CLAIMS

1. A TSG-6-Link Module (LM) multimer, comprising:

a first TSG-6-LM linked directly or indirectly via a linker to a first multimerization domain; and

a second TSG-6-LM linked directly or indirectly via a linker to a second multimerization domain, wherein:

the first and second multimerization domains interact to form a multimer containing two or more TSG-6 link modules;

the link module comprises the sequence of amino acids set forth in SEQ ID NO: 207, 360, 361, 416, 417 or a sequence of amino acids having at least 95% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO: 207, 360, 361, 416, 417 or 418; and

the TSG-6-LM multimer has a binding affinity to hyaluronan (HA) with an association constant of at least 10^7 M^{-1}.

2. The TSG-6-LM multimer of claim 1, wherein the first and second link module are the same or different.

3. The TSG-6-LM multimer of claim 1 or claim 2, wherein:

the TSG-6 link module comprises an amino acid replacement at an amino acid position corresponding to 20, 34, 41, 54, 56, 72 or 84 set forth in SEQ ID NO:360, whereby the TSG-6 link module is modified to reduce or eliminate binding to heparin; and

a corresponding amino acid residue is identified by alignment with the TSG-6-LM set forth in SEQ ID NO:360.

4. The TSG-6-LM multimer of claim 3, wherein the amino acid replacement is selected from among Asp (D), Glu (E), Ser (S), Thr (T), Asn (N), Gln (Q), Ala (A), Val (V), Ile (I), Leu (L), Met (M), Phe (F), Tyr (Y) and Trp (W).

5. The TSG-6-LM multimer of claim 3 or claim 4, wherein the TSG-6 -LM comprises an amino acid replacement corresponding to amino acid replacement K20A, K34A or K41A in a TSG-6-LM set forth in SEQ ID NO:360.

6. The TSG-6-LM multimer of any of claims 1-3, wherein the TSG-6 link module comprises the amino acid replacements K20A, K34A and K41A in a TSG-6-LM set forth in SEQ ID NO:360.

7. The TSG-6-LM multimer of any of claims 1-6, wherein the link module comprises the sequence of amino acids set forth in SEQ ID NO:361 or 416 or a sequence of amino acids having at least 95% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO: 361 or 416 and that specifically binds HA.

8. The TSG-6-LM multimer of any of claims 1-7, wherein the multimerization domain is selected from among an immunoglobulin constant region (Fc), a leucine zipper, complementary hydrophobic regions, complementary hydrophilic regions, compatible protein-protein interaction domains, free thiols that forms an intermolecular disulfide bond between two molecules, and a protuberance-into-cavity and a compensatory cavity of identical or similar size that form stable multimers.

9. The TSG-6-LM multimer of any of claims 1-8, wherein the multimerization domain is an Fc domain or a variant thereof that effects multimerization.

10. The TSG-6-LM multimer of any of claims 1-9, wherein the first and second polypeptide comprise a TSG-6-LM and an immunoglobulin Fc domain.

11. The TSG-6-LM multimer of any of claims 1-10, comprising the sequence of amino acids set forth as amino acids 24-349 of SEQ ID NO: 212 or 215 or a sequence of amino acids that exhibits at least 95% amino acid sequence identity to amino acids 24-349 of SEQ ID NO:212 or 215.

12. The TSG-6-LM multimer of any of claims 1-10, wherein the TSG-6-LM multimer comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217, or a sequence of nucleotides that exhibits at least 95% sequence identity to the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217.

13. The TSG-6-LM multimer of any of claims 1-12 that has a binding affinity to HA with an association constant (Ka) of at least 1×10^8 M^{-1}.

14. A nucleic acid molecule encoding the TSG-6-LM multimer of any of claims 1-13.

15. The nucleic acid molecule of claim 14, comprising the sequence of nucleotides set forth in any of SEQ ID NO:211, 214 or 217, or a sequence of nucleotides that exhibits at least 85% sequence identity to the sequence of nucleotides set forth in any of SEQ ID NO: 211, 214 or 217.

16. A vector, comprising the nucleic acid molecule of claim 14 or claim 15.

17. An isolated cell or cell culture, comprising the nucleic acid molecule of 14 or claim 15.

18. A method of producing a TSG-6-LM multimer, comprising:

introducing the nucleic acid molecule of 14 or claim 15into a cell;

culturing the cell under conditions whereby the fusion polypeptide is expressed by the cell; and

recovering the TSG-6-LM multimer.

19. A kit or combination, comprising:

the TSG-6-LM multimer of any of claims 1-13; and

an anti-hyaluronan agent.

