

WHAT IS CLAIMED:

1. A modified membrane type serine protease 1 (MTSP-1) polypeptide, comprising the amino acid modification G151N or H and additional modifications, whereby the modified MTSP-1 polypeptide cleaves a target site in complement protein C3 that inactivates C3 to thereby inhibit or reduce complement activation, wherein:

amino acid modifications are selected from among replacements, insertions, and/or deletions in the unmodified MTSP-1 polypeptide;

residues are numbered by chymotrypsin numbering;

corresponding residues are determined by alignment and chymotrypsin numbering;

the unmodified MTSP-1 polypeptide consists of the sequence of amino acids set forth in any of SEQ ID NOs: 1-4, or the unmodified MTSP-1 polypeptide comprises the protease domain that consists of SEQ ID NO: 2 or 4; and

the amino acid modifications, by chymotrypsin number, correspond to:

I41D/C122S/G151N/Q192T

I41S/F99L/C122S/G151N/Q192V

I41E/F99L/C122S/G151N/Q192T

I41D/Y59F/D96E/F99L/C122S/G151N/Q192T

I41D/Y59F/C122S/G151N/Q192T

I41S/D60bT/F60eS/Y60gW/D96K/F97G/ins97aV/T98P/F99L/C122S/G151H/Q175L/Q192D

I41T/F97W/F99L/C122S/G151N/Q175M/Q192G/D217L

I41G/F97S/F99L/C122S/G151N/Q175L/Q192G/D217I

C122S/G151N/G193A

H40R/I41H/C122S/G151N

H40R/I41H/Y60gL/F97D/F99L/C122S/G151N/Q175M/D217I/K224S

H40R/I41H/Y60gH/F97D/F99L/C122S/G151N/Q175A/Q192H/D217I/K224R

H40R/I41H/Y60gL/F97D/F99L/C122S/G151N

H40K/I41M/F97D/F99L/C122S/G151N/Q175M/Q192D/D217N/K224R

H40R/I41H/F97D/F99L/C122S/G151N/L153R

H40R/I41H/F97D/C122S/G151N/L153R/V202M

H40R/I41H/F97D/C122S/G133D/G151N

I41R/F97W/F99L/C122S/G151N/Q192G

F97D/ins97aV/T98P/F99L/C122S/G151N/Q192G

I41D/ins97aE/F97T/T98G/F99L/C122S/G151N/Q175L/Q192E/Q209L

Q38R/I41S/D60bH/F60eV/ins97aE/F97T/T98G/F99L/C122S/G151N/Q175L/Q192E

ins97aV/F97D/T98P/F99L/C122S/G151N/Q175L/Q192G

Q38E/I41S/D60bH/F60eI/ins97aE/F97T/T98G/F99L/C122S/G151N/Q175L/Q192V

Q38R/I41S/D60bH/F60eI/ins97aE/F97T/T98G/F99L/C122S/G151N/Q175L/Q192E

Q38E/I41S/D60bV/F60eK/ins97aE/F97T/T98G/F99L/C122S/G151N/Q175L/Q192I

Q38R/I41E/ins97aE/F97T/T98G/F99L/C122S/G151N/Q175L/Q192T

Q38R/I41T/ins97aE/F97T/T98G/F99L/C122S/G151N/Q175L/Q192G

Q38S/I41S/F60eR/F97T/ins97aE/T98G/F99L/C122S/G151N/Q175L/Q192S

Q38L/I41T/D60bR/F60eL/Y60gM/F97T/ins97aE/T98G/F99L/C122S/G151N/Q175L/Q192G

Q38F/I41S/D60bF/F60eR/Y60gF/F97T/ins97aE/T98G/F99L/C122S/G151N/Q175L/Q192V

Q38V/I41S/D60bT/F60eT/F97D/ins97aV/T98P/F99L/C122S/G151N/Q175H/Q192S

Q38T/I41S/D60bV/F60eR/ins97aV/F97D/T98P/F99L/C122S/G151N/Q175R/Q192V
Q38Y/I41A/D60bL/F60eQ/ins97aV/F97D/T98P/F99L/C122S/G151N/Q175M/Q192A
Q38L/I41T/D60bA/F60eL/ins97aV/F97D/T98P/F99L/C122S/G151H/Q175M/Q192T
Q38R/I41S/D60bY/F60eD/ins97aV/F97D/T98P/F99L/C122S/G151N/Q175M/Q192A
Q38W/I41S/D60bG/F60eI/F97D/ins97aV/T98P/F99L/C122S/G151N/Q175A/Q192D
Q38T/I41S/D60bG/F60eM/ins97aV/F97D/T98P/F99L/C122S/G151N/Q175S/Q192S
I41T/D60bW/F60eH/F97D/ins97aV/T98P/F99L/C122S/G151N/Q175L/Q192G
Q38E/I41S/D60bV/F60eK/Y60gW/F97T/ins97aE/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38E/I41S/D60bV/F60eK/Y60gW/ins97aE/F97T/T98G/F99L/C122S/G151H/Q175L/Q192D
I41S/D60bT/F60eS/Y60gW/ins97aV/F97D/T98P/F99L/C122S/G151H/Q175L/Q192E
Q38K/I41G/F60eG/Y60gW/D96P/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41E/D60bS/F60eV/Y60gF/D96W/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38V/I41G/F60eG/Y60gW/D96P/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41A/D60bT/F60eG/Y60gW/D96V/F97N/T98G/F99L/C122S/G151H/Q175L/Q192D
