

CLAIMS

1. A method of detecting human folate receptor 1 (FOLR1) in a sample comprising:
 - (a) capturing said folate receptor 1 (FOLR1) with an immunocapture reagent bound to a solid support;
 - (b) eluting FOLR1 from the solid support;
 - (c) digesting the eluted FOLR1; and
 - (d) performing liquid chromatography-mass spectrometry (LC/MS) analysis on the digested FOLR1, wherein said FOLR1 is detected by monitoring the chromatographic separation and mass spectrometric response of at least one signature FOLR1 peptide, wherein the immunocapture reagent comprises an antibody or antigen-binding fragment which binds to FOLR1, and wherein the FOLR1 is shed FOLR1, wherein the method can detect at least 0.5 ng/mL FOLR1 in a sample.
2. The method of claim 1, wherein the level of FOLR1 in the sample is quantitated by said LC/MS analysis.
3. The method of claim 2, wherein the level of FOLR1 in the sample is quantitated by comparing the level of FOLR1 in the sample to a reference level of FOLR1.
4. The method of any one of claims 1-3, wherein binding of the antibody or antigen-binding fragment to FOLR1 is not competitively inhibited by binding of IMGN853 to FOLR1.
5. The method of any one of claims 1-3, wherein binding of the antibody or antigen-binding fragment to FOLR1 is not competitively inhibited by binding of huMov19 to FOLR1.
6. The method of any one of claims 1-3, wherein binding of the antibody or antigen-binding fragment to FOLR1 is not competitively inhibited by binding of a second antibody or antigen-binding fragment to FOLR1, wherein the second antibody or antigen-binding fragment comprises: a variable light chain (VL) complementarity determining region (CDR)-1 of SEQ ID NO: 59; a VL CDR-2 of SEQ ID NO: 60; a VL CDR-3 of SEQ ID NO: 61; a variable heavy chain (VH) CDR-1 of SEQ ID NO: 62; a VH CDR-2 of SEQ ID NO: 64; and a VH CDR-3 of SEQ ID NO: 65.
7. The method of any one of claims 1-3, wherein binding of the antibody or antigen-binding fragment to FOLR1 is not competitively inhibited by binding of a second antibody or antigen-binding fragment to FOLR1, wherein the second antibody or antigen-binding fragment comprises a variable heavy chain (VH) having the sequence of SEQ ID NO: 56 and a variable light chain (VL) having the sequence of SEQ ID NO: 57 or SEQ ID NO: 58.
8. The method of any one of claims 1-3, wherein binding of the antibody or antigen-binding fragment to FOLR1 is not competitively inhibited by binding of a second antibody or antigen-binding fragment to FOLR1, wherein the second antibody or antigen-binding fragment comprises (i) a heavy chain comprising the same amino acid sequence as the amino acid

sequence of the heavy chain encoded by the plasmid deposited with the American Type Culture Collection (ATCC) as PTA-10772 and (ii) a light chain comprising the same amino acid sequence as the amino acid sequence of the light chain encoded by the plasmid deposited with the ATCC as PTA-10774.

9. The method of any one of claims 1-8, wherein binding of the antibody or antigen-binding fragment to FOLR1 is not inhibited by binding of folic acid to FOLR1.

10. The method of any one of claims 1-9, wherein the antibody is muFR1-9.

11. The method any one of claims 1-9, wherein the antibody is muFR1-13.

12. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment comprises a variable heavy chain (VH) complementarity determining region (CDR)-1 of SEQ ID NO: 1; a VH CDR-2 of SEQ ID NO: 2; a VH CDR-3 of SEQ ID NO: 3; a variable light chain (VL) complementarity determining region (CDR)-1 of SEQ ID NO: 13; a VL CDR-2 of SEQ ID NO: 14, and a VL CDR-3 of SEQ ID NO: 15.

13. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment comprises a variable heavy chain (VH) complementarity determining region (CDR)-1 of SEQ ID NO: 4; a VH CDR-2 of SEQ ID NO: 5; a VH CDR-3 of SEQ ID NO: 6; a variable light chain (VL) complementarity determining region (CDR)-1 of SEQ ID NO: 16; a VL CDR-2 of SEQ ID NO: 17, and a VL CDR-3 of SEQ ID NO: 18.

14. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment comprises a variable heavy chain (VH) having the sequence of SEQ ID NO: 25 and a variable light chain (VL) having the sequence of SEQ ID NO: 29.

15. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment comprises a variable heavy chain (VH) having the sequence of SEQ ID NO: 26 and a variable light chain (VL) having the sequence of SEQ ID NO: 30.

16. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment comprises a heavy chain having the sequence of SEQ ID NO: 33 and a light chain having the sequence of SEQ ID NO: 37.

17. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment comprises a heavy chain having the sequence of SEQ ID NO: 34 and a light chain having the sequence of SEQ ID NO: 38.

18. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment binds to human FOLR1 with a K_d of 1.0 nM to 10 nM.

19. The method of any one of claims 1-9, wherein the antibody or antigen-binding fragment binds to human FOLR1 with a K_d of 0.5 nM to 5 nM.

20. The method of any one of claims 1-19, wherein the solid support comprises a mass spectrometric immunoassay (MSIA) microcolumn.