20. The kit or combination of claim 19, further comprising reagents for detection of the TSG-6-LM multimer.

21. The kit or combination of claim 19 or claim 20, wherein the anti-hyaluronan agent is a hyaluronan degrading enzyme or is an agent that inhibits hyaluronan synthesis.

22. The kit or combination of claim 21, wherein the anti-hyaluronan agent is a hyaluronan-degrading enzyme.

23. The kit or combination of claim 22, wherein the hyaluronan degrading enzyme is a hyaluronidase.

24. The kit or combination of claim 23, wherein the hyaluronan-degrading enzyme is a PH20 hyaluronidase or truncated form thereof lacking a C-terminal glycosylphosphatidyl-inositol (GPI) attachment site or a portion of the GPI attachment site.

25. The kit or combination of claim 24, wherein the PH20 or truncated form thereof comprises the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47, 48,

150-170 and 183-189 or a sequence of amino acids that exhibits at least 95% sequence identity to any of SEQ ID NOS: 4-9, 47, 48, 150-170 and 183-189.

26. The kit or combination of any of claims 19-25, wherein the anti-hyaluronan agent is a hyaluronan-degrading enzyme that is modified by conjugation to a polymer.

27. The kit or combination of claim 26, wherein the polymer is PEG and the hyaluronan degrading enzyme is PEGylated.

28. The TSG-6-LM multimer of any of claims 1-13 for use for detection of hyaluronan in a biological sample.

29. The TSG-6-LM multimer of any of claims 1-13 for use for predicting efficacy of treatment of a subject with an anti-hyaluronan agent.

CLAIMS:

1. A TSG-6-Link Module (LM) multimer, comprising:

a first TSG-6-LM linked directly or indirectly via a linker to a first multimerization domain; and

a second TSG-6-LM linked directly or indirectly via a linker to a second multimerization domain, wherein:

the first and second multimerization domains interact to form a multimer containing two or more TSG-6 link modules; and

the TSG-6-LM multimer has a binding affinity to hyaluronan (HA) with an association constant of at least 10^7 M^{-1}.

2. The TSG-6-LM multimer of claim 1, wherein the first and second link module are the same or different.

3. The TSG-6-LM multimer of claim 1 or claim 2, wherein the link module comprises the sequence of amino acids set forth in SEQ ID NO: 207, 360, 417 or 418 or a sequence of amino acids comprising at least 85% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO: 207, 360, 417 or 418 that specifically binds HA.

4. The TSG-6-LM multimer of any of claims 1-3, wherein the TSG-6 link module is modified to reduce or eliminate binding to heparin.

5. The TSG-6-LM multimer of claim 4, wherein binding to heparin is reduced at least 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold or more.

6. The TSG-6-LM multimer of claim 4 or claim 5, wherein the TSG-6 link module comprises an amino acid replacement at an amino acid position corresponding to amino acid residue 20, 34, 41, 54, 56, 72 or 84 set forth in SEQ ID NO:360, whereby a corresponding amino acid residue is identified by alignment to a TSG-6-LM set forth in SEQ ID NO:360.

7. The TSG-6-LM multimer of claim 6, wherein the amino acid replacement is to a non-basic amino acid residue selected from among Asp (D), Glu (E), Ser (S), Thr (T), Asn (N), Gln (Q), Ala (A), Val (V), Ile (I), Leu (L), Met (M), Phe (F), Tyr (Y) and Trp (W).

8. The TSG-6-LM multimer of any of claims 4-7, wherein the TSG-6 -LM comprises an amino acid replacement corresponding to amino acid replacement K20A, K34A or K41A in a TSG-6-LM set forth in SEQ ID NO:360 or the replacement at the corresponding residue in another TSG-6-LM.

9. The TSG-6-LM multimer of any of claims 4-8, wherein the TSG-6 link module comprises amino acid replacements corresponding to amino acid replacements K20A, K34A and K41A in a TSG-6-LM set forth in SEQ ID NO:360 or the replacement at the corresponding residue in another TSG-6-LM.

10. The TSG-6-LM multimer of any of claims 4-9, wherein the link module comprises the sequence of amino acids set forth in SEQ ID NO:361 or 416 or a sequence of amino acids comprising at least 85% amino acid sequence identity to the sequence of amino acids set forth in SEQ ID NO: 361 or 416 that specifically binds HA.

11. The TSG-6-LM multimer of any of claims 1-10, wherein the multimerization domain is selected from among an immunoglobulin constant region (Fc), a leucine zipper, complementary hydrophobic regions, complementary hydrophilic regions, compatible protein-protein interaction domains, free thiols that forms an intermolecular disulfide bond between two molecules, and a protuberance-into-cavity and a compensatory cavity of identical or similar size that form stable multimers.

