paediatric population encompasses several subsets. The applied age classification of paediatric patients is:

- 5 • pre-term and term neonates from 0 to 27 days;
- infants (or toddlers) from 1 month to 23 months;
- children from 2 years to 11 years; and
- adolescents from 12 to less than 18 years.

(see: [http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-](http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/2014_c338_01/2014_c338_01_en.pdf)

10 [1/2014_c338_01/2014_c338_01_en.pdf](http://ec.europa.eu/health/sites/health/files/files/eudralex/vol-1/2014_c338_01/2014_c338_01_en.pdf))

The haemophilia may be congenital haemophilia A (cHA) or acquired haemophilia A (aHA). Congenital haemophilia is an inherited bleeding disorder characterized by an absent or reduced level of clotting Factor VIII. Acquired haemophilia is an autoimmune condition in which there is sudden
15 production of autoantibody inhibitors in an individual without any personal or family history of bleeding. The body produces autoantibodies against endogenous Factor VIII in haemophilia A.

An 'inhibitor patient' is defined as one who in their medical history has developed inhibitory antibodies ('inhibitor') in response to the application of an exogenous therapeutic blood factor (in the case of
20 congenital haemophilia) or who has developed inhibitors to their own endogenous FVIII (in the case of acquired haemophilia). They may currently present with or without inhibitors but in the latter case will probably not have been tolerised via ITI and remain capable generating inhibitors if re-presented with FVIII. Such a subject may also be considered to be patient that is non-naïve to a Factor VIII (FVIII) therapy.

25

An inhibitor patient may have less than 5 Bethesda units of FVIII inhibitor (antibody) activity. The antibody inhibitors in a patient's blood are quantified using the Bethesda method and it is common parlance to use 'Bethesda units' when talking about the level of inhibitors in a patient. A value of greater than 5 Bethesda units of FVIII inhibitor is considered a high titre of inhibitor (see:
30 https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-clinical-investigation-recombinant-human-plasma-derived-factor-viii-products-revision-2_en.pdf).

The composition for use in the treatment of haemophilia A in a subject of the first aspect of the invention may also be used for a subject that has greater than or equal to 5 Bethesda units of FVIII
35 inhibitor (antibody) activity. The composition for use in the treatment of haemophilia A in a subject of the first aspect of the invention may also be used for a subject that has less than 5 Bethesda units of FVIII inhibitor (antibody) activity.

A Bethesda unit (BU) is a measure of blood coagulation inhibitor activity. According to Practical
40 Haemostasis, "1 Bethesda Unit (Bu) is defined as the amount of inhibitor in a plasma sample which

will neutralise 50% of 1 unit of Factor VIII:C in normal plasma after 2hr incubation at 37°C." (Schumacher, Harold Robert (2000). Handbook of Hematologic Pathology. Informa Health Care, p. 583). 1 unit of Factor VIII is equal to 1 IU/mL Factor VIII.

- 5 The Bethesda assay is based on measuring residual FVIII activity remaining after dilutions of test plasma. The assay requires a comparison between a test mixture of test plasma and normal plasma and a control mixture of normal plasma and buffer incubated for 2 hours at 37°C. The percent residual activity in the test mixture is converted to Bethesda units (BU).
- 10 A Bethesda assay is performed as follows:
 1. Doubling dilutions of test (patient) plasma [usually 1/2 - 1/1024] are made in Imadazole buffered saline and incubated with an equal volume of a normal plasma pool at 37°C. In the Nijmegen modification, the dilutions of patient plasma are made in Factor VIII deficient plasma.
 - 15 2. A control mixture consisting of an equal volume of normal plasma mixed with Imadazole buffered saline (or in the case of the Nijmegen modification immunodepleted factor VIII deficient plasma) is prepared. The normal plasma pool will contain ~ 100% [100 IU/dL] Factor VIII. This mixture actually has a starting concentration of 50% [50 IU/dL] Factor VIII (because you have performed a 50:50 dilution with buffer) but this does not matter because the same source and volume is added to all incubation mixtures. The use of the control compensates for the deterioration in Factors VIII and V during the incubation period.
 - 20 3. At the end of the incubation period the residual factor VIII is assayed using a standard 1-stage APTT based assay using the incubated control as the 100% [100 IU/dL] standard.
 - 25 4. The inhibitor concentration is calculated from a graph of residual factor VIII activity versus inhibitor units. The dilution of test plasma that gives a residual factor VIII nearest to 50% but within the range 30-60% is chosen for calculation of the inhibitor. It is also possible to calculate the inhibitor titre for each dilution and take the average. Any residual factor VIII <25% [25 IU/dL] or >75% [75 IU/dL] should NOT be used for the calculation of inhibitor level.
 - 30 5. If the residual factor VIII activity is between 80-100% [80-100 IU/dL] the sample does not contain an inhibitor.
 - 35 6. Derive the inhibitor titre from the graph and multiply by the dilution to give the final titre. A positive control plasma of known inhibitor titre should be included.

Levels of activity in the blood coagulation cascade may be measured by any suitable assay, for example the Whole Blood Clotting Time (WBCT) test, the Activated Partial Thromboplastin Time (APTT) or ROTEM. In the One stage and Two stage/Chromogenic assays, the blood samples have to be prepared by centrifugation to remove cellular fragments, mostly because the assay method involves spectrophotometry so the sample needs to be clear. The global clotting assays below assess the time course of the physical formation of a clot and are thus closer to 'real life' as all the components that contribute to a clot, e.g. the platelets, are included.

The Whole Blood Clotting Time (WBCT) test measures the time taken for whole blood to form a clot in an external environment, usually a glass tube or dish. WBCT can be assessed with 2ml of whole blood taken immediately after collection and divided into two glass tubes. These two tubes are then placed into a 37°C water bath and checked approximately every 20-30 seconds by gently tilting. A clot is determined when the tube can be inverted horizontally and there is no run-off of plasma and a solid clot is retained.

The Activated Partial Thromboplastin Time (APTT) test measures a parameter of part of the blood clotting pathway. It is abnormally elevated in haemophilia and by intravenous heparin therapy. The APTT requires a few millilitres of blood from a vein. The APTT time is a measure of one part of the clotting system known as the "intrinsic pathway". The APTT value is the time in seconds for a specific clotting process to occur in the laboratory test. This result is always compared to a "control" sample of normal blood. If the test sample takes longer than the control sample, it indicates decreased clotting function in the intrinsic pathway. General medical therapy usually aims for a range of APTT of the order of 45 to 70 seconds, but the value may also be expressed as a ratio of test to normal, for example 1.5 times normal. A high APTT in the absence of heparin treatment can be due to haemophilia, which may require further testing.

ROTEM (rotational thromboelastometry) uses a ROTEM Delta 2.7.2 system to assess the coagulability of the blood samples via the NATEM assay (activated by re-calcification only). For the measurement 20uL of CaCl₂ and 340uL of citrated whole blood sample is placed in the apparatus. The assay is performed within 15 minutes of taking the fresh blood sample. The assay delivers a panoply of statistics during the formation of the clot, including the Clotting Time (CT – the time for the blood to start clotting), the Clot Formation Time (CFT – the time to maximum clot firmness) among others.

The invention provides a composition which enables the subject to maintain a whole blood clotting time of less than 20 minutes, less than 15 minutes, or suitably, less than 12 minutes.

The following describes the Chromogenic assay (sometimes called the "Two-stage Assay") for assessing FVIII concentration.

FVIII plasma activity can be determined using a Chromogenix Coamatic Factor VIII chromogenic assay (Diapharma, K822585) with modifications to the supplied method as follows:

- 5 i. The inclusion of an amount of naïve plasma in the FVIII standard preparations to achieve comparability with plasma sample dilutions,
- ii. The use of FVIII standards specific to each test article (Nuwiq™ or Factane™),
- iii. The inclusion of additional FVIII activity values within two standard curve ranges.