Q38K/I41G/D60bT/F60eG/Y60gW/D96P/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38K/I41G/D60bT/F60eG/Y60gW/D96L/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38E/I41S/D60bV/F60eK/Y60gW/D96P/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38E/I41S/D60bW/F60eG/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38M/I41S/F60eH/Y60gW/D96Y/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bV/F60eR/Y60gF/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38E/I41S/D60bV/F60eK/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/F60eR/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
I41S/D60bT/F60eR/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/D60bT/F60eR/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/F60eR/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/F60eR/D96M/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/F60eR/Y60gW/F97N/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/F60eR/Y60gW/D96M/T98G/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/F60eR/Y60gW/D96M/F97N/F99L/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/F60eR/Y60gW/D96M/F97N/T98G/C122S/G151N/Q175L/Q192D
Q38Y/I41S/D60bT/F60eR/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q192D
Q38Y/I41S/D60bT/F60eR/Y60gW/D96M/F97N/T98G/F99L/C122S/G151N/Q175L
I41G/F97A/F99L/C122S/G151H/Q175L/Q192G/D217L
I41G/F97S/F99L/C122S/G151N/Q175I/Q192T/D217T
I41S/F97E/F99L/C122S/G151N/Q175G/Q192R/D217A
I41G/F97L/F99L/C122S/G151N/Q175A/Q192V/D217R
I41G/F99L/C122S/G151N/Q175I/Q192T/D217T
I41G/F97S/F99L/C122S/G151N/Q192T/D217T
I41G/F97S/F99L/C122S/G151N/Q175I/D217T
I41G/F97S/F99L/C122S/G151N/Q175I/Q192T
I41E/F97V/F99L/C122S/G151N/Q175L/Q192S/D217H
I41D/F97T/F99L/C122S/G151N/Q175S/Q192T/D217A
I41G/F99L/C122S/G151N/Q192T/D217T
I41G/F99L/C122S/G151N/Q175I/Q192T
F97V/F99L/C122S/G151N/Q175L/Q192S/D217H

I41E/F99L/C122S/G151N/Q175L/Q192S/D217H
I41E/F97V/F99L/C122S/G151N/Q192S/D217H
I41E/F97V/F99L/C122S/G151N/Q175L/D217H
I41E/F97V/F99L/C122S/G151N/Q175L/Q192S
I41D/F99L/C122S/G151N/Q175S/Q192T/D217A
I41D/F97T/F99L/C122S/G151N/Q192T/D217A
I41D/F97T/F99L/C122S/G151N/Q175S/D217A
I41D/F97T/F99L/C122S/G151N/Q175S/Q192T
I41G/F97A/F99L/C122S/G151N/Q175M/Q192S
I41G/F97V/F99L/C122S/G151N/Q192T/D217V
I41D/F99L/C122S/G151N/Q192T/D217A
I41D/F99L/C122S/G151N/Q175S/Q192T
I41D/F97T/F99L/C122S/G151N/Q192T
I41D/F99L/C122S/G151N/Q192T
Q38K/I41G/Y60gG/F99L/C122S/G151N/Q192H
Q38K/I41D/Y60gG/F99L/C122S/G151N
Q38K/I41G/Y60gW/F99L/C122S/G151N/Q192A
Q38K/I41S/Y60gW/F99L/C122S/G151N/Q192G
Q38F/I41S/Y60gA/C122S/G151N/Q192R
I41E/C122S/G151N/Q175L/Q192A
I41S/F99M/C122S/G151N/Q175L/Q192G
I41E/F99L/C122S/G151N/Q175L/Q192A
I41S/F99L/C122S/G151H/Q175L/Q192V
I41G/F99L/C122S/G151N/Q192A
I41S/F99M/C122S/G151N/Q175G/Q192R
I41E/F99L/C122S/G151N/Q175R/Q192H
I41S/F99L/C122S/G151N/Q175P/Q192V
I41E/F99L/C122S/G151N/Q175G/Q192T
I41S/C122S/G151N/Q175D/Q192R
I41G/F99L/C122S/G151N/Q175R/Q192A
I41D/F99L/C122S/G151N
I41S/F99L/C122S/G151N/Q175P
I41E/F99L/C122S/G151N/Q175G
I41D/G43A/F99L/C122S/G151N/Q192T/P232S/K239R
I41D/G43A/D96E/F99L/C122S/G151N/Q192T
I41D/D96E/F99L/C122S/G151N/Q192T
I41D/D96E/C122S/G151N/Q192T
I41D/D96E/F99L/C122S/G151N/Q192T/D217E
I41D/F99L/M117T/C122S/G151N/Q192T/A204D/D217E
I41D/F99L/C122S/G151N/Q192T/D217E
I41D/F99L/C122S/I136M/G151N/Q192T/D217L/K224R
I41D/F60eI/D96E/F99L/C122S/G151N/Q192T
I41D/C122S/G151N/Q192T/D217E
Q38W/I41S/D96R/ins97aA/C122S/G151N/Q192A
L33M/Q38F/I41S/D96A/ins97aW/C122S/G151N/Q192S
Q38W/I41V/D96R/ins97aA/C122S/G151N

