CLAIMS

What is claimed is:

1) An aptamer that binds to tissue factor pathway inhibitor (TFPI) wherein the aptamer competes, either directly or indirectly, for binding to TFPI with a reference antibody selected from the group consisting of: AD4903.

2) An aptamer that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs.: 4, 1, 2, 3, 5, 6, 7, 8, 9 and 10.

3) The aptamer of claim 1, wherein the TFPI is human TFPI.

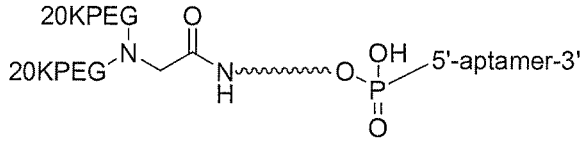
4) The aptamer of claim 2 or 3, wherein the aptamer has a dissociation constant for human TFPI of 100 nM or less.

5) The aptamer of claim 2, 3 or 4, wherein the aptamer comprises at least one chemical modification.

6) The aptamer of claim 5, wherein the modification is selected from the group consisting of: a chemical substitution at a sugar position, a chemical substitution at an internucleotide linkage, and a chemical substitution at a base position.

7) The aptamer of claim 5, wherein the modification is selected from the group consisting of: incorporation of a modified nucleotide; a 3' cap; a 5' cap; conjugation to a high molecular weight, non-immunogenic compound; conjugation to a lipophilic compound; incorporation of a CpG motif; and incorporation of a phosphorothioate or phosphorodithioate into the phosphate backbone.

8) An aptamer or a salt thereof that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises the following structure:



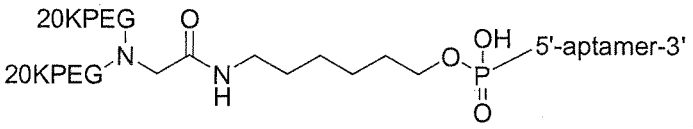
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wherein HN ~~~~ PO_3H is from a 5'-amine linker phosphoramidite, and

wherein the aptamer comprises the nucleic acid sequence of mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 2), wherein "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-O Methyl containing nucleotide, and wherein 20KPEG is a mPEG moiety having a molecular weight of 20 kDa.

9) The aptamer of claim 8, wherein the linker is selected from the group consisting of: 2-18 consecutive CH_2 groups, 2-12 consecutive CH_2 groups, 4-8 consecutive CH_2 groups and 6 consecutive CH_2 groups.

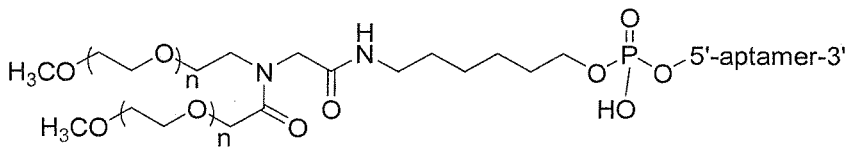
10) An aptamer or a salt thereof that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises the following structure:



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wherein the aptamer comprises the nucleic acid sequence of mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 2), wherein "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-O Methyl containing nucleotide, and wherein 20KPEG is a mPEG moiety having a molecular weight of 20 kDa.

11) An aptamer or a salt thereof that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises the following structure:



,

wherein the aptamer comprises the nucleic acid sequence of mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 2), wherein "n" is approximately 450, "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-O Methyl containing nucleotide.

12) An aptamer that binds to human tissue factor pathway inhibitor (TFPI) wherein the aptamer competes for binding to TFPI with a reference aptamer selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10.

13) An aptamer that binds to human tissue factor pathway inhibitor (TFPI) and comprises a stem and loop motif having the nucleotide sequence of SEQ ID NO: 4, wherein:
a) any one or more of nucleotides 1, 2, 3, 4, 6, 8, 11, 12, 13, 17, 20, 21, 22, 24, 28, 30 and 32 may be modified from a 2'-OMe substitution to a 2'-deoxy substitution;
b) any one or more of nucleotides 5, 7, 15, 19, 23, 27, 29 and 31 may be modified from a 2'-OMe uracil to either a 2'-deoxy uracil or a 2'-deoxy thymine;
c) nucleotide 18 may be modified from a 2'-OMe uracil to a 2'-deoxy uracil; and/or
d) any one or more of nucleotides 14, 16 and 25 may be modified from a 2'-deoxy cytosine to either a 2'-OMe cytosine or a 2'-fluoro cytosine.

