

CLAIMS:

1. A method of identifying a subject for treatment of a tumor with a modified hyaluronidase, comprising:
 - a) measuring the expression or level of hyaluronan in a biopsy of a tumor previously obtained from a subject; and
 - b) selecting a subject in which hyaluronan is expressed in at least 30% of the tumoral area of the tumor biopsy, thereby identifying the subject from whom the sample was obtained as a subject for treatment with the modified hyaluronidase, wherein modification of the hyaluronidase is conjugation to a polymer.
2. The method of claim 1, wherein a subject is selected if at least 70% of the tumoral area of the tumor biopsy expresses hyaluronan.
3. The method of claim 1, wherein hyaluronan is measured by immunohistochemistry.
4. The method of claim 2, wherein hyaluronan is measured by immunofluorescence.
5. The method of any of claims 1-4, wherein hyaluronan is detecting using an HA-binding protein.
6. The method of any of claims 1-5, wherein the tumor is a solid tumor.
7. The method of claim any of claims 1-6, wherein the tumor is associated with a cancer.
8. The method of claim 7, wherein the cancer is selected from among any one or more of a late-stage cancer, a metastatic cancer and an undifferentiated cancer.
9. The method of claim 7 or claim 8, wherein the cancer is selected from among any one or more of ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, mesothelioma, breast cancer, brain cancer and colon cancer.
10. The method of any of claims 1-9, wherein the hyaluronidase is a soluble hyaluronidase.
11. The method of claim 10, wherein the soluble hyaluronidase is a soluble PH20.
12. The method of claim 11, wherein the hyaluronidase is a soluble form of a PH20 that is selected from among an ovine, mouse, monkey, bovine, bacterial and human PH20.

13. The method of claim 11 or claim 12, wherein the soluble form of PH20 is a C-terminally truncated PH20 that lacks all or a portion of a C-terminal glycosylphosphatidylinositol (GPI) anchor.

14. The method of any of claims 10-13, wherein the soluble hyaluronidase is selected from among:

a) a polypeptide consisting of a sequence of amino acids set forth in SEQ ID NO:1 that contains amino acid residues 36-464 of SEQ ID NO:1 and the residues up to a C-terminal amino acid residue that is selected from among an amino acid residue 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482 and 483 of SEQ ID NO:1; or

b) a polypeptide that has a sequence of amino acids that exhibits at least about 91% amino acid sequence identity with a sequence of amino acids set forth in a), whereby the soluble hyaluronidase is soluble, N-glycosylated and neutral active.

15. The method of any of claims 10-14, wherein the soluble hyaluronidase consists of the sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1, or a sequence of amino acids has at least about 91% amino acid sequence identity with a sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1.

16. The method of any of claims 10-15, wherein the soluble hyaluronidase is selected from among a polypeptide consisting of the sequence of amino acids set forth in any of SEQ ID NOS: 4-9 and 46-48, or a sequence that exhibit at least 91% sequence identity to any of SEQ ID NOS: 4-9 and 46-48.

17. The method of any of claims 1-16, wherein the polymer conjugated to the hyaluronidase comprises a pegylation moiety (PEG).

18. The method of claim 17, wherein the PEG moiety results from reaction with a PEG reagent selected from among methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (5 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (30 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (30 kDa); methoxy-poly(ethylene glycol)-butyraldehyde (mPEG-butyraldehyde) (30 kDa), methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (30 kDa); (methoxy-

poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (10 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (20 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (40 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (60 kDa branched); biotin-poly(ethylene glycol)-N-hydroxysuccinimide ester (biotin-PEG-NHS) (5 kDa biotinylated); poly(ethylene glycol)-p-nitrophenyl carbonate (PEG-p-nitrophenyl-carbonate) (30 kDa); and poly(ethylene glycol)-propionaldehyde (PEG-propionaldehyde) (30 kDa).

19. The method of claim 17 or claim 18, wherein the PEG is a branched or linear PEG.

20. The method of any of claims 17-19, wherein the PEG has a weight of 30 or about 30 kilodaltons.

CLAIMS:

1. A method of identifying a subject for treatment with a modified hyaluronidase, comprising:
 - a) measuring the expression or level of a marker in a sample previously obtained from a subject, wherein the marker is associated with a hyaluronan-associated disease or condition;
 - b) comparing the expression or level of the marker to expression or level in a control sample or standard; and
 - c) if the marker is different from the control sample or standard, identifying the subject from whom the sample was obtained as a subject for treatment with the modified hyaluronidase, wherein modification of the hyaluronidase is conjugation to a polymer.
2. The method of claim 1, wherein the sample in a) comprises a tissue, a cell or a bodily fluid.
3. The method of claim 1 or claim 2, wherein the sample is a tumor biopsy.
4. The method of any of claims 1-3, wherein the control sample in b) is selected from among:
 - a) an analogous sample previously obtained from another subject that is a normal subject or a subject known to express low hyaluronan in the sample; and
 - b) a cell line.
5. The method of any of claims 1-4, wherein the expression or level of the marker is elevated compared to the control sample or standard.
6. The method of any of claims 1-5, wherein the marker is elevated and the elevation of the marker is by greater than 0.5-fold compared to the control sample.
7. The method of any of claims 1-6, wherein detection of expression or level of the marker is effected by immunohistochemistry, histology, ELISA, an ELISA-like assay, Western Blot, flow cytometry, PCR or RT-PCR.
8. The method of any of claims 1-7, wherein the marker is hyaluronan (HA), a hyaluronidase or a hyaluronan synthase.
9. The method of any of claims 1-8, wherein the marker is a hyaluronan synthase that is hyaluronan synthase 2 (HAS2).