Q38Y/I41T/D96M/ins97aS/C122S/G151N
I41S/F99L/C122S/G151N/Q175M/Q192G/D217V
I41S/F97L/F99L/C122S/G151N/Q192G/D217V
I41S/F97S/F99L/C122S/G151N/Q175L/Q192A/D217L
I41G/F97R/F99L/C122S/G151N/Q175L/Q192S/D217V, or
I41T/F97L/F99L/C122S/G151N/Q175S/Q192S/D217W, or
I41R/F97T/Ins97aE/T98G/F99L/C122S/G151N/Q175L/Q192E, or the same modifications
except C122S is not modified and is C122C.

2. The modified MTSP-1 polypeptide of claim 1, wherein the target site in C3 comprises residues 737-744 of SEQ ID NO:9.

3. The modified MTSP-1 polypeptide of claim 2, wherein the cleavage is between residues 740 and 741 of SEQ ID NO:9, whereby cleavage occurs at

Q H A R ↓ A S H L.

4. The modified MTSP-1 polypeptide of any of claims 1-3, wherein the unmodified MTSP-1 polypeptide consists of the sequence of amino acids set forth in any of SEQ ID NOs:1-4.

5. The modified MTSP-1 polypeptide of any of claims 1-3, wherein the unmodified MTSP-1 polypeptide consists of the sequence of amino acids set forth in SEQ ID NO:2 or SEQ ID NO:4.

6. The modified MTSP-1 polypeptide of claims 1-5, further comprising a replacement at one or more of positions corresponding to I41, F99, and Q192.

7. The modified MTSP-1 polypeptide of any of claims 1-6, comprising a replacement at a position corresponding to I41, wherein the replacement is S, D, R, or E.

8. The modified MTSP-1 polypeptide of any of claims 1-7, comprising a replacement at a position corresponding to Q192, wherein the replacement is T, D, or E.

9. The modified MTSP-1 polypeptide of any of claims 1-5, comprising modifications corresponding to I41E/F99L/C122S/G151N/Q192T, or I41D/C122S/G151N/Q192T, or I41S/F99L/C122S/G151N/Q192V, or I41R/F97T/Ins97aE/T98G/F99L/C122S/G151N/Q175L/Q192E, or I41D/Y59F/D96E/F99L/C122S/G151N/Q192T, or I41D/Y59F/C122S/G151N/Q192T, or I41S/D60bT/F60eS/Y60gW/D96K/F97G/ins97aV/T98P/F99L/C122S/G151H/Q175L/Q192D, or I41T/D60bW/F60eH/F97D/ins97aV/T98P/F99L/C122S/G151N/Q175L/Q192G, or I41S/F99L/C122S/G151N/Q175M/Q192G/D217V, or comprising the same modifications except wherein C122S is C122C.

10. The modified MTSP-1 polypeptide of any of claims 1-5, comprising modifications corresponding to any of:

I41R/F97T/Ins97aE/T98G/F99L/C122S/G151N/Q175L/Q192E, or

I41E/F99L/C122S/G151N/Q192T, or

I41D/C122S/G151N/Q192T, or

I41S/F99L/C122S/G151N/Q192V, or

I41D/Y59F/D96E/F99L/C122S/G151N/Q192T, or

I41D/Y59F/C122S/G151N/Q192T, or

I41T/F97W/F99L/C122S/G151N/Q175M/Q192G/D217L, or

I41G/F97S/F99L/C122S/G151N/Q175L/Q192G/D217I, or

I41T/F97L/F99L/C122S/G151N/Q175S/Q192S/D217W, or

or comprising the same modifications except C122S is not modified and is C122C.