21. The method of any one of claims 1-19, wherein the solid support comprises magnetic beads.

22. The method of any one of claims 1-21, wherein at least one wash step is performed prior to eluting FOLR1 from the solid support.

23. The method of claim 22, wherein two or more wash steps are performed prior to eluting FOLR1 from the solid support.

24. The method of claim 22 or 23, where the wash step comprises contacting the FOLR1 bound to the solid support with washing buffers, a salt solution, and a detergent.

25. The method of any one of claims 1-24, wherein FOLR1 is eluted from the solid support with an acidic solution.

26. The method of claim 25, wherein the FOLR1 is reduced and alkylated prior to digesting the FOLR1.

27. The method of any one of claims 1-26, wherein FOLR1 is digested with Trypsin/Lys-C.

28. The method of any one of claims 1-27, wherein digesting the FOLR1 with trypsin produces a peptide comprising the sequence of SEQ ID NO: 42.

29. The method of any one of claims 1-28, wherein digesting the FOLR1 with trypsin produces a peptide comprising the sequence of SEQ ID NO: 43.

30. The method of any one of claims 1-29, wherein digesting the FOLR1 with trypsin produces a peptide comprising the sequence of SEQ ID NO: 44.

31. The method of any one of claims 1-30, wherein digesting the FOLR1 with trypsin produces a peptide comprising the sequence of SEQ ID NO: 45.

32. The method of any one of claims 1-31, wherein at least two signature peptides of FOLR1 are selected and monitored at the LC/MS analysis step.

33. The method of any one of claims 1-32, wherein at least three signature peptides of FOLR1 are selected and monitored at the LC/MS analysis step.

34. The method of any one of claims 1-33, wherein at least four signature peptides of FOLR1 are selected and monitored at the LC/MS analysis step.

35. The method of claim 34, wherein the signature peptides comprise:

(a) a peptide comprising the sequence of SEQ ID NO: 42;

(b) a peptide comprising the sequence of SEQ ID NO: 43;

(c) a peptide comprising the sequence of SEQ ID NO: 44; and

- (d) a peptide comprising the sequence of SEQ ID NO: 45.
- 36. The method of any one of claims 1-35, wherein said sample comprises a bodily fluid.
- 37. The method of claim 36, wherein said bodily fluid is plasma.
- 38. The method of claim 36, wherein said bodily fluid is serum.
- 39. The method of claim 36, wherein said bodily fluid is ascites fluid.
- 40. The method of any one of claims 1-35, wherein the sample comprises a peripheral blood sample.
- 41. The method of any one of claims 1-40, wherein the sample is obtained from a patient having cancer.
- 42. The method of claim 41, wherein said cancer is selected from the group consisting of: ovarian, brain, breast, uterine, endometrial, pancreatic, renal, lung cancer, and cancer of the peritoneum.
- 43. The method of claim 42, wherein the cancer is ovarian cancer.
- 44. The method of any one of claims 1-43, wherein detecting FOLR1 is not inhibited by IMGN853 present in the sample.
- 45. The method of any one of claims 1-43, wherein detecting FOLR1 is not inhibited by huMov19 present in the sample.
- 46. The method of any one of claims 1-43, wherein detecting FOLR1 is not inhibited by an antibody or antigen-binding fragment present in the sample, wherein said antibody or antigen-binding fragment comprises: a variable light chain (VL) complementarity determining region (CDR)-1 of SEQ ID NO: 59; a VL CDR-2 of SEQ ID NO: 60; a VL CDR-3 of SEQ ID NO: 61; a variable heavy chain (VH) CDR-1 of SEQ ID NO: 62; a VH CDR-2 of SEQ ID NO: 64; and a VH CDR-3 of SEQ ID NO: 65.
- 47. The method of any one of claims 1-43, wherein detecting FOLR1 is not inhibited by an antibody or antigen-binding fragment present in the sample, wherein said antibody or antigen-binding fragment comprises a variable heavy chain (VH) having the sequence of SEQ ID NO: 56 and a variable light chain (VL) having the sequence of SEQ ID NO: 57 or SEQ ID NO: 58.
- 48. The method of any one of claims 1-43, wherein detecting FOLR1 is not inhibited by an antibody or antigen-binding fragment present in the sample, wherein said antibody or antigen-binding fragment comprises (i) a heavy chain comprising the same amino acid sequence as the amino acid sequence of the heavy chain encoded by the plasmid deposited with the American Type Culture Collection (ATCC) as PTA-10772 and (ii) a light chain comprising the

same amino acid sequence as the amino acid sequence of the light chain encoded by the plasmid deposited with the ATCC as PTA-10774.

49. The method of any one of claims 1-48, wherein detecting FOLR1 is not inhibited by folic acid present in the sample.

~~50. The method of any one of claims 1-49, which can detect at least 0.5 ng/mL FOLR1 in a sample.~~

50. ~~[[51.]]~~ The method of any one of claims 1-49, which can detect at least 0.3 ng/mL FOLR1 in a sample.

51. ~~[[52.]]~~ The method of any one of claims 1-49, which can detect at least 0.25 ng/mL FOLR1 in a sample.

52. ~~[[53.]]~~ The method of any one of claims 1-~~51~~[[52]], wherein the signal-to-noise ratio is at least 5.

53. ~~[[54.]]~~ The method of any one of claims 1-~~51~~[[52]], wherein the signal-to-noise ratio is at least 10.