14) An aptamer that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises a primary nucleic acid sequence selected from the group consisting of SEQ ID NOs.: 4, 1, 2, 3, 5, 6, 7, 8, 9 and 10.

CLAIMS

What is claimed is:

1) An aptamer that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs.: 4, 1, 2, 3, 5, 6, 7, 8, 9 and 10.

2) The aptamer of claim 1, wherein the TFPI is human TFPI.

3) The aptamer of claim 1 or 2, wherein the aptamer has a dissociation constant for human TFPI
of 100 nM or less.

4) The aptamer of claim 1, 2 or 3, wherein the aptamer comprises at least one chemical modification.

5) The aptamer of claim 4, wherein the modification is selected from the group consisting of: a chemical substitution at a sugar position, a chemical substitution at an internucleotide linkage, and a chemical substitution at a base position.

6) The aptamer of claim 4, wherein the modification is selected from the group consisting of: incorporation of a modified nucleotide; a 3' cap; a 5' cap; conjugation to a high molecular weight, non-immunogenic compound; conjugation to a lipophilic compound; incorporation of a CpG motif; and incorporation of a phosphorothioate or phosphorodithioate into the phosphate backbone.

7) The aptamer of claim 6, wherein the high molecular weight, non-immunogenic, compound is polyethylene glycol.

8) The aptamer of claim 6 or 7, wherein the 3' cap is an inverted deoxythymidine cap.

9) The aptamer of any one of claims 1 to 8 or a salt thereof comprising the nucleic acid sequence set forth below:
mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA (SEQ ID NO: 1) (ARC26835), wherein "dN" is a deoxynucleotide and "mN" is a 2'-OMe containing nucleotide.

10) The aptamer of any one of claims 1 to 8 or a salt thereof comprising the nucleic acid sequence set forth below:
mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 2) (ARC17480), wherein "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-OMe containing nucleotide.

11) The aptamer of any one of claims 1 to 8 or a salt thereof comprising the nucleic acid sequence set forth below:
NH_2-mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 3) (ARC19498), wherein "NH_2" is from a 5'-hexylamine linker phosphoramidite, "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-OMe containing nucleotide.

12) The aptamer of any one of claims 1 to 8 or a salt thereof comprising the nucleic acid sequence set forth below:
PEG40K-NH-mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 4) (ARC19499), wherein "NH" is from a 5'-hexylamine linker phosphoramidite, "PEG" is a polyethylene glycol, "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-OMe containing nucleotide.

13) A pharmaceutical composition comprising a therapeutically effective amount of the aptamer of any one of claims 1 to 12 or a salt thereof, and a pharmaceutically acceptable carrier or diluent.

14) A method for treating, preventing, delaying the progression or ameliorating a bleeding disorder mediated by TFPI, comprising administering to a subject the composition of claim 13.

15) The method of claim 14, wherein the subject is a mammal.

16) The method of claim 15, wherein the mammal is a human.

17) The method of any one of claims 14 to 16, wherein the bleeding disorder is selected from the group consisting of: prophylaxis and/or on-demand therapy for coagulation factor deficiencies, congenital or acquired, mild/moderate/severe, including Hemophilia A (Factor VIII-deficiency), Hemophilia B (Factor IX deficiency) and Hemophilia C (Factor XI deficiency); Hemophilia A or B with inhibitors; other factor deficiencies (V, VII, X, XIII, prothrombin, fibrinogen); deficiency of α2-plasmin inhibitor; deficiency of plasminogen activator inhibitor 1; multiple factor deficiency; functional factor abnormalities (*e.g.*, dysprothrombinemia); joint hemorrhage (hemarthrosis) including but not limited to ankle, elbow and knee; spontaneous bleeding in other locations (muscle, gastrointestinal, mouth, *etc.*); hemorrhagic stroke; intracranial hemorrhage; lacerations and other hemorrhage associate with trauma; acute traumatic coagulopathy; coagulopathy associated with cancer (*e.g.*, acute promyelocytic leukemia); von Willebrand's Disease; disseminated intravascular coagulation; liver disease; menorrhagia and thrombocytopenia.