Preparation of Nuwiq™ and Factane™ FVIII standard stock solutions:

- 10 A vial of each test article can be reconstituted to 100 IU/ml with purified water, stored frozen in small aliquots at -70°C and an aliquot thawed at 37°C on the day of the assay. The stock solution appropriate to the study test article is used for the analysis of the corresponding plasma samples.

The outline assay method was as follows:

- 15 1. A vial of Technoclone Factor VIII-deficient plasma (native; Diapharma 5154007) freshly reconstituted in 1ml purified water,
2. FVIII standard working stock solution (1 IU/mL) freshly prepared by the addition of 0.010mL of appropriate FVIII standard stock solution (100 IU/ml) to 0.990 mL FVIII-deficient plasma,
- 20 3. Coamatic kit Factor reagent, S-2765 + I-2581 substrate and buffer working solution prepared according to kit instructions and pre-warmed to 37°C,
4. 20% acetic acid stop solution was prepared,
5. A standard curve prepared from FVIII working stock solution (1 IU/ml) using a FVIII range appropriate to the study samples (see Tables 1 and 2),
6. One aliquot of each test plasma sample thawed quickly at 37°C.
- 25 7. 25µl thawed test plasma samples diluted with 2000µl of buffer working solution.
8. 50µl of diluted FVIII standards and diluted test plasma samples added to the wells of a 96-well plate according to a plate map and incubated for 4 minutes at 37°C.
9. 50µl of Factor reagent added to each well and incubated for 2 minutes at 37°C (High range standard curve) or 4 minutes at 37°C (Low range standard curve) .
- 30 10. 50µl of S-2765 + I-2581 substrate added to each well and incubated for 2 minutes at 37°C (High range standard curve) or 10 minutes at 37°C (Low range standard curve).

11. 50µl of 20% acetic acid stop solution added to each well. The colour in the wells turned a shade of yellow and the optical density was measured by a microplate reader at absorbance 405nm.
- 5 12. FVIII standard activity (IU/ml) plotted against absorbance at 405nm using a best-fit linear curve.
13. Test plasma sample absorbance read against the standard curve and the FVIII activity reported in IU/ml.
- 10 14. The mean (and median where indicated in individual studies) FVIII activity result calculated from the 3 mouse test plasma samples at each time-point and the data subjected to pharmacokinetic analysis.

Further tests for assessing FVIII concentration include:

Chromogenic FVIII Activity Assay

- 15 The Biophen FVIII:C Assay Kit Ref#221406 was used with plasma samples diluted 1:10 in assay buffer and run against both a Nuwiq™ and a human plasma reference standard curve. Each curve was generated by serial dilution of FVIII in canine FVIII deficient plasma, then 1:10 dilution in assay buffer. The standard range in both curves was 0.003-0.4U/mL, with linear range being 0.13-20 1.00U/mL. Assay was performed as per kit protocol.

One-Stage Factor VIII Assay – Siemens BCS-XP System

- 25 Samples were measured against a canine FVIII reference curve, generated using normal canine pooled plasma diluted in Owren's Veronal Buffer containing 2.5% canine FVIII deficient plasma. The range of the curve is 5-200%. Plasma samples were diluted 1:10 in Owren's Veronal Buffer, mixed with FVIII deficient plasma, then Actin FS was added. After an incubation of 3min, activation with CaCl₂ was initiated and time to clot was measured at 405nm.
- 30 In accordance with the second aspect described above, the method of treating haemophilia A in a subject comprises the step of administering a composition comprising a colloidal particle. The colloidal particle comprises (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl
- 35 inositol (PI). The second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer. The subject has previously generated antibody inhibitors to Factor VIII (FVIII).

The biocompatible hydrophilic polymer may be selected from the group consisting of polyalkylethers, polylactic acids and polyglycolic acids, preferably, the biocompatible hydrophilic polymer is polyethylene glycol (PEG). The polyethylene glycol may have a molecular weight of between about 500 to about 5000 Daltons, preferably approximately 2000 Daltons or 5000 Daltons.

5

The phosphatidyl choline (PC) may be 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).

The second amphipathic lipid may be N-(Carbonyl-methoxypolyethyleneglycol)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG) such as N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000) or N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000).

10

The colloidal particle may further comprise (iii) a non-ionic surfactant.

15

The composition may further comprise a Factor VIII (FVIII) molecule. Alternatively, the method may comprise a further step of separately or subsequently administering a composition comprising a Factor VIII (FVIII) molecule.

20

The composition comprising the colloidal particle and the Factor VIII may be administered as part of a treatment regimen. The composition comprising the colloidal particle and the Factor VIII may be administered and/or re-dosed at intervals to allow the blood concentration of FVIII to be maintained at a consistent level, providing a sustained, constant and predictable therapeutic effect without the need to wait to re-dose until the concentration of FVIII in the blood of the patient reaches sub-therapeutic or therapeutically irrelevant levels, suitably every 2, 3, 4, 5, 6, 7, 14, 21 days, such as 2 to 21 days, 4 to 14 days, 4 to 7 days.

25

Such a treatment regimen reduces the amount of FVIII required to treat a patient suffering from haemophilia A.

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The haemophilia may be congenital haemophilia A (cHA) or acquired haemophilia A (aHA).

The subject may be a paediatric patient.

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The invention also includes uses of a composition comprising a colloidal particle in the manufacture of a medicament for the treatment of haemophilia A in a subject wherein the subject has previously generated antibody inhibitors to Factor VIII (FVIII).

40

In accordance with the third aspect described above, the kit comprises (i) a composition comprising a colloidal particle and (ii) a composition comprising a Factor VIII (FVIII) molecule for use in the

treatment of haemophilia A in a subject. The subject has previously generated antibody inhibitors to a critical intrinsic blood factor. The colloidal particle is be composed (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI). The second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.

Lyophilised formulations of the invention may be supplied as separate dosage forms along with a suitable diluent, adjuvant or excipient provided also, e.g. a physiologically acceptable buffer. The colloidal particle and/or Factor VIII (FVIII) of the kit may be provided as a lyophilised formulation. Alternatively, the Factor VIII (FVIII) of the kit may be provided as a lyophilised formulation and colloidal particle may be provided as a solution for reconstitution of the Factor VIII (FVIII). As described herein, such compositions may additionally comprise Factor VIII as a separate dosage form, or formulated with the colloidal particles as described herein. The lyophilised form of may be provided in a 500 IU vial. The colloidal particle and/or Factor VIII (FVIII) of the kit may be provided in aqueous form ready for use.

The colloidal particle may further comprise (iii) a non-ionic surfactant.

The kit optionally comprises instructions for use also.

In accordance with the fourth aspect described above, the kit comprises (i) a composition comprising a colloidal particle and (ii) a composition comprising a Factor VIII (FVIII) molecule for separate, simultaneous or subsequent use in the treatment of haemophilia A in a subject. The subject has previously generated antibody inhibitors to a critical intrinsic blood factor. The colloidal particle is be composed (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI). The second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.

Lyophilised formulations of the invention may be supplied as separate dosage forms along with a suitable diluent, adjuvant or excipient provided also, e.g. a physiologically acceptable buffer. The colloidal particle and/or Factor VIII (FVIII) of the kit may be provided as a lyophilised formulation. Alternatively, the Factor VIII (FVIII) of the kit may be provided as a lyophilised formulation and colloidal particle may be provided as a solution for reconstitution of the Factor VIII (FVIII). As described herein, such compositions may additionally comprise Factor VIII as a separate dosage form, or formulated with the colloidal particles as described herein. The lyophilised form of may be provided in a 500 IU vial. The colloidal particle and/or Factor VIII (FVIII) of the kit may be provided in aqueous form ready for use.