11. The modified MTSP-1 polypeptide of any of claims 1-5, comprising modifications corresponding to I41D/C122S/G151N/Q192T or I41D/G151N/Q192T or I41E/F99L/C122S/G151N/Q192T or I41E/F99L/G151N/Q192T, wherein the unmodified MTSP-1 polypeptide consists of the protease domain of SEQ ID NO:2 or SEQ ID NO:4.

12. The modified MTSP-1 polypeptide of any of claims 1-5, wherein the modified MTSP-1 polypeptide comprises the sequence of amino acid residues set forth in any of SEQ ID NOs: 21, 42-47, 77, 81, 160, 161, 165, 168, 179, 187, 198, 199, 204, 270, 271, 285, 287, 297, 302-305, 318, 319, 323-325, 327, 330-335, 341, 381, 438, 451, 452, 454, 455, 457-461, 476-478, 481-491, 497, 498, 500, 502, 506, 509-511, 519, 522, 525-528, 530-532, 540, 542-544, 548, 549, 556-559, 563, 565, 568, 570, 571, 576-582, 586, 587, 590, 592, 594, 596, 598, 600-609, 635, 638, 642, 643, 686-690, or the same sequences of amino acid residues, except the residue at position C122 is C (Cys).

13. The modified MTSP-1 polypeptide of any of claims 1-5, wherein the modified MTSP-1 polypeptide comprises the sequence of amino acid residues set forth in SEQ ID NO:42.

14. The modified MTSP-1 polypeptide of any of claims 1-5, wherein the modified MTSP-1 polypeptide comprises the sequence of amino acid residues set forth in SEQ ID NO:42, except that the residue at position 122, by chymotrypsin numbering, is cysteine (C).

15. The modified MTSP-1 polypeptide of any of claims 1-14, wherein the unmodified MTSP-1 polypeptide consists of the sequence of amino acid residues set forth in SEQ ID NO: 2 or 4, or consists of the sequence of amino acid residues set forth in SEQ ID NO:42, except that the residue at position 122, by chymotrypsin numbering, is cysteine (C).

16. The modified MTSP-1 polypeptide of any of claims 1-15 that is modified by a polymer to increase serum half-life or to reduce immunogenicity or both.

17. The modified MTSP-1 polypeptide of claim 16 that is PEGylated.

18. The modified MTSP-1 polypeptide of claim 14 that is PEGylated.

19. The modified MTSP-1 polypeptide of any of claims 15-18, comprising a PEG that has a molecular weight from 3 kD to 50 kD.

20. The modified MTSP-1 polypeptide of claim 19, comprising a PEG that has a molecular weight of 40 kD.

21. The modified MTSP-1 polypeptide of any of claims 16-20, comprising a branched PEG.

22. The modified MTSP-1 polypeptide of any of claims 17-21, wherein the PEG is linked via a cysteine residue in the modified MTSP-1 polypeptide.

23. The modified MTSP-1 polypeptide of any of claims 17-22, comprising a PEG-maleimide linked via a cysteine residue in the modified MTSP-1 polypeptide.

24. A modified MTSP-1 polypeptide comprising the sequence of amino acid residues set forth in SEQ ID NO:42, except that the residue at position 122, by chymotrypsin numbering, is cysteine (C);

the modified MTSP-1 polypeptide is PEGylated; and
the PEG is linked via the cysteine residue in the modified MTSP-1
polypeptide.

25. The modified MTSP-1 polypeptide of claim 24, wherein the PEG is a branched PEG.

26. The modified MTSP-1 polypeptide of claim 24 or claim 25, wherein the PEG is a PEG-maleimide.

27. The modified MTSP-1 polypeptide of any of claims 24-26, wherein the PEG has a molecular weight from 3 kDa to 50 kDa.

28. The modified MTSP-1 polypeptide of any of claims 24-27 that is a mature MTSP-1 polypeptide.

29. A fusion protein, comprising a modified MTSP-1 polypeptide or a catalytically active portion of a modified MTSP-1 polypeptide of any of claims 1-28 that is linked to a multimerization domain or a protein transduction domain (PTD) or to a Small Ubiquitin-like Modifier (SUMO) tag.

