CLAIMS

1. An isolated isotype IgG human monoclonal antibody that binds to a site comprising the Kunitz 2 domain of human tissue factor pathway inhibitor (TFPI), wherein the antibody comprises a heavy chain and a light chain and where in the heavy chain is comprised of an amino acid sequence of SEQ ID NO:1, and wherein said amino acid sequence comprises one or more amino acid substitutions to SEQ ID NO: 1 that increase affinity of the antibody for TFPI

2. The isolated monoclonal antibody of claim 1, wherein the substitution is at a position selected from the group consisting of Q1, R30, S31, G33, M34, S35, S50, S55, and K99 and combinations thereof.

3. The isolated monoclonal antibody of claim 1, wherein substitution is selected from the group consisting of Q1E, R30S, S31P, S31V, G33A, G33K, G33P, M34L, M34K, S35L, S35D, S50A, S55A, S55G, S55R, K99V, and K99L and combinations thereof.

4. The isolated monoclonal antibody of claim 1, wherein the heavy chain amino acid sequence of SEQ ID NO: 1 comprises two or more substitutions selected from the group consisting of Q1E, R30S, S31P, S31V, G33A, G33K, G33P, ~~M341~~ M34L, M34K, S35L, S35D, S50A, S55A, S55G, S55R, K99V, and K99L and combinations thereof.

5. The human monoclonal antibody of claim 1, wherein the light chain comprises an amino acid sequence of SEQ ID NO:2, wherein said amino acid sequence comprises one or more amino acid substutions to SEQ ID NO:2 that increase affinity of the antibody for TFPI.

6. The isolated monoclonal antibody of claim 5, wherein the substitution is at a position selected from the group consisting of D1, I2, A13, S21, N26, R28, N29, A32, H33, Y48, Y49, N51, N52, G56, E80, D91, D92, P95 and V96 and combinations thereof.

7. The isolated monoclonal antibody of claim 5, wherein the substitution is selected from the group consisting of D1S, I2, A13S, S21T, N26A, R28P, N29K, A32N, H33Y, Y48F, Y49R, N51S, N51V, N52G, G56D, E80M, D91L, D91R, D91W, D91K, D92S, G93S, V94T, P95V, P95A, V96G, V96M and V96W, and combinations thereof.

8. The isolated monoclonal antibody of claim 5, wherein the light chain amino acid sequence shown in SEQ ID NO: 2 comprises two or more substitutions selected from the group consisting of D1S, I2, N26A, R28P, A32N, H33Y, Y48F, Y49R, N51S, N51V, N52G, G56D, D91L, D91R, D91W, D91K, V94T, P95V, P95A, V96G, V96M and V96W and combinations thereof.

9. An isolated isotype IgG human monoclonal antibody that binds to a site comprising the Kunitz 2 domain of human tissue factor pathway inhibitor (TFPI), wherein the antibody comprises:

   (a) a heavy chain comprising an amino acid sequence of SEQ ID NO:3, wherein said heavy chain amino acid sequence comprises one or more amino acid substitutions to SEQ ID NO:3 that increase affinity of the antibody for TFPI; and

   (b) a light chain comprising an amino acid sequence of SEQ ID NO:4, wherein said light chain amino acid sequence comprises one or more amino acid modifications to SEQ ID NO:4 that increase affinity of the antibody for TFPI.

10. The isolated monoclonal antibody of claim 9, wherein the heavy chain substitution comprises a substitution selected from the group consisting of Q1E, N32D, N32E, S33G, S37N, G44S, I53T, K55Y, S57K, S57R, K58M, N61G, N61T, D62I, D62R, D62Q, D62L, D62S, D62V, D62N, D62K, H103D, H103G, H107M, G109A and Y112D and combinations thereof.

11. The isolated monoclonal antibody of claim 9, wherein the light chain substitution comprises the substitution selected from the group consisting M4I, M4L, V3OL, F31I, F31M, F31Y, F31H, S32L, S32R, S32Y, D33F, D33R, N351, N35L, N35T, N35V, T36N, Y37F, N39D, L42Q, K44R, Q50R, L51R, Y54F, K55L, G56D, G56A, G56V, S57Y, A60D, S61C, D97M, D97T and S98H, and combinations thereof.