The colloidal particle may further comprise (iii) a non-ionic surfactant.

The kit optionally comprises instructions for use also.

5

In accordance with the fifth aspect described above, the dosage form of a pharmaceutical composition comprises a colloidal particle comprising (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl
10 serine (PS) and a phosphatidyl inositol (PI), wherein said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer for use in the treatment of haemophilia in a subject. The subject has previously generated antibody inhibitors to Factor VIII (FVIII).

15 The colloidal particle may further comprise a non-ionic surfactant.

The dosage form may be provided as suitable containers or vials containing the appropriate dose for a patient, for example as a 250 IU, 500 IU, 750 IU or 1000 IU vial. The dosage form may also be provided as a tablet or in liquid form. The dosage form may also be in lyophilised form.

20

A surprising technical effect demonstrated by the invention is achieved by masking the epitopes of FVIII that would ordinarily provoke an immune response and the subsequent production of anti-FVIII antibodies.

25 Without wishing to be bound by theory it is thought that these benefits derive from the non-covalent association of PEG Lip to the A3 domain of FVIII, thus shielding epitopes in the light chain domains of FVIII from recognition by the body's immune system; and/or preventing the endocytosis of FVIII by dendritic cells.

30 This effect is more pronounced in recombinant FVIII molecules, which are typically not administered with VWF which would naturally protect these epitopes in wild-type FVIII.

In addition to protection of the epitopes, the association with PEG Lip may also extend the half-life of FVIII by protecting FVIII from the normal proteolytic clearance mechanisms, extending the dosing
35 interval and reducing the total exposure of the patient to FVIII over time.

A surprising observation is described as follows:

A clinical trial was devised to examine the use of PEG Lip + FVIII in patients with inhibitors, where it
40 was hoped that the combination would prevent existing antibodies from attacking FVIII. As well as

patients with existing antibodies, some patients were included who had a history of developing antibodies when presented with FVIII but were not currently exhibiting antibodies.

Surprisingly it was found that these patients did not develop new antibodies when presented with the PEGLip + FVIII combination with a colloidal particle to FVIII molecule ratio of 15:1 to 16:1, implying that the PEGLip was able to shield the epitopes from dendritic / B-cells and/or prevent the endocytosis of FVIII.

An experiment was also conducted in haemophilia A dogs involved IV dosing with PEGLip + human FVIII as a control with a colloidal particle to FVIII molecule ratio of 15:1 to 16:1. Such experiments are usually difficult as the animal's immune system naturally reacts to the foreign (human) protein by producing antibodies. Surprisingly in this case the animal did not produce antibodies to the human protein when it was administered in the presence of PEGLip.

The following samples are made and tested according to the invention:

1. A series of colloidal particles (PEGLip) comprising a higher ratios of DSPE-PEG to POPC, wherein the PEG is PEG-2000.
2. A series of colloidal particles (PEGLip) comprising a ratios of DSPE-PEG to POPC, wherein the PEG is PEG-5000.
3. Colloidal particles according to point 1 and point 2 further comprising polysorbate 80.
4. A series of colloidal particles (F-PEGLip) comprising higher ratios of DSPE-PEG to POPC and/or high molecular weight PEG.

In certain embodiments, the following formulations are provided:

15 to 16:1 formulation

PEGLip particles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG(2000)) in a 97:3 molar ratio in a 50mM sodium citrate buffer in a 9% suspension formulated with FVIII (Nuwiq™, Octapharma AG) in a ratio of PEGLip particle to FVIII molecule of between 15 to 16:1.

7 to 8:1 formulation

PEGLip particles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG(2000)) in a 97:3 molar ratio in a 50mM sodium citrate buffer formulated in a 9% suspension with FVIII (Nuwiq™, Octapharma AG) in a ratio of PEGLip particle to FVIII molecule of between 7 to 8:1.

In alternative embodiments, the following formulations are provided:

15 to 16:1 formulation

PEGLip particles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG(2000)) in a 97:3 molar ratio and polysorbate 80 in a 9:1 w/w ratio (POPC + DSPE-PEG(2000):polysorbate 80) in a 50mM sodium citrate buffer in a 9% suspension formulated with FVIII (Nuwiq™, Octapharma AG) in a ratio of PEGlip particle to FVIII molecule of between 15 to 16:1.

10 7 to 8:1 formulation

PEGLip particles composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG(2000)) in a 97:3 molar ratio and polysorbate 80 in a 9:1 w/w ratio (POPC + DSPE-PEG(2000):polysorbate 80) in a 50mM sodium citrate buffer formulated in a 9% suspension with FVIII (Nuwiq™, Octapharma AG) in a ratio of PEGlip particle to FVIII molecule of between 7 to 8:1.

In one particular embodiment of the invention, there is provided a composition for use in the treatment of haemophilia A in a subject who has previously generated antibody inhibitors to a Factor VIII, as follows:

20

• colloidal particles composed of a first amphipathic lipid comprising a phosphatidyl choline moiety and a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI), in a 97:3 molar ratio (9:1 w/w), for example a 97:3 molar ratio of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000) or a weight-corrected ratio if an equivalent molar ratio of a heavier PEGylated lipid is used for example a w/w ratio of between 4:1 and 5:1 of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000).

• a diluent, such as a buffer (suitably at a physiologically acceptable pH, e.g. pH 6.7), for example a citrate buffer, optionally at a concentration of 50mM.

35 In an alternative embodiment, there is provided a composition for use in the treatment of haemophilia A in a subject who has previously generated antibody inhibitors to Factor VIII, as follows:

• colloidal particles composed of a first amphipathic lipid comprising a phosphatidyl choline moiety and a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI), in a 97:3 molar ratio (9:1 w/w), for example

40

- 5 a 97:3 molar ratio of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000) or a weight-corrected ratio if an equivalent molar ratio of a heavier PEGylated lipid is used for example a w/w ratio of between 4:1 and 5:1 of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000). The colloidal particle further comprising a non-ionic surfactant selected from the group consisting of a polyoxyethylene sorbitan, a polyhydroxyethylene stearate and a polyhydroxyethylene laurylether, for example polyoxyethylene (20) sorbitan monooleate
- 10
- a diluent, such as a buffer (suitably at a physiologically acceptable pH, e.g. pH 6.7), for example a citrate buffer, optionally at a concentration of 50mM.

15 Preferred features for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

The present invention will now be described with reference to the following examples which are present for the purposes of illustration only and should not be construed as being limitations on the invention. Reference is also made to the following drawings in which:

20

Figure 1 shows the effect of PEGlip and F-PEGlip on coagulation in ex-vivo blood of severe haemophiliacs with inhibitors.

25

Figure 2 shows the effect of PEGlip-FVIII on coagulation in ex-vivo blood of severe haemophiliacs with inhibitors.

Figure 3 shows the effect of PEGlip-FVIII on coagulation in ex-vivo blood of severe haemophiliacs with inhibitors.

30 EXAMPLES

The following examples use a technique known as rotational thromboelastometry (ROTEM) to assess various parameters of the clotting cascade and clot formation. The following abbreviations are used.