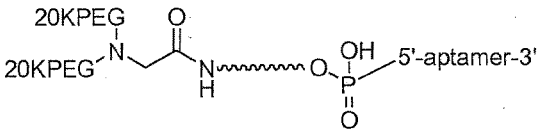
18) The method of any one of claims 14 to 17, wherein the composition is administered prior to, during and/or after a medical procedure.

19) The method of any one of claims 14 to 18, wherein the composition is administered in combination with another drug.

20) The method of any one of claims 14 to 19, wherein the composition is administered in combination with another therapy.

21) A kit comprising the aptamer of any one of claims 1 to 12.

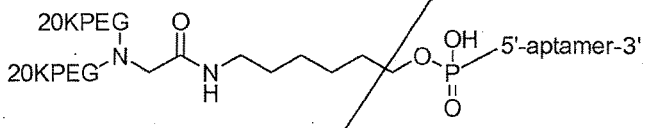
22) An aptamer or a salt thereof that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises the following structure:



wherein HN ~~~~ PO_3H is from a 5'-amine linker phosphoramidite, and wherein the aptamer comprises the nucleic acid sequence of mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 2), wherein "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-O Methyl containing nucleotide, and wherein 20KPEG is a mPEG moiety having a molecular weight of 20 kDa.

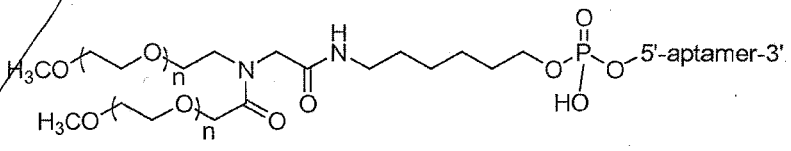
23) The aptamer of claim 22, wherein the linker is selected from the group consisting of: 2-18 consecutive CH_2 groups, 2-12 consecutive CH_2 groups, 4-8 consecutive CH_2 groups and 6 consecutive CH_2 groups.

24) An aptamer or a salt thereof that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises the following structure:



wherein the aptamer comprises the nucleic acid sequence of mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 2), wherein "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-O Methyl containing nucleotide, and wherein 20KPEG is a mPEG moiety having a molecular weight of 20 kDa.

25) An aptamer or a salt thereof that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises the following structure:



wherein the aptamer comprises the nucleic acid sequence of mG-mG-mA-mA-mU-mA-mU-mA-dC-mU-mU-mG-mG-dC-mU-dC-mG-mU-mU-mA-mG-mG-mU-mG-dC-mG-mU-mA-mU-mA-mU-mA-3T (SEQ ID NO: 2), wherein "n" is approximately 450, "3T" is an inverted deoxythymidine, "dN" is a deoxynucleotide and "mN" is a 2'-O Methyl containing nucleotide.

26) A reversal agent comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 15 (ARC23085), SEQ ID NO: 16 (ARC23087); SEQ ID NO: 17 (ARC23088) and SEQ ID NO: 18 (ARC23089).

27) A method of producing an aptamer that binds to tissue factor pathway inhibitor (TFPI), the method comprising the step of chemically synthesizing a nucleic acid having a nucleic acid sequence according to a nucleic acid sequence of any one of claims 1 to 12 or 22 to 25.

28) The method of claim 27 wherein the method further comprises formulating a pharmaceutical composition by mixing the synthesized nucleic acid sequence, or a salt thereof, with a pharmaceutically acceptable carrier or diluent.

29) A method of producing a reversal agent, the method comprising the step of chemically synthesizing a nucleic acid having a nucleic acid sequence according to a nucleic acid sequence of claim 26.

30) The method of claim 28 wherein the method further comprises formulating a pharmaceutical composition by mixing the synthesized nucleic acid sequence or a salt thereof, with a pharmaceutically acceptable carrier or diluent.

31) An aptamer that binds to a human tissue factor pathway inhibitor (TFPI) polypeptide having the amino acid sequence of SEQ ID NO: 11, wherein the aptamer modulates TFPI-mediated inhibition of blood coagulation, and wherein the aptamer competes for binding to TFPI with a reference aptamer comprising a nucleic acid sequence selected from the group consisting of: SEQ ID NO: 4 (ARC19499), SEQ ID NO: 1 (ARC26835), SEQ ID NO: 2 (ARC17480), SEQ ID NO: 3 (ARC19498), SEQ ID NO: 5 (ARC19500), SEQ ID NO:6

(ARC19501), SEQ ID NO: 7 (ARC31301), SEQ ID NO: 8 (ARC18546), SEQ ID NO: 9 (ARC19881) and SEQ ID NO: 10 (ARC19882).