12. The TSG-6-LM multimer of any of claims 1-11, wherein the multimerization domain is an Fc domain or a variant thereof that effects multimerization.

13. The TSG-6-LM multimer of any of claims 1-12, wherein the first and second polypeptide comprise a TSG-6-LM and an immunoglobulin Fc domain.

14. The TSG-6-LM multimer of any of claims 1-13, comprising the sequence of amino acids set forth as amino acids 24-349 of SEQ ID NO: 212 or 215 or a sequence of amino acids that exhibits at least 85% amino acid sequence identity to amino acids 24-349 of SEQ ID NO:212 or 215.

15. The TSG-6-LM multimer of any of claims 1-13, wherein the TSG-6-LM multimer comprises a sequence of amino acids encoded by the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217, or a sequence of nucleotides that exhibits at least 85% sequence identity to the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217.

16. The TSG-6-LM multimer of any of claims 1-15 that has a binding affinity to HA with an association constant (Ka) of at least $1 \times 10^8 M^{-1}$.

17. A nucleic acid molecule encoding the TSG-6-LM multimer of any of claims 1-16, wherein:

the nucleic acid molecule encodes a fusion polypeptide containing a TSG-6-LM linked directly or indirectly via a linker to a multimerization domain; and

the multimerization domain is a polypeptide that interacts with itself to form a stable protein-protein interaction, whereby the encoded protein forms a multimer containing at least two TSG-6 link modules.

18. The nucleic acid molecule of claim 17, comprising the sequence of nucleotides set forth in any of SEQ ID NO:211, 214 or 217, or a sequence of nucleotides that exhibits at least 85% sequence identity to the sequence of nucleotides set forth in any of SEQ ID NO: 211, 214 or 217.

19. A vector, comprising the nucleic acid molecule of claim 17 or claim 18.

20. An isolated cell or cell culture, comprising the nucleic acid molecule of claim 17 or claim 18.

21. A method of producing a TSG-6-LM multimer, comprising:

introducing the nucleic acid molecule of claim 17 or claim 18 into a cell;

culturing the cell under conditions whereby the fusion polypeptide is expressed by the cell; and

recovering the TSG-6-LM multimer.

22. (Amended) A kit or combination, comprising:

the TSG-6-LM multimer of any of claims 1-16; and

an anti-hyaluronan agent.

23. The kit or combination of claim 22, further comprising reagents for detection of the TSG-6-LM multimer.

24. The kit or combination of claim 22 or claim 23, wherein the anti-hyaluronan agent is a hyaluronan degrading enzyme or is an agent that inhibits hyaluronan synthesis.

25. The kit or combination of claim 24, wherein the anti-hyaluronan agent is a hyaluronan-degrading enzyme.

26. The kit or combination of claim 25, wherein the hyaluronan degrading enzyme is a hyaluronidase.

27. The kit or combination of claim 26, wherein the hyaluronan-degrading enzyme is a PH20 hyaluronidase or truncated form thereof lacking a C-terminal glycosylphosphatidylinositol (GPI) attachment site or a portion of the GPI attachment site.

28. The kit or combination of claim 27, wherein the PH20 or truncated form thereof comprises the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47, 48, 150-170 and 183-189 or a sequence of amino acids that exhibits at least 85% sequence identity to any of SEQ ID NOS: 4-9, 47, 48, 150-170 and 183-189.

29. The kit or combination of any of claims 22-28, wherein the anti-hyaluronan agent is a hyaluronan-degrading enzyme that is modified by conjugation to a polymer.

30. The kit or combination of claim 29, wherein the polymer is PEG and the hyaluronan degrading enzyme is PEGylated.

31. Use of the TSG-6-LM multimer of any of claims 1-16 for manufacture of a composition for predicting efficacy of treatment of a subject for treatment of a tumor with an anti-hyaluronan agent, comprising:

contacting a tissue or body fluid sample previously obtained from a subject who has a tumor or cancer with the composition; and

detecting binding of the TSG-6-LM multimer to the sample, thereby determining the amount of hyaluronan in the sample, wherein if the amount of hyaluronan in the sample is at or above a predetermined threshold level, selecting the subject for treatment with an anti-hyaluronan agent.