35

CT	Clotting Time	The time to the initiation of clot formation, taken as the time from initiation of analysis until clot firmness is 2mm as measured on a ROTEG plot
CFT	Clot Formation Time	a measure of the amplification of the clotting cascade, taken as the time in which clot firmness increases from 2 to 20mm
CT + CFT		the sum of these values. Both CT and CFT are measured in seconds
MCF	Maximal Clot Firmness	an assessment of the ultimate strength/firmness of the fibrin clot, measured in mm
Alpha		a measure of the rate of clot formation, assessed as the angle between the center line and the tangent to the curve through the 2mm amplitude point on a ROTEG plot

Table 1

Comparison of PEGLip and experimental formulation F-PEGLip

Ingredient (g per 100g)	PEGLip (PLP-00)	F-PEGLip (PLP-01)
POPC	8.333	6.68
mPEG-2000-DSPE	0.926	0.76
Polysorbate 80	0	0.85
Sodium Citrate Dihydrate	1.47	1.47
Water	89.271	90.24
Total	100	100
pH	6.5 - 7.2	6.5 - 7.2
POPC:mPEG-2000-DSPE (molar)	97:3	97:3
POPC:mPEG-2000-DSPE (w/w)	9:1	9:1
Total lipid:non-ionic surfactant (w/w)	n/a	9:1

5 **Example 1:**

Comparison of the clot-forming ability of rFVIII alone and in combination with PEGLip (PLP-00) in haemophilic platelet-rich human plasma (PRP) containing inhibitors.

Preparation of the PRP model:10 **Preparation of plasma pool**

Plasma aliquots from severe haemophilic patients ('SHP' single donors, 2ml/ aliquot) were thawed at 37°C for 4 min and pooled. Inhibitor plasma was added to SHP pool to prepare inhibitor SHP (I-SHP) with a final inhibitor concentration of 10 Bethesda Units (BU)/ml. In some experiments, a donor inhibitor plasma containing 7 BU/ml was used without further dilution in SHP.

15

Platelet wash

Platelet concentrate aliquots (0.5ml/tube) were supplemented with platelet activation inhibitors (PGE1- 50ng/ml, citric acid 5mM), incubated 1 min with rolling and additional 7 min at rest at room temp (15-25°C), and then centrifuged at 800g for 5 min at room temp. Platelet pellet was re-suspended in 4ml phosphate buffered saline (PBS) supplemented with platelet activation inhibitors and re-centrifuged as above.

Preparation of inhibitor-PRP (I-PRP) model

The washed platelet pellet was re-suspended in inhibitor-SHP (I-SHP) to a physiological count (250 platelets/ nL) to create inhibitor platelet-rich plasma (I-PRP). I-PRP aliquots were pooled. For the experiments where the products were incubated for 10 minutes with the I-PRP, inhibitor plasma from a single individual (148 BU/ml) was used. For the experiment where a 30 minute exposure to the I-PRP preceded ROTEG analysis, inhibitor plasma from three different donors (148 BU/ml, 106 BU/ml and 7 BU/ml) was used.

Results:

Experiment (a): This experiment studied the FVIII-dependent coagulation activity in inhibitor-positive plasma, a short time after injection. I-PRP was spiked with free rFVIII (Kogenate, Bayer) or PEGLiP:rFVIII at either 1.0 or 1.5IU FVIII/ml, 26:1 and 17:1 PEGLiP particles to FVIII molecules, respectively. ROTEM analysis was initiated after 10 min. The results are shown in Table 2:

Table 2

Comparison of 26:1 and 17:1 PEGLiP:rFVIII at 1.0 and 1.5 IU/ml respectively, assessed 10 minutes after exposure to anti-FVIII antibodies (Average $\pm$ SD)

Substrate	I-PRP		I-PRP	
Donor inhibitor level	148 BU/ml		148 BU/ml	
Experimental inhibitor level	10 BU/ml		10 BU/ml	
rFVIII conc.	1.0 IU/ml		1.5 IU/ml	
PEGLiP:FVIII*	n/a	26:1	n/a	17:1
CT (s)	2955 $\pm$ 916	1769 $\pm$ 279	1331 $\pm$ 121	888 $\pm$ 47
CFT (s)	2203 $\pm$ 201	1443 $\pm$ 224	840 $\pm$ 444	540 $\pm$ 197
CT+CFT (s)	5158 $\pm$ 1117	3211 $\pm$ 503	2171 $\pm$ 323	1428 $\pm$ 149
MCF (mm)	24 $\pm$ 1	40 $\pm$ 4	47 $\pm$ 1	52 $\pm$ 0

*PEGLiP particles : molecules of FVIII

Table 3

Comparison of PEGLiP:rFVIII vs. free rFVIII, assessed 30 minutes after exposure to PRP dosed with inhibitor plasma from three different donors (Average $\pm$ SD)

Substrate	I-PRP		I-PRP		I-PRP	
Inhibitor Donor	Donor 1		Donor 2		Donor 3	
Repeats	14 repeats		3 repeats		3 repeats	
Donor Inhibitor level	148 BU/ml		106 BU/ml		7 BU/ml	
Inhibitor level in experiment	10 BU/ml		10 BU/ml		7 BU/ml	
rFVIII conc.	2.0IU/ml		2.0IU/ml		1.5IU/ml	
mLiP:FVIII*	n/a	13:1	n/a	13:1	n/a	17:1
Test solution	free rFVIII	PEGLiP:rFVIII	free rFVIII	PEGLiP:rFVIII	free rFVIII	PEGLiP:rFVIII
CT (s)	1889 $\pm$ 796	1642 $\pm$ 496	1681 $\pm$ 548	1319 $\pm$ 336	1914 $\pm$ 470	1568 $\pm$ 203
CFT (s)	1076 $\pm$ 604	707 $\pm$ 342	1327 $\pm$ 685	580 $\pm$ 191	670 $\pm$ 233	415 $\pm$ 54
CT+CFT (s)	2878 $\pm$ 1157	2349 $\pm$ 794	3009 $\pm$ 1094	1899 $\pm$ 487	2584 $\pm$ 665	1983 $\pm$ 235
MCF (mm)	49 $\pm$ 14	53 $\pm$ 7	47 $\pm$ 12	53 $\pm$ 14	50 $\pm$ 1	55 $\pm$ 2
Alpha (deg.)	15 $\pm$ 16	27 $\pm$ 9	8 $\pm$ 14	30 $\pm$ 7	20 $\pm$ 18	38 $\pm$ 5

*PEGLiP particles : molecules of FVIII

5

Experiment (b): This experiment studied the FVIII dependent coagulation activity when the injected FVIII was exposed to inhibitory antibodies for a longer period. I-PRP was spiked with free rFVIII (Kogenate, Bayer) or PEGLiP:rFVIII (PLP-00) at either 1.5 or 2.0IU FVIII/ml, 17:1 and 13:1 PEGLiP particles to FVIII molecules, respectively. Inhibitory plasma from three different donors was used.

10

Discussion:

When assessed after 10 minutes, the PEGLiP:rFVIII formulation demonstrated a faster initiation of clotting (CT, 33-40% reduction), a faster amplification (CFT, 34-35% reduction) and a firmer, better quality clot (MCF, 11 to 67% increase) than rFVIII alone. When assessed after 30 minutes, the PEGLiP:rFVIII formulation demonstrated a faster initiation of clotting (CT, 13-22% reduction), a faster amplification (CFT, 34-56% reduction) and a firmer, better quality clot (MCF, 8 to 13% increase) than rFVIII alone. The large increase in the rate of clot formation (alpha, 80-275% increase) was particularly impressive.

15

20

In the longer incubation experiments, PEGLiP increased the efficacy of rFVIII against inhibition from multiple sources.

Conclusions:

Use of a combination of PEGLiP and rFVIII results in more efficient clot formation in the presence of FVIII inhibitory antibodies than FVIII alone as exemplified by:

- 5 - Faster onset of clot formation
- A more rapid amplification of the clotting cascade
- A better quality, stronger clot
- These enhancements are still evident after 30 minutes
- These enhancements are seen against inhibitors from three different sources, implying that
- 10 PEGLiP is effective against antibodies with a range of epitope specificities and indicating its potential utility in a wide population of patients.