10. The method of any of claims 1-9, wherein the marker is hyaluronan.
11. The method of claim 10, wherein hyaluronan is detecting using an HA-binding protein.
12. The method of claim 10 or claim 11, wherein a subject is selected for treatment if moderate to high hyaluronan is measured.
13. The method of any of claims 10-12, wherein the sample is a tumor and a subject is selected for treatment if moderate to high hyaluronan is measured.
14. The method of claim 13, wherein moderate to high hyaluronan is present if at least 30% of the tumoral area expresses hyaluronan.
15. The method of claim any of claims 1-14, wherein the disease or condition is selected from among disc pressure, cancer and edema.
16. The method of claim 15, wherein the disease or condition is cancer, and the cancer is a tumor or a solid tumor.
17. The method of claim 15 or claim 16, wherein the disease or condition is cancer selected from among any one or more of a late-stage cancer, a metastatic cancer and an undifferentiated cancer.
18. The method of any of claims 15-17, wherein the disease or condition is cancer selected from among any one or more of ovarian cancer, in situ carcinoma (ISC), squamous cell carcinoma (SCC), prostate cancer, pancreatic cancer, non-small cell lung cancer, mesothelioma, breast cancer, brain cancer and colon cancer.
19. The method of any of claims 1-18, wherein the hyaluronidase is a soluble hyaluronidase.
20. The method of claim 19, wherein the soluble hyaluronidase is a soluble PH20.
21. The method of claim 20, wherein the PH20 is selected from among an ovine, mouse, monkey, bovine, bacterial and human PH20.

22. The method of claim 20 or claim 21, wherein the soluble form of PH20 is a soluble PH20 that lacks all or a portion of a C-terminal GPI anchor.

23. The method of any of claims 1-22, wherein the soluble hyaluronidase is selected from among:

a) a polypeptide that is encoded by a nucleic acid molecule encoding a C-terminally truncated polypeptide of the polypeptide set forth in SEQ ID NO:1, wherein the C-terminal amino acid residues is selected from among an amino acid residue 467 to 483 of SEQ ID NO:1; or

b) a polypeptide that has at least about 91% amino acid sequence identity with a sequence of amino acids set forth in a), whereby the soluble hyaluronidase is soluble, N-glycosylated and neutral active.

24. The method of any of claims 1-23, wherein the soluble hyaluronidase has the sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1, or has at least about 91% amino acid sequence identity with a sequence of amino acids set forth as amino acids 36-467, 36-468, 36-469, 36-470, 36-471, 36-472, 36-473, 36-474, 36-475, 36-476, 36-477, 36-478, 36-479, 36-480, 36-481, 36-482, or 36-483 of SEQ ID NO:1.

25. The method of any of claims 1-24, wherein the soluble hyaluronidase is selected from among a polypeptide containing the sequence of amino acids set forth in any of SEQ ID NOS: 4-9 and 46-48, and variants thereof that exhibit at least 91% sequence identity to any of SEQ ID NOS: 4-9 and 46-48.

26. The method of any of claims 1-25, wherein the polymer conjugated to the hyaluronidase comprises a pegylation moiety (PEG).

27. The method of claim 83, wherein the PEG moiety results from reaction with a PEG reagent selected from among methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (5 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl butanoate (mPEG-SBA) (30 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl α -methylbutanoate (mPEG-SMB) (30 kDa); methoxy-poly(ethylene glycol)-butyraldehyde (mPEG-butyraldehyde) (30 kDa), methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (20 kDa); methoxy-poly(ethylene glycol)-succinimidyl propionate (mPEG-SPA) (30 kDa); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (10 kDa branched); (methoxy-poly(ethylene glycol))₂-N-hydroxysuccinimide ester (mPEG₂-NHS) (20 kDa branched); (methoxy-poly(ethylene glycol))

2-N-hydroxysuccinimide ester (mPEG₂-NHS) (40 kDa branched); (methoxy-poly(ethylene glycol)) 2-N-hydroxysuccinimide ester (mPEG₂-NHS) (60 kDa branched); biotin-poly(ethylene glycol)-N-hydroxysuccinimide ester (biotin-PEG-NHS) (5 kDa biotinylated); poly(ethylene glycol)-p-nitrophenyl carbonate (PEG-p-nitrophenyl-carbonate) (30 kDa); and poly(ethylene glycol)-propionaldehyde (PEG-propionaldehyde) (30 kDa).

28. The method of claim 26 or claim 27, wherein the PEG is a branched or linear PEG.

29. The method of any of claims 26-28, wherein the PEG has a weight of 30 or about 30 kilodaltons.