32. The use of claim 31, wherein the predetermined threshold level is high HA.

33. The use of claim 31 or claim 32, wherein:

the predetermined threshold level is at least or above 0.025 μg HA/ml of sample, 0.030 μg/ml, 0.035 μg/ml, 0.040 μg/ml, 0.045 μg/ml, 0.050 μg/ml, 0.055 μg/ml, 0.060 μg/ml, 0.065 μg/ml, 0.070 μg/ml, 0.08 μg/ml, 0.09 μg/ml. 0.1 μg/ml, 0.2 μg/ml, 0.3 μg/ml or higher; or

the sample is a tumor tissue sample, and the predetermined threshold is an HA score of at least +2 (HA^{+2}) or at least +3 (HA^{+3}); or

the sample is a tumor tissue sample, and the predetermined threshold level is at least a percent HA positive pixels in tumor (cells and stroma) to total stain in tumor tissue of at least 10%, 10% to 25% or greater than 25%.

34. The use of claim 33, wherein the predetermined threshold level is an HA score of at least +3 (HA^{+3}) (high levels).

35. Use of the TSG-multimer of any of claims 1-16 for manufacture of a composition for predicting efficacy of treatment of a subject with an anti-hyaluronan agent according to a method comprising:

contacting a tissue or body fluid sample previously obtained from a subject who is or has been treated with the composition; and

detecting binding of the TSG-6-LM multimer to the sample, thereby determining the amount of hyaluronan in the sample, wherein detection of a decrease in hyaluronan compared to before treatment with the anti-hyaluronan agent or before the previous dose of the anti-hyaluronan agent indicates that the treatment is effective.

36. The use of claim 35, wherein the anti-hyaluronan agent is a hyaluronan degrading enzyme or is an agent that inhibits hyaluronan synthesis.

37. The use of claim 36, wherein the anti-hyaluronan agent is a hyaluronan-degrading enzyme.

38. The use of claim 37, wherein the hyaluronan degrading enzyme is a hyaluronidase.

39. The use of claim 38, wherein the hyaluronan-degrading enzyme is a PH20 hyaluronidase or truncated form thereof lacking a C-terminal glycosylphosphatidylinositol (GPI) attachment site or a portion of the GPI attachment site.

40. The use of claim 39, wherein the PH20 or truncated form thereof comprises the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47, 48, 150-170 and 183-189 or a sequence of amino acids that exhibits at least 85% sequence identity to any of SEQ ID NOS: 4-9, 47, 48, 150-170 and 183-189.

41. The use of any of claims 36-40, wherein the anti-hyaluronan agent is a hyaluronan-degrading enzyme that is modified by conjugation to a polymer.

42. The use of claim 41, wherein the polymer is PEG and the hyaluronan degrading enzyme is PEGylated.

43. The use of any of claims 35-42, wherein the subject has a tumor or cancer.

44. Use of the TSG-multimer of any of claims 1-16 for manufacture of a composition for diagnosis of a hyaluronan-associated disease or condition, comprising:

contacting a tissue or body fluid sample previously obtained from a subject with the TSG-6-LM multimer; and

detecting binding of the TSG-6-LM multimer or a hyaluronan-binding region thereof that binds to the sample, thereby determining the amount of hyaluronan in the sample, wherein the subject is diagnosed with a hyaluronan-associated disease or condition if the amount of hyaluronan is above a predetermined level or above the hyaluronan level of a reference sample.

45. The use of claim 44, wherein the hyaluronan-associated disease or condition is a tumor or cancer.

46. The use of claim 44 or claim 45, wherein the predetermined or reference level is the median level of hyaluronan present in healthy or normal tissue or fluid samples from a population of control subjects.

47. The use of any of claims 44-46, wherein:

the predetermined threshold level is greater than 0.015 μg HA/ml of sample; or

the sample is a tumor or tissue sample, and the predetermined threshold level is an HA score of HA^{+1}, HA^{+2} or HA^{+3}.

48. The use of any of claims 31-47, wherein the TSG-6-LM multimer comprises a TSG-6 link module (LM) or a sufficient portion thereof that specifically binds HA.

49. The use of claim 48, wherein the TSG-6-LM multimer is modified to reduce or eliminate binding to heparin.

50. The use of claim 49, wherein binding to heparin is reduced at least 1.2-fold, 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 30-fold, 40-fold, 50-fold, 100-fold or more.

51. (Amended) The use of claim 49 or claim 50, wherein TSG-6-LMcomprises an amino acid replacement selected from among an amino acid replacement corresponding to amino acid replacement K20A, K34A and K41A in a TSG-6-LM set forth in SEQ ID NO:360 or the replacement at the corresponding residue in another TSG-6-LM.

52. The use of any of claims 31-51, wherein the TSG-6-LM is a multimer comprising a first HA-binding domain linked directly or indirectly via a linker to a multimerization domain and a second HA-binding domain linked directly or indirectly via a linker to a multimerization domain, wherein the first and second multimerization domains interact to form a multimer containing two or more HA-binding domains.