Example 2:

Comparison of whole blood clotting time of PEGLiP-rFVIII and free rFVIII in whole blood containing
15 anti-FVIII antibodies.

This study used whole human blood in three separate experiments to examine the effectiveness of the PEGLiP product (PLP-00) in enhancing clotting efficacy in whole blood containing inhibitors. This importantly adds more of the components of the clotting cascade and also simulates a model of
20 acquired haemophilia. The three experiments were:

- a) An examination of the ability of PEGLiP to enhance the clotting capability of FVIII added to whole blood containing inhibitors
- b) A quantification of the effect size achieved by adding PEGLiP, with implications for FVIII dosing
- 25 c) An examination of the effects of varying the ratio of PEGLiP particles to FVIII molecules on the magnitude of clotting enhancement.

Blood Sampling:

Blood samples were collected from a healthy donor in a citrated tube. Blood was allowed to stand
30 for 15 min at room temperature prior to use and then was analysed within 4 hours of collection.

Whole blood ('WB', 250µl) was mixed with severe haemophilic patient plasma (SHP) or inhibitor plasma (50µl) to a final inhibitor concentration of ~15 BU/ml to create whole blood (WB) or inhibitor-whole blood (I-WB) substrates, and incubated for 10 min at 37°C. Then the test article (rFVIII (Kogenate, Bayer) or PEGLiP:rFVIII) and CaCl₂ solution were added and ROTEM analysis was
35 started immediately.

Results:

Experiment (a): This trial compared the clotting parameters of whole blood (WB) or inhibitor-whole blood (I-WB), and the clotting improvements obtained with either rFVIII alone (Kogenate, Bayer) or
40 rFVIII plus PEGLiP, at a ratio of 13:1 PEGLiP particles to rFVIII molecules (13:1 PEGLiP-FVIII). The experiment was repeated twice and the average results of the two repeats are shown in Table 4:

Table 4

Comparison 13:1 PEGLiP:rFVIII 1.0IU/ml with free rFVIII 1.0IU/ml in an I-WB model (average of two repeats $\pm$ SD)

Substrate	WB + SHP	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)
Inhibitor level in experiment	n/a	15 BU/ml	15 BU/ml	15 BU/ml
rFVIII conc	n/a	n/a	1.0 IU/ml	1.0 IU/ml
PEGLiP:FVIII *	n/a	n/a	n/a	13:1
CT	691 $\pm$ 36	4751 / >5603	1223 $\pm$ 30	1128 $\pm$ 238
CFT	249 $\pm$ 122	>990 / NA	1793 $\pm$ 247	843 $\pm$ 217
CT+CFT	940 $\pm$ 158	NA	3016 $\pm$ 277	1970 $\pm$ 21
MCF	59 $\pm$ 2	4 / NA	42 $\pm$ 2	53 $\pm$ 7

5 *PEGLiP particles : molecules of FVIII

Discussion:

The results show that the addition of inhibitors to the whole blood severely delay the clotting cascade. This is rectified to some extent with the addition of rFVIII. Supplying rFVIII with PEGLiP in a 13:1 ratio of PEGLiP particles to rFVIII molecules shortens the time to clot even further than FVIII alone and produces a better quality clot (increased MCF).

10

Experiment (b): This trial compared the relative clotting efficacy in inhibitor-whole blood (I-WB) of a combination of 13:1 PEGLiP:rFVIII at 1IU/ml to free rFVIII at concentrations of 1.0 IU/ml and 4.0 IU/ml.

15

Table 5

Comparison of 13:1 PEGLiP-rFVIII 1.0IU/ml with free rFVIII 1.0IU/ml and 4.0IU/ml in an I-WB model (average $\pm$ SD)

Substrate	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)
Inhibitor level in experiment	15 BU/ml	15 BU/ml	15 BU/ml	15 BU/ml
rFVIII conc	n/a	1.0 IU/ml	4.0 IU/ml	1.0 IU/ml
PEGLiP:FVIII*	n/a	n/a	n/a	13:1
CT	>5880	1745	641	841
CFT	NA	1663	343	377
CT+CFT	NA	3408	984	1218
MCF	NA	39	53	62
Alpha (deg)	NA	NA	42	38

*PEGLiP particles : molecules of FVIII

5

Discussion: The results show that by administering PEGLiP with rFVIII in a 13:1 formulation, the efficacy of a 1IU/ml concentration of rFVIII is improved such that it approaches the efficacy of a concentration four times as great. This implies that administering PEGLiP in combination with rFVIII at these ratios in HA inhibitor patients could be FVIII-sparing, leading to smaller and/or less frequent injections.

10

Experiment (c): Two trials were performed, where the ratio of PEGLiP particles to rFVIII was 13:1 or 1:1. The results are given in Table 6, below.

15 Table 6

Comparison of 13:1 and 1:1 PEGLiP:rFVIII 1.0IU/ml and free rFVIII 1.0IU/ml

Substrate	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)	WB + Inhibitor plasma (I-WB)
Inhibitor level in experiment	15 BU/ml	15 BU/ml	15 BU/ml	15 BU/ml
rFVIII concn	n/a	1.0 IU/ml	1.0 IU/ml	1.0 IU/ml
PEGLiP:FVIII*	n/a	n/a	13:1	1:1
CT	>3971	1719	431	824
CFT	NA	655	328	507
CT+CFT	NA	2374	759	1331
MCF	NA	47	57	53
Alpha (deg)	NA	NA	43	30

*PEGLiP particles: molecules of FVIII

Discussion:

Both formulations that contained PEGLiP showed enhanced clotting processes over the formulation with rFVIII alone. The formulation with a greater proportion of PEGLiP (13:1) showed greater
5 enhancement compared to the 1:1 formulation. In particular, the alpha parameter, which provides an indication of thrombin burst at the start of the clotting process shows enhancement with the 13:1 formulation over the 1:1 formulation.

Conclusions:

- 10 (a) PEGLiP in association with rFVIII can enhance the efficacy of rFVIII in the presence of inhibitors in whole blood. This implies a potential utility in acquired haemophilia as well as congenital haemophilia.
- (b) The size of the enhancement is nearly equivalent to a quadrupling of the dose of rFVIII, with important implications for the size and frequency of rFVIII dosing
- 15 (c) Because PEGLiP additionally binds native FVII (and later, FVIIa) and associates this with the platelets, reducing the concentration of PEGLiP particles to FVIII molecules reduces this capability, reducing both the time to clot initiation and thrombin burst, reducing the amplification of the cascade.

20 **Example 3:**

Ex-vivo studies of the effect of PEGLiP + FVIII on coagulation in a model of severe haemophiliac blood with inhibitors.

This study used whole human blood to examine the effectiveness of F-PEGLiP product (PLP-01) in
25 enhancing clotting efficacy in whole blood containing inhibitors.

Method:

A simulated solution of severe haemophilia A blood with inhibitors was created by dosing a sample of normal Whole Blood (WB) drawn from a healthy volunteer with 70BU/ml FVIII deficient plasma
30 with inhibitors (70BU/ml, George King Biomedical). Sufficient inhibitor plasma was added and the mixed incubated to deplete the blood of FVIII and to leave 15 Bethesda Units/ml as a simulation of Inhibitor Blood (IB).

Samples of WB, IB or IB spiked with a test article (see Table 7), and were subjected to analysis by
35 ROTEM, using a low amount of tissue factor activator.