30. The fusion protein of claim 29, wherein the polypeptide is linked to a multimerization domain that is an Fc domain.

31. A nucleic acid molecule, comprising a sequence of nucleotides encoding a modified MTSP-1 polypeptide of any of claims 1-28 or fusion protein of claim 29 or claim 30.

32. A vector, comprising the nucleic acid molecule of claim 31.

33. The vector of claim 32 that is a eukaryotic vector.

34. The vector of claim 32 that is a herpes virus simplex vector, a vaccinia virus vector, an adenoviral vector, an adenovirus associated vector (AAV), a retroviral vector, or an insect vector.

35. An isolated cell or a cell culture, comprising the nucleic acid molecule of claim 31, or a vector any of claims 32-34, wherein the isolated cell is not a zygote.

36. Use of a modified membrane type serine protease 1 (MTSP-1) polypeptide of any of claims 1-28 or the fusion protein of claim 29 or claim 30 in the preparation of a medicament for the treatment of a disease or pathological condition mediated by or involving complement activation, wherein inhibition of complement activation effects treatment or amelioration of the disease or pathological condition.

37. The use of claim 36, wherein the complement-mediated disease or condition is selected from among sepsis, Rheumatoid arthritis (RA), an ocular disease, membranoproliferative glomerulonephritis (MPGN), Multiple Sclerosis (MS), Myasthenia gravis (MG), asthma, inflammatory bowel disease, immune complex (IC)-mediated acute inflammatory tissue injury, Alzheimer's Disease (AD), and Ischemia-reperfusion injury.

38. The use of claim 36, wherein the disease or condition is an ocular disease or is rejection or inflammation due to a transplanted organ.

39. The use of claim 38, wherein the disease or condition is a diabetic retinopathy or a macular degeneration.

40. The use of claim 39, wherein the disease or condition is a macular degeneration.

41. The use of claim 39, wherein the macular degeneration is age-related macular degeneration (AMD).
42. The use of claim 38, wherein the disease or condition is delayed renal graft function (DGF).
43. The use of any of claims 36-42 wherein the modified MTSP-1 polypeptide is PEGylated.
44. The use of any of claims 36-43, wherein the modified MTSP-1 polypeptide comprises the sequence of amino acid residues set forth in SEQ ID NO:42 or the modified MTSP-1 polypeptide comprises the sequence of amino acid residues of SEQ ID NO:42, except that residue 122 is cysteine (C).
45. The use of any of claims 36-44, wherein the modified MTSP-1 polypeptide comprises the modifications I41D/C122S/G151N/Q192T or I41D/G151N/Q192T.
46. The use of any of claims 36-45, wherein the modified MTSP-1 polypeptide is PEGylated.
47. A method of making a modified MTSP-1 polypeptide, comprising:
 - introducing a nucleic acid or vector encoding the modified MTSP-1 polypeptide of any of claims 1-28 or fusion protein of claim 29 or claim 30 into a cell;
 - culturing the cell under conditions, whereby the polypeptide is expressed; and
 - isolating the expressed polypeptide.
48. The method of claim 47, wherein nucleic acid encoding the modified MTSP-1 polypeptide is operatively linked to a nucleic acid encoding a Small Ubiquitin-like Modifier (SUMO) tag.
49. Use of the nucleic acid molecule of claim 31 in the preparation of a medicament for the treatment of a disease or pathological condition mediated by or involving complement activation, wherein inhibition of complement activation effects treatment or amelioration of the disease or pathological condition.
50. The use of claim 49, wherein the complement-mediated disease or condition is selected from among sepsis, Rheumatoid arthritis (RA), an ocular disease, membranoproliferative glomerulonephritis (MPGN), Multiple Sclerosis (MS), Myasthenia gravis (MG), asthma, inflammatory bowel disease, immune complex (IC)-mediated acute inflammatory tissue injury, Alzheimer's Disease (AD), and Ischemia-reperfusion injury.
51. The use of claim 49, wherein the disease or condition is an ocular disease or is rejection or inflammation due to a transplanted organ.
52. The use of claim 51, wherein the disease or condition is a diabetic retinopathy or a macular degeneration.
53. The use of claim 52, wherein the disease or condition is age-related macular degeneration (AMD).
54. A pharmaceutical composition, comprising the modified MTSP-1 polypeptide of any of claims 1-28 or fusion protein of claim 29 or claim 30, or the nucleic acid molecule of claim 31, or the vector of any of claims 32-34, in a pharmaceutically acceptable vehicle.
55. A modified membrane type serine protease 1 (MTSP-1) polypeptide of any of claims 1-28 or fusion protein of claim 29 or claim 30, or the nucleic acid molecule of claim 31, or the vector of any of claims 32-34 for use in therapy.