53. The use of claim 52, wherein the first and second HA-binding domain is a TSG-6 link module, a variant thereof or a sufficient portion thereof that specifically binds to HA.

54. The use of claim 52 or claim 53, wherein the multimerization domain is selected from among an immunoglobulin constant region (Fc), a leucine zipper, complementary hydrophobic regions, complementary hydrophilic regions, compatible protein-protein interaction domains, free thiols that forms an intermolecular disulfide bond between two molecules, and a protuberance-into-cavity and a compensatory cavity of identical or similar size that form stable multimers.

55. The use of any of claims 52-54, wherein the multimerization domain is an Fc domain or a variant thereof that effects multimerization.

56. The use of any of claims 52-55, wherein the TSG-6 link module and an immunoglobulin Fc domain comprise a fusion protein (TSG-6-LM-Fc).

57. The use of claim 56, wherein the TSG-6-LM-Fc has the sequence of amino acids set forth as amino acids 24-349 of SEQ ID NO: 212 or 215 or a sequence of amino acids that exhibits at least 85% amino acid sequence identity to amino acids 24-349 of SEQ ID NO:212 or 215 and specifically binds HA.

58. The use of claim 56 or claim 57, wherein the TSG-6-LM-Fc comprises the sequence of amino acids encoded by the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217, or a sequence of nucleotides that exhibits at least 85% sequence identity to the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217.

59. The use of any of claims 31-58, wherein the TSG-6-LM multimer specifically binds to HA with a binding affinity that has a dissociation constant (Kd) of at least less than or less than 1×10^{-7} M.

60. The use of any of claims 31-59, wherein the TSG-6-LM multimer is conjugated to a detectable moiety that is detectably labeled or that can be detected.

61. The use of any of claims 31-60, wherein:

the sample is a stromal tissue sample; or

the sample is a fluid sample that is a blood, serum, urine, sweat, semen, saliva, cerebral spinal fluid, or lymph sample.

62. The use of any of claims 31-61, wherein the sample is a stromal tissue sample from a tumor.

63. The use of any of claims 31-62, wherein the tumor is of a cancer selected from among breast cancer, pancreatic cancer, ovarian cancer, colon cancer, lung cancer, non-small cell lung cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), thyroid cancer, cervical cancer, uterine cancer, prostate cancer, testicular cancer, brain cancer, bladder cancer, stomach cancer, hepatoma, melanoma, glioma, retinoblastoma, mesothelioma, myeloma, lymphoma, and leukemia.

64. The use of any of claims 31-63, wherein the tumor is of a cancer that is a late-stage cancer, a metastatic cancer and an undifferentiated cancer.

65. The use of any of claims 31-64, wherein hyaluronan is detected by a solid phase binding assay by histochemistry, or by *in vivo* imaging.

66. Use of the TSG-6-LM multimer of any of claims 1-16 for manufacture of a composition for selecting a subject for treatment of a tumor with anti-hyaluronan agent.

67. A pharmaceutical composition comprising the TSG-6-LM multimer of any of claims 1-16 for use in selecting a subject for treatment of a tumor with an anti-hyaluronan agent.

68. Use of the TSG-6-LM multimer of any of claims 1-16 for manufacture of a composition for predicting efficacy of treatment of a subject with an anti-hyaluronan agent.

69. A pharmaceutical composition comprising the TSG-6-LM multimer of any of claims 1-16 for predicting efficacy of treatment of a subject with an anti-hyaluronan agent.

70. The use of any of claims 66-69, wherein the TSG-6-LM-Fc comprises the sequence of amino acids set forth as amino acids 24-349 of SEQ ID NO:212 or 215, or a sequence of amino acids that exhibits at least 85% sequence identity to the sequence of amino acids 24-349 set forth in SEQ ID NO: 212 or 215 and specifically binds HA, or an HA-binding domain thereof or a sufficient portion thereof to specifically bind to HA.

71. The use of any of claims 66-70 wherein the TSG-6-LM-Fc comprises the sequence of amino acids encoded by the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217, or a sequence of nucleotides that exhibits at least 85% sequence identity to the sequence of nucleotides set forth in any of SEQ ID NOS: 211, 214 or 217.

72. The use of any of claims 66-70, wherein the anti-hyaluronan agent is a hyaluronan degrading enzyme or is an agent that inhibits hyaluronan synthesis.

73. The use of claim 72, wherein the anti-hyaluronan agent is a hyaluronan-degrading enzyme.