Results:**Table 7**

Test Article	FVIII IU/ml	F-PEGLip mg/ml	Ratio Liposomes:FVIII	CT	CFT	CT+CFT
Whole blood (WB)			N/A	507	137	644
Inhibitor Blood (IB)			N/A	1,234	943	2,177
IB + FVIII control	91	0	N/A	1,129	740	1,869
IB + PLP-00 control	0	90	N/A	1,415	922	2,337
IB + PLP-00 + FVIII	91	35	10:1	1,274	950	2,224
IB + PLP-00 + FVIII	72	84	29:1	1,487	1,153	2,640
IB + PLP-00 + FVIII	25	88	86:1	1,111	1,214	2,325
IB + PLP-01 + FVIII	81	62	19:1	1217	687	1,904
IB + PLP-01 + FVIII	72	63	22:1	855	377	1,232

- 5 Spiking whole blood with inhibitors to FVIII to create a model of inhibitor blood resulted in extended clotting time in inhibitor whole blood (I-WB). Clotting time was not restored with either FVIII or PEG Lip alone. When FVIII was co-administered with F-PEGLip (PLP-01) coagulation was restored with reduced clotting time. A reduced clotting time in a HA with inhibitor model with FVIII + F-PEGLip (PLP-01) was observed. See also Figure 1.

10

Conclusion:

Both PLP-00 and PLP-01 show a trend of more effective restoration of coagulation in inhibitor blood at higher PEG Lip:FVIII ratios.

15 **Example 4:**

Ex-vivo studies of the effect of PEG Lip:FVIII on coagulation in blood of severe haemophiliacs with inhibitors.

- 20 These experiments evaluated the effects of the addition of FVIII, PEG Lip (PLP-00), varying ratios of PEG Lip(PLP-00):FVIII (10:1, 29:1, 86:1) or a 28:1 mixture of F-PEGLip:FVIII (PLP-01) to a citrate anti-coagulated whole blood sample from a haemophilic A dog with low titre anti-FVIII antibodies against both human (5.6 BU) and canine (3.2 BU) FVIII. Samples of the test product were added to inhibitor blood, mixed gently, then added to a ROTEM cup, followed by 10 µl CaCl₂. Coagulation was followed for 60 minutes using the NATEM programme.

25

Test Articles:

FVIII: 1000IU/ml FVIII: Nuwiq (Octapharma, 500IU vial reconstituted with 0.5ml sterile water)

PLP-00: 90mg/ml PEG Lip: 9% PEGylated liposomes in 50mM citrate buffer pH 6.7 (batch 19-740)

PLP-01: 68mg/ml F-PEGLip: 6.8% Tweenylated PEGylated liposomes in 50mM citrate buffer pH 6.7 (batch 09-01-2020)

Control: 50mM sodium citrate buffer pH 6.7

5 **Results:**

Table 8

Test Articles	FVIII IU/ml	F-PEGLip mg/ml	Ratio Liposomes:FVIII	CT	CFT	CT+CFT
Inhibitor Blood (IB)	#N/A	#N/A	N/A	5333	n/c	5333
IB + FVIII control	91	0	N/A	4343	n/c	4343
IB + PLP-00 control	0	90	N/A	4376	n/c	4376
IB + PLP-00 + FVIII	91	35	10:1	4984	n/c	4984
IB + PLP-00 + FVIII	72	84	29:1	638	338.5	976.5
IB + PLP-00 + FVIII	25	88	86:1	647	334	981
IB + PLP-01 + FVIII	48	55	28:1	669	437	1106

n/c no clotting

Prior to any treatment, the inhibitor blood of the subject did not clot within the required timescale.

- 10 This was not resolved when the inhibitor blood was spiked with FVIII alone or with PLP-00 alone. Similarly, when the inhibitor blood was spiked with a 10:1 mixture of PLP-00 + FVIII, there was no correction to the coagulation time.

- 15 However, mixtures of 29:1 and 86:1 PLP-00 + FVIII and 28:1 PLP-01 + FVIII all significantly reduced the coagulation times of inhibitor blood. See also Figure 2.

- 20 In conclusion, the addition of PLP-00 to FVIII in ratios of 29:1 and above prevented the inhibition of the action of FVIII by the inhibitors in the blood. However low levels of PLP-00 (10:1) were unable to protect the FVIII from inhibition. This implies there is a critical ratio of PEGlip:FVIII between 10:1 and 29:1 where PEGlip provides protection against antibody inhibitors.

A second formulation of PEGylated liposomes incorporating additional PEG (F-PEGLip/PLP-01) also provided protection for FVIII against inhibitor antibody degradation at a 28:1 PLP-01:FVIII ratio.

25 **Example 5:**

Clinical study of PEGLiP + FVIII in inhibitor patients

Studies are underway to demonstrate that PEGLiP-FVIII at a determined ratio restores coagulation in inhibitor patients.

- 30 Inhibitor-prone HA patients did not generate inhibitors when injected with PEGLiP+FVIII (PLP-00), while still showing coagulation correction.

Four severe haemophilic patients with a history of generating antibody inhibitors to FVIII presented to the trial. Due to their history, these patients are unable to receive replacement FVIII as a prophylactic therapy due to the risk of them generating inhibitors. In the trial three of these patients initially presented without inhibitors and one presented with a low titre (<5BU). All patients were

5 dosed with PEGlip + FVIII at 22mg/kg PEGlip and 35IU/kg recombinant humanised FVIII (a colloidal particle:FVIII ratio of 15:1 to 16:1). Over the first week of assessment, all patients experienced significant coagulation correction, enabling them to be dosing every 6 days on average (range 4 – 7 days) over the following 6 weeks. Despite re-dosing on a once weekly basis over 6 weeks, none of the three inhibitor-prone patients who presented without inhibitors generated inhibitors to the

10 treatment. The one patient who presented with low titre inhibitors experienced a small, clinically insignificant rise during the first stage which actually reduced during the repeated dosing on the second phase. It is proposed that the inability of the PEGlip-FVIII to stimulate inhibitor generation in inhibitor-prone individuals will make the product exceptionally suitable for the treatment of previously untreated patients or minimally treated patients to prevent the generation of inhibitors in these

15 vulnerable individuals.

Table 9

Severe HA Patients with Inhibitors (N=4)	Mean (SD)	Median (Min; Max)
Frequency of PEGlip-FVIII injections determined in Stage A (days)	6.0 (1.4)	6.5 (4.0; 7.0)
Frequency of PEGlip-FVIII injections administered during Stage B (days)	5.1 (1.2)	5.2 (3.6; 6.3)
History of spontaneous bleeding episodes during 24 weeks prior to enrollment (episodes/month)	0.9 (0.4)	0.8 (0.5; 1.3)
Spontaneous bleeding episodes evaluated during Stage B (episodes/month) - 3 of the 4 patients did not experience any spontaneous bleeds	0.5 (0.9)	0.0 (0.0; 0.9)
Anti-FVIII antibodies (Bethesda Units) - Screening	1.1 (2.2)	0.0 (0.0; 4.4)
Anti-FVIII antibodies (Bethesda Units) - End of Stage A	2.0 (4.0)	0.0 (0.0; 8.0)
Anti-FVIII antibodies (Bethesda Units) - End of Stage B	1.7 (3.4)	0.0 (0.0; 6.8)

Conclusion:

20 A ratio of between 15:1 to 16:1 of PEGlip to FVIII both lowers the risk of bleeding events in patients with inhibitors to FVIII, as well as in patients who are prone to developing inhibitors to FVIII, without stimulating the production of further, significant amounts of inhibitors.

Summary of Examples

25 Ratios of PEGlip:FVIII of 10:1 and below (Example 1, Example 2 - experiment c, Example 3 and Example 4) are less effective at improving coagulation than higher ratios (13:1 and above). Clinical experiments show that a ratio of 15:1 to 16:1 not only prevents bleeds in inhibitor patients with haemophilia A but that this formulation is both effective in the presence of inhibitors and prevents the generation of inhibitors in inhibitor-prone patients.