32) An aptamer that binds to a region on a human tissue factor pathway inhibitor (TFPI) polypeptide comprising one or more portions of SEQ ID NO: 11, wherein the one or more portions is selected from the group consisting of: amino acid residues 148-170, amino acid residues 150-170, amino acid residues 155-175, amino acid residues 160-180, amino acid residues 165-185, amino acid residues 170-190, amino acid residues 175-195, amino acid residues 180-200, amino acid residues 185-205, amino acid residues 190-210, amino acid residues 195-215, amino acid residues 200-220, amino acid residues 205-225, amino acid residues 210-230, amino acid residues 215-235, amino acid residues 220-240, amino acid residues 225-245, amino acid residues 230-250, amino acid residues 235-255, amino acid residues 240-260, amino acid residues 245-265, amino acid residues 250-270, amino acid residues 255-275, amino acid residues 260-276, amino acid residues 148-175, amino acid residues 150-175, amino acid residues 150-180, amino acid residues 150-185, amino acid residues 150-190, amino acid residues 150-195, amino acid residues 150-200, amino acid residues 150-205, amino acid residues 150-210, amino acid residues 150-215, amino acid residues 150-220, amino acid residues 150-225, amino acid residues 150-230, amino acid residues 150-235, amino acid residues 150-240, amino acid residues 150-245, amino acid residues 150-250, amino acid residues 150-255, amino acid residues 150-260, amino acid residues 150-265, amino acid residues 150-270, amino acid residues 150-275, amino acid residues 150-276, amino acid residues 190-240, amino acid residues 190-276, amino acid residues 240-276, amino acid residues 242-276, amino acid residues 161-181, amino acid residues 162-181, amino acid residues 182-240, amino acid residues 182-241, and amino acid residues 182-276.

33) An aptamer that binds to human tissue factor pathway inhibitor (TFPI) and exhibits one or more of the following properties:

a) competes for binding to TFPI with any one of SEQ ID NOs: 1-10;

b) inhibits TFPI inhibition of Factor Xa;

c) increases thrombin generation in hemophilia plasma;

d) inhibits TFPI inhibition of the intrinsic tenase complex;

e) restores normal hemostasis, as measured by thromboelastography (TEG) in whole blood and plasma;
f) restores normal clotting, as indicated by shorter clot time, more rapid clot formation or more stable clot development, as measured by thromboelastography (TEG) or rotational thromboelastometry (ROTEM) in whole blood and plasma; or
g) decreases the clot time, as measured by dilute prothrombin time (dPT), tissue factor activated clotting time (TF-ACT) or any other TFPI-sensitive clot-time measurement.

34) An aptamer that binds to human tissue factor pathway inhibitor (TFPI) wherein the aptamer competes for binding to TFPI with a reference aptamer selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10.

35) An aptamer that binds to tissue factor pathway inhibitor (TFPI) wherein the aptamer competes, either directly or indirectly, for binding to TFPI with a reference antibody selected from the group consisting of: AD4903.

36) An aptamer that binds to human tissue factor pathway inhibitor (TFPI) and comprises a stem and loop motif having the nucleotide sequence of SEQ ID NO: 4, wherein:
a) any one or more of nucleotides 1, 2, 3, 4, 6, 8, 11, 12, 13, 17, 20, 21, 22, 24, 28, 30 and 32 may be modified from a 2'-OMe substitution to a 2'-deoxy substitution;
b) any one or more of nucleotides 5, 7, 15, 19, 23, 27, 29 and 31 may be modified from a 2'-OMe uracil to either a 2'-deoxy uracil or a 2'-deoxy thymine;
c) nucleotide 18 may be modified from a 2'-OMe uracil to a 2'-deoxy uracil; and/or
d) any one or more of nucleotides 14, 16 and 25 may be modified from a 2'-deoxy cytosine to either a 2'-OMe cytosine or a 2'-fluoro cytosine.

37) An aptamer that binds to tissue factor pathway inhibitor (TFPI), wherein the aptamer comprises a primary nucleic acid sequence selected from the group consisting of SEQ ID NOs.: 4, 1, 2, 3, 5, 6, 7, 8, 9 and 10.