12. The isolated monoclonal antibody of claim 9, wherein the heavy chain comprises two or more substitutions selected from the group consisting of Q1E, S3OR, N32D, N32E, S33G, S37N, G44S, I53T, K55Y, S57K, S57R, K58M, N61G, N61T, D62I, D62R, D62Q, D62L, D62S, D62V, D62N, D62K, H103D, H103G, H107M, G109A and Y112D, and combinations thereof.

13. The isolated monoclonal antibody of claim 9, wherein the light chain comprises two or more substitutions selected from the group consisting of M4I, M4L, V3OL, F31I, F31M, F31Y, F31H, S32L, S32R, S32Y, D33F, D33R, N351, N35L, N35T, N35V, T36N, Y37F, N39D, L42Q, K44R, Q50R, L51R, Y54F, K55L, G56D, G56A, G56V, S57Y, A60D, S61C, D97M, D97T and S98H, and combinations thereof.

14. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway inhibitor, wherein the antibody comprises:

(a) a human heavy chain comprising:

(i) a CDR 1 region with an amino acid sequence FTFRSYGMS, optionally comprising a substitution of R30S or S35D or both;

(ii) a CDR2 region with an amino acid sequence SIRGSSSSTYYADSVKG, optionally comprising a substitution of S55R or S56G or both;

(iii) a CDR3 region with an amino acid sequence KYRYWFDY, optionally comprising a substitution of K99L.

(b) a human light chain comprising:

(i) a CDR1 region with an amino acid sequence SGDNLRNYYAH, optionally comprising a substitution of R28P or N29K;

(ii) a CDR2 region with an amino acid sequence YYDNNRPS, optionally comprising at least one substitution selected from the group consisting of Y48F and N51V and combinations thereof;

(iii) a CDR3 region with an amino acid sequence QSWDDGVPV, optionally comprising a substitution of S89A, D91W, D92S, G93S, and V94T, and combinations thereof.

15. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway inhibitor (TFPI) wherein the antibody comprises:

(a) a human heavy chain comprising:

(i) a CDR 1 region with the amino acid sequence of DSVSSNSAAWS;

(ii) a CDR2 region with the amino acid sequence of IIYKRSKWYNDYAVSVKS;

(iii) a CDR3 with the amino acid sequence of WNSDKHWGFDY;

and

(b) a human light chain comprising:

(i) a CDR1 region with the amino acid sequence of RSSQSLVFSDGNTYLN;

(ii) a CDR2 region with the amino acid sequence of KGSNRAS,

(iii) a CDR3 with the amino acid sequence shown of QQYDSYPLT.

16. The isolated human monoclonal IgG antibody of claim 1, wherein

(a) the human heavy chain comprises SEQ ID NO: 3 and

(b) the human light chain comprises SEQ ID NO: 4.

17. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway (TFPI) inhibitor, wherein the antibody comprises at least one amino acid substitution:

(a) a human heavy chain comprising:

(i) a CDR 1 region with the amino acid sequence of DSVSSNSAAWS, optionally comprising a substitution of N32 relative to SEQ ID NO: 3;

(ii) a CDR2 region with the amino acid sequence of IIYKRSKWYNDYAVSVKS, optionally comprising a substitution selected from the group consisting of K55 and D62 relative to SEQ ID NO: 3;

(iii) a CDR3 region that binds specifically to TFPI and has the amino acid sequence of WHSDKHWGFDY, optionally comprising a substitution at Y112 relative to SEQ ID NO: 3, and

(iv) optionally, an amino acid substitution at G44 relative to SEQ ID NO: 3;

(b) a human light chain comprising:

(i) a CDR1 region with the amino acid sequence of RSSQSLVFSDGNTYLN, optionally comprising a substitution of a position selected from the group consisting of S32 and N35 and combinations thereof relative to SEQ ID NO: 4;

(ii) a CDR2 region with the amino acid sequence of KGSNRAS;

(iii) a CDR3 region that binds specifically to TFPI and has the amino acid sequence of QQYDSYPLT.

18. The isolated human monoclonal IgG antibody of claim 17, wherein

(a) the human heavy chain comprises SEQ ID NO: 3 and

(b) the human light chain comprises SEQ ID NO: 4.

19. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway inhibitor (TFPI), wherein the antibody comprises:

(a) a human heavy chain comprising:

(i) a CDR1 region with the amino acid sequence of DSVSSNSAAWS, comprising a substitution of N32D relative to SEQ ID NO: 3a CDR2 region with the amino acid sequence of IIYKRSKWYNDYAVSVKS, comprising at least one substitution relative to SEQ ID NO: 3 selected from the group consisting of K55Y and D62R and combinations thereof;

(ii) a CDR3 region that has the amino acid sequence of WHSDKHWGFDY comprising a substitution relative of Y112D relative to SEQ ID NO: 3 , and

(iii) a substitution relative to SEQ ID No. 3 of G44S; and

(b) a human light chain comprising:

(i) a CDR1 region with the amino acid sequence of RSSQSLVFSDGNTYLN, comprising at least one substitution selected from the group consisting of S32R and N35I relative to SEQ ID NO: 4 and combinations thereof,

(ii) a CDR2 region with the amino acid sequence of KGSNRAS,

(iii) a CDR3 region that binds specifically to TFPI and has the amino acid sequence of QQYDSYPLT.

20. The isolated human monoclonal IgG antibody of claim 19, wherein

(a) the human heavy chain comprises the amino acid sequence shown in SEQ ID NO: 3; and

(b) the human light chain comprises the amino acid sequence shown in SEQ ID NO: 4.

21. The antibody of claim 1, wherein the amino acid substitution at position 1 of the heavy chain relative to SEQ ID NO: 1 is Q1E.

22. The antibody of claim 9, wherein the amino acid substitution at position 1 of the heavy chain relative to SEQ ID NO: 3 is Q1E.

23. A pharmaceutical composition comprising a therapeutically effective amount of the monoclonal antibody of any of claims 1-22 and a pharmaceutically acceptable carrier.

CLAIMS

1. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway inhibitor, wherein the antibody comprises:

(a) a human heavy chain comprising:

a CDR 1 region with the amino acid sequence shown in SEQ ID NO: 35, optionally comprising a substitution relative to SEQ ID NO: 1 of S35D;

a CDR2 region with the amino acid sequence of shown in SEQ ID NO: 36, optionally comprising a substitution relative to SEQ ID NO: 1 of S55R;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 37, optionally comprising a substitution relative to SEQ ID NO: 1 of K99L.

(b) a human light chain comprising:

a CDR1 region with the amino acid sequence shown in SEQ ID NO: 38;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 39, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting of Y48F and N51V and combinations thereof;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 40, comprising a substitution relative to SEQ ID NO: 2 of D91W.

2. The isolated human monoclonal IgG antibody of claim 1, wherein

the human heavy chain comprises SEQ ID NO: 7 and

the human light chain comprises SEQ ID NO: 14.

3. An isolated human monoclonal IgG antibody that binds specifically to human tissue factor pathway inhibitor, wherein the antibody comprises:

(a) a human heavy chain comprising:

a CDR 1 region with the amino acid sequence shown in SEQ ID NO: 35, comprising at least one substitution relative to SEQ ID NO: 1 selected from the group consisting of R30S and S35D and combinations thereof;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 36, comprising at least one substitution relative to SEQ ID NO: 1 selected from the group consisting of S55R and S56G and combinations thereof;

a CDR3 region with an amino acid sequence shown in SEQ ID NO: 37, optionally comprising a substitution relative to SEQ ID NO: 1 of K99L, and

(b) a human light chain comprising:

a CDR1 region with the amino acid sequence shown in SEQ ID NO: 38, comprising substitution relative to SEQ ID NO: 2 of N29K;

a CDR2 region with the amino acid sequence shown in SEQ ID NO: 39, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting Y48F and N51V and combinations thereof;

a CDR3 region with the amino acid sequence shown in SEQ ID NO: 40, comprising at least one substitution relative to SEQ ID NO: 2 selected from the group consisting of S89A, D91W, D92S, G93S, and V94T and combinations thereof.

4. The isolated human monoclonal IgG antibody of claim 3, wherein

the substitutions relative to SEQ ID NO: 1 in the heavy chain are comprised of R30S, S35D, S55R, S56G, and K99L.

5. The isolated human monoclonal IgG antibody of claim 4, wherein

the substitutions relative to SEQ ID NO: 2 in the light chain are comprised of N29K, Y48F, N51V, S89A, D91W, D92S, G93S, and V94T.

6. The isolated human monoclonal IgG antibody of claim 3, wherein

(a) the human heavy chain comprises the amino acid sequence as shown in SEQ ID NO: 10; and

(b) the human light chain comprises the amino acid sequence as shown in SEQ ID NO: 17.