Example 6:

Ex-vivo studies of the effect of PEG Lip:FVIII on coagulation in blood of severe haemophiliacs with inhibitors.

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These experiments evaluated the effects of the addition of FVIII, PEG Lip (PLP-00), varying ratios of PEG Lip:FVIII (PLP-00; 10:1, 15:1, 20:1, 25:1, 30:1, 90:1) and varying ratios of F-PEG Lip:FVIII (PLP-01; 10:1, 20:1, 30:1) to a citrate anti-coagulated whole blood sample from a haemophilic A dog with low titre anti-FVIII antibodies against both human (5.6 BU) and canine (3.2 BU) FVIII. Samples of the test product were added to inhibitor blood, mixed gently, then added to a ROTEM cup, followed by 10 µl CaCl₂. Coagulation was followed for 60 minutes using the NATEM programme. The effectiveness was judged by the Clotting Time that was achieved with each treatment as a percentage of the untreated haemophilic inhibitor blood.

Test Articles:

FVIII: 1000IU/ml FVIII: Nuwiq (Octapharma, 500IU vial reconstituted with 0.5ml sterile water)

PLP-00: 90mg/ml PEG Lip: 9% PEGylated liposomes in 50mM citrate buffer pH 6.7 (batch 19-740)

PLP-01: 68mg/ml F-PEG Lip: 6.8% Tweenylated PEGylated liposomes in 50mM citrate buffer pH 6.7 (batch 09-01-2020)

Control: 50mM sodium citrate buffer pH 6.7

Results:**Table 10**

Effect of PEG Lip (PLP-00) and FlexPEG Lip (PLP-01) in combination with FVIII in reducing

Clotting Time in ex vivo severe haemophilic blood with inhibitors

Test Articles	FVIII IU/ml	PLP-00/PLP-01 mg/ml	Liposomes:FVIII	CT as % IB Control
Control – Inhibitor Blood (IB)	#N/A	#N/A	N/A	100%
IB + FVIII control	91	0	N/A	96%
IB + PEG Lip control	0	90	N/A	82%
IB+PEG Lip-FVIII	91	36	10:1	96%
IB+PEG Lip-FVIII	91	56	15:1	39%
IB+PEG Lip-FVIII	91	74	20:1	29%
IB+PEG Lip-FVIII	81	83	25:1	25%
IB+PEG Lip-FVIII	70	84	30:1	18%
IB+PEG Lip-FVIII	25	88	90:1	12%
IB+FlexPEG Lip-FVIII	48	19	10:1	100%
IB+FlexPEG Lip-FVIII	48	39	20:1	100%
IB+FlexPEG Lip-FVIII	48	57	30:1	38%

Prior to any treatment, the inhibitor blood of the subject did not clot within the required timescale. This was not resolved when the inhibitor blood was spiked with FVIII alone or with PLP-00 alone. Similarly, when the inhibitor blood was spiked with a 10:1 mixture of PLP-00 + FVIII, there was no
 5 correction to the coagulation time.

However, mixtures of $\geq 15:1$ PLP-00 + FVIII and 30:1 PLP-01 + FVIII all significantly reduced the coagulation times of inhibitor blood. See also Figure 3.

10 In conclusion, the addition of PLP-00 to FVIII in ratios of 15:1 and above prevented the inhibition of the action of FVIII by the inhibitors in the blood. However low levels of PLP-00 (10:1) were unable to protect the FVIII from inhibition. This implies there is a critical ratio of PEGLiP:FVIII between 10:1 and 15:1 where PEGLiP begins to provide protection against antibody inhibitors. A ratio of 90:1 provides little benefit over a ratio of 30:1, implying that there is an optimum PEGLiP-sparing ratio
 15 between 15:1 and 30:1

A second formulation of PEGylated liposomes incorporating additional PEG (F-PEGLiP/PLP-01) also provided protection for FVIII against inhibitor antibody degradation at a 30:1 PLP-01:FVIII ratio, although the lower limit of effectiveness of this formulation is higher than 20:1, a ratio at which PLP-
 20 00 still provides some efficacy.

Example 7:

Clinical study of PEGLiP + FVIII in inhibitor patients

25 A phase 2 clinical trial was undertaken to demonstrate that PEGLiP-FVIII at a determined ratio restores coagulation in inhibitor patients. The study built upon the findings described in Example 5.

Severe HA patients who either had inhibitors at screening, or whose clinical record showed them to be prone to develop inhibitors on exposure to FVIII did not increase their inhibitor titre or generate
 30 inhibitors, respectively, when injected with PEGLiP+FVIII (PLP-00+simoctocog alfa), while still showing coagulation correction and a reduction in bleeding frequency.

Thirteen severe haemophilic patients with a history of generating antibody inhibitors to FVIII presented to the trial. Due to their history, eight of these patients are unable to receive replacement
 35 FVIII as a prophylactic therapy due to the risk of them generating inhibitors. The other 5 patients presented at screening with active inhibitor titres (mean 2.4 Bethesda Units). All patients were dosed with PEGLiP + FVIII (simoctocog alfa) at 22mg/kg PEGLiP and 35IU/kg recombinant humanised FVIII (a colloidal particle:FVIII ratio of 15:1 to 16:1). Over the first week of assessment, all patients experienced significant coagulation correction, enabling them to be dosing every 5.5 days on
 40 average (range 3.0 – 7.4 days) over the following 6 weeks, representing a significant extension in dosing interval (normal dosing interval for simoctocog alfa is every other day or 2-3 times per week).

Despite this lower frequency of dosing, bleeding events were reduced significantly in the patients, with average monthly bleed rates dropping from an average of 1 per month (12.3 per annum) to 0.3 per month (3.2 per annum).

- 5 Despite re-dosing on a once weekly basis over 6 weeks, none of the eight inhibitor-prone patients who presented without inhibitors generated inhibitors to the treatment. In addition, the five patients who presented with inhibitors did not experience a significant rise in inhibitor titre during the 6 weeks prophylactic stage of that trial. It is proposed that as well as a treatment for inhibitor-presenting and inhibitor-prone patients, the inability of the PEGlip-FVIII to stimulate inhibitor generation in inhibitor-prone individuals will additionally make the product exceptionally suitable for the treatment of previously untreated patients or minimally treated patients to prevent the generation of inhibitors in these vulnerable individuals.

Table 11

	Inhibitor patients No active inhibitors	Inhibitor patients Active inhibitors at screening	Total inhibitor patients
n =	8	5	13
Bethesda titres (units)			
At screening			
Mean (SD) units	0.0 (0)	2.4 (1.3)	0.9 (1.4)
Median [Min; Max] units	0.0 [0; 0]	2.0 [1.3; 4.4]	0.0 [0.0; 4.4]
At end of stage A			
Mean (SD) units	0.0 (0)	2.5 (3.1)	1.0 (2.2)
Median [Min; Max] units	0.0 [0; 0]	1.1 [1.0; 8.0]	0.0 [0.0; 8.0]
At end of stage B			
Mean (SD) units	0.0 (0)	2.4 (2.5)	0.9 (1.9)
Median [Min; Max] units	0.0 [0; 0]	1.3 [0.9; 6.8]	0.0 [0.0; 6.8]
Change (Stage B- screening)	0	0	0.0
(p-value)	n/a	P=0.975	p>0.999
Frequency of PEGlip-FVIII injections administered during Stage B (days)			
Mean (SD)			5.5 (1.3)
Median [Min; Max]			5.5 [3.0; 7.4]
Bleeding Episodes per month 24 weeks prior to enrolment			
Mean (SD)	0.9 (0.3)	1.2(0.2)	1.0 (0.3)
Median [Min; Max]	1.0 [0.5; 1.3]	1.3 [0.8;1.3]	1.0 [0.5; 1.3]
During Stage B			
Mean (SD)	0.2 (0.7)	0.3 (0.4)	0.3 (0.6)
Median [Min; Max]	0.0 [0.0; 1.9]	0.0 [0.0; 0.8]	0.0 [0.0; 1.9]
Mean (Stage B – Screening)	-0.7	-0.9	-0.8
(p-value)			p<0.005

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CLAIMS

1. A composition comprising a colloidal particle comprising (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a
5 phosphatidyl serine (PS) and a phosphatidyl inositol (PI),
wherein said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.
for use in the treatment of haemophilia A in a subject,
wherein the subject has previously generated antibody inhibitors to Factor VIII (FVIII).
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2. The composition for use of claim 1, wherein the biocompatible hydrophilic polymer is selected from the group consisting of polyalkylethers, polylactic acids and polyglycolic acids.
3. The composition for use of claim 1 or claim 2, wherein the biocompatible hydrophilic polymer
15 is polyethylene glycol (PEG).
4. The composition for use of claim 3, wherein the polyethylene glycol has a molecular weight of between about 500 to about 5000 Daltons.
- 20 5. The composition for use of claim 4, wherein the polyethylene glycol has a molecular weight of about 2000 Daltons or about 5000 Daltons.
6. The composition for use of any one of claims 1 to 5, wherein the phospholipid is N-(Carbonyl-methoxypolyethyleneglycol)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG).
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7. The composition for use of any one of claims 1 to 6, wherein the phospholipid is N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG2000) or N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000).
30
8. The composition for use of any one of claims 1 to 7, wherein the phosphatidyl choline (PC) is 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).
9. The composition for use of any one of claims 1 to 8, wherein the composition comprises the
35 first amphipathic lipid and the second amphipathic lipid in a molar ratio of from 90 to 99:10 to 1.
10. The composition for use of claim 9, wherein the composition comprises the first amphipathic lipid and the second amphipathic lipid in a molar ratio of 97:3.

11. The composition for use of any one of claims 1 to 10, wherein the colloidal particle further comprises (iii) a non-ionic surfactant selected from the group consisting of a polyoxyethylene sorbitan, a polyhydroxyethylene stearate and a polyhydroxyethylene laurylether.
- 5 12. The composition for use of claim 11, wherein the non-ionic surfactant is polyoxyethylene (20) sorbitan monooleate.
13. The composition for use of claim 11 or claim 12, wherein the colloidal comprises the first
10 amphipathic lipid and the second amphipathic lipid to the non-ionic surfactant in a ratio of from 30:1 to 2:1 w/w.
14. The composition for use of claim 1, wherein the composition comprises the first amphipathic
lipid to the second amphipathic lipid to the non-ionic surfactant in a ratio of from 10 to 40:1:0 to 4
w/w.
- 15 15. The composition for use of any one of claims 1 to 14, wherein the composition further
comprises a Factor VIII (FVIII) molecule.
16. The composition for use of claim 15, wherein the composition comprises the colloidal
20 particle and the Factor VIII (FVIII) molecule in a stoichiometric ratio of from 1 to 90:1.
17. The composition for use of claim 15 or claim 16, wherein the composition comprises the
colloidal particle and the Factor VIII (FVIII) molecule in a stoichiometric ratio of 10 to 20:1 or 5 to
10:1.
- 25 18. The composition for use of any one of claims 1 to 17, wherein the haemophilia A is congenital
haemophilia A (cHA).
19. The composition for use of any one of claims 1 to 17, wherein the haemophilia A is acquired
30 haemophilia A (aHA).
20. The composition for use of any one of claims 1 to 19, wherein the composition further
comprises a therapeutically active compound.
- 35 21. The composition for use of any one of claims 1 to 20, wherein the composition further
comprises an excipient, diluent or adjuvant.
22. The composition for use of any one of claims 1 to 21, wherein the subject is a paediatric
subject.
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23. A method of treating haemophilia A in a subject comprising the step of:
administering a composition comprising a colloidal particle comprising (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a
5 phosphatidyl serine (PS) and a phosphatidyl inositol (PI),
wherein said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer, and
wherein the subject has previously generated antibody inhibitors to Factor VIII (FVIII).
- 10 24. The method of claim 23, wherein the biocompatible hydrophilic polymer is selected from the group consisting of polyalkylethers, polylactic acids and polyglycolic acids.
25. The method of claim 23 or claim 24, wherein the biocompatible hydrophilic polymer is polyethylene glycol (PEG).
- 15 26. The method of claim 25, wherein the polyethylene glycol has a molecular weight of between about 500 to about 5000 Daltons.
27. The method of claim 26, wherein the polyethylene glycol has a molecular weight of about
20 2000 Daltons or about 5000 Daltons.
28. The method of any one of claims 23 to 27, wherein the phosphatidyl choline (PC) is 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC).
- 25 29. The method of any one of claims 23 to 28, wherein the phospholipid is N-(Carbonyl-methoxypolyethyleneglycol)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG).
30. The method of any one of claims 23 to 29, wherein the phospholipid is N-(Carbonyl-methoxypolyethyleneglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-
30 PEG2000) or N-(Carbonyl-methoxypolyethyleneglycol-5000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG5000).
31. The method of any one of claims 23 to 30, wherein the colloidal particle further comprises
35 (iii) a non-ionic surfactant.
32. The method of any one of claims 23 to 31, wherein the composition further comprises a Factor VIII (FVIII) molecule.
33. The method of any one of claims 23 to 31, wherein the method comprises a further step of
40 separately or subsequently administering a composition comprising a Factor VIII (FVIII) molecule.

34. The method of any one of claims 23 to 33, wherein the haemophilia A is congenital haemophilia A (cHA).
- 5 35. The method of any one of claims 23 to 33, wherein the haemophilia A is acquired haemophilia A (aHA).
36. The method of any one of claims 23 to 35, wherein the subject is a paediatric subject.
- 10 37. A kit comprising (i) a composition comprising a colloidal particle and (ii) a composition comprising a Factor VIII (FVIII) molecule for use in the treatment of haemophilia A in a subject, wherein the subject has previously generated antibody inhibitors to Factor VIII (FVIII),
wherein the colloidal particle comprises (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid
15 moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI),
wherein said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.
- 20 38. The kit of claim 37, wherein the colloidal particle further comprises (iii) a non-ionic surfactant.
39. A kit comprising (i) a composition comprising a colloidal particle and (ii) a composition comprising a Factor VIII (FVIII) molecule for separate, simultaneous or subsequent use in the
25 treatment of haemophilia A in a subject, wherein the subject has previously generated antibody inhibitors to Factor VIII (FVIII),
wherein the colloidal particle comprises (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a phosphatidyl ethanolamine (PE), a phosphatidyl
30 serine (PS) and a phosphatidyl inositol (PI),
wherein said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer.
40. The kit of claim 39, wherein the colloidal particle further comprises (iii) a non-ionic
35 surfactant.
41. A dosage form of a pharmaceutical composition comprising a colloidal particle comprising (i) a first amphipathic lipid comprising a phosphatidylcholine (PC) moiety and (ii) a second amphipathic lipid comprising a phospholipid moiety selected from the group consisting of a
40 phosphatidyl ethanolamine (PE), a phosphatidyl serine (PS) and a phosphatidyl inositol (PI),

wherein said second amphipathic lipid comprises a phospholipid moiety derivatised with a biocompatible hydrophilic polymer
for use in the treatment of haemophilia A in a subject,
wherein the subject has previously generated antibody inhibitors to Factor VIII (FVIII).

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42. The dosage form of claim 41, wherein the colloidal particle further comprises (iii) a non-ionic surfactant.