

CLAIMS

WHAT IS CLAIMED IS:

1. A method of treating cancer in a patient comprising administering a pharmaceutical composition comprising an anti-folate receptor α (FR α) active agent to a cancer patient with a soluble FR α level equal to or greater than a target soluble FR α level, wherein the active agent is an immunoconjugate comprising an anti-FR α antibody or antigen-binding fragment thereof conjugated to a cytotoxic agent.
2. The method of claim 1, wherein the patient's soluble FR α has been detected in a sample obtained from the patient prior to the administration.
3. The method of claim 1 or 2, wherein a cancer sample obtained from the patient does not have a high FR α immunohistochemistry (IHC) score.
4. The method of claim 1 or 2, wherein a cancer sample obtained from the patient has a high FR α IHC score.
5. The method of claim 1 or 2, wherein no FR α IHC score has been obtained from the patient.
6. A method of treating cancer in a patient comprising (i) administering a pharmaceutical composition comprising an anti-FR α active agent to the patient if the patient has a soluble FR α level equal to or greater than a target soluble FR α level and/or a cancer sample obtained from the patient has a high FR α IHC score and (ii) administering chemotherapy to the patient if the patient does not have a soluble FR α level equal to or greater than a target soluble FR α level and a cancer sample obtained from the patient does not have a high FR α IHC score, wherein the active agent is an immunoconjugate comprising an anti-FR α antibody or antigen-binding fragment thereof conjugated to a cytotoxic agent.

7. The method of claim any one of claims 1-6, further comprising determining the level of soluble FR α in sample obtained from the patient prior to the administering of the active agent.
8. The method of any one of claims 3, 4, 6, and 7, further comprising determining the FR α IHC score in a cancer sample obtained from the patient prior to the administration.
9. A method for identifying a cancer in a patient as likely to respond to an anti-FR α active agent, the method comprising assaying for soluble FR α in a sample obtained from the patient and optionally determining the FR α IHC score in a tumor sample obtained from the patient, wherein the presence of a soluble FR α level equal to or greater than a target soluble FR α level and/or a high FR α IHC score indicates the cancer is likely to respond to the anti-FR α active agent, optionally wherein the method further comprises administering a pharmaceutical composition comprising the anti-FR α active agent to the patient if the cancer is likely to respond, wherein the active agent is an immunoconjugate comprising an anti-FR α antibody or antigen-binding fragment thereof conjugated to a cytotoxic agent.
10. The method of claim 9, wherein a level of soluble FR α that is not equal to or greater than a target soluble FR α level and an IHC score that is not high indicates the cancer is not likely to respond to a pharmaceutical composition comprising the anti-FR α active agent.
11. The method of claim 9, wherein a level of soluble FR α that is not equal to or greater than a target soluble FR α level and an IHC score that is not high indicates the cancer is likely to be more responsive to chemotherapy than to a pharmaceutical composition comprising the anti-FR α active agent.
12. The method of any one of claims 1-11, wherein the patient's level of soluble FR α is assessed using liquid chromatography-mass spectrometry (LC/MS), enzyme-linked immunosorbent assay (ELISA), and/or or meso scale discovery (MSD).

13. The method of any one of claims 1-12, wherein the target soluble FR α level is about 0.5 ng/mL, about 0.6 ng/mL, about 0.7 ng/mL, about 0.75 ng/mL, about 0.8 ng/mL, about 0.9 ng/mL, about 1 ng/mL, about 1.1 ng/mL, about 1.2 ng/mL, about 1.25 ng/mL, about 1.3 ng/mL, about 1.4 ng/mL, about 1.5 ng/mL, about 1.6 ng/mL, about 1.7 ng/mL, about 1.75 ng/mL, about 1.8 ng/mL, about 1.9 ng/mL, about 2.0 ng/mL, about 2.1 ng/mL, about 2.2 ng/mL, about 2.25 ng/mL, about 2.3 ng/mL, about 2.4 ng/mL, about 2.5 ng/mL, about 2.6 ng/mL, about 2.7 ng/mL, about 2.75 ng/mL, about 2.8 ng/mL, about 2.9 ng/mL, about 3.0 ng/mL, about 3.1 ng/mL, about 3.2 ng/mL, about 3.25 ng/mL, about 3.3 ng/mL, about 3.4 ng/mL, about 3.5 ng/mL, about 3.6 ng/mL, about 3.7 ng/mL, about 3.75 ng/mL, about 3.8 ng/mL, about 3.9 ng/mL, about 4.0 ng/mL, about 4.1 ng/mL, about 4.2 ng/mL, about 4.25 ng/mL, about 4.3 ng/mL, about 4.4 ng/mL, about 4.5 ng/mL, about 4.6 ng/mL, about 4.7 ng/mL, about 4.75 ng/mL, about 4.8 ng/mL, about 4.9 ng/mL, or about 5 ng/mL.
14. The method of any one of claims 1-13, wherein the target soluble FR α level is the average soluble FR α level in patients with ovarian, primary peritoneal, or fallopian tube cancer with a tumor with medium (50-74% cells positive) or high (at least 75% cells positive) membrane FR α levels as determined by percent staining (PS) 2+ staining intensity.
15. The method of any one of claims 3, 4, and 6-13, wherein a high IHC score refers to at least 75% of tumor cells with percent staining (PS) 2+ staining intensity.
16. The method of any one of claims 3, 4, and 6-13, wherein a high IHC score refers to at least 50% of tumor cells with PS2+ staining intensity.
17. The method of any one of claims 1-16, wherein the cancer is a solid tumor.

18. The method of any one of claims 1-17, wherein the cancer is selected from the group consisting of: ovarian cancer, uterine cancer, endometrial cancer, pancreatic cancer, renal cancer, lung cancer, peritoneal cancer, breast cancer, and fallopian tube cancer.
19. The method of claim 18, wherein the cancer is ovarian cancer, optionally wherein the ovarian cancer is platinum-resistant or platinum-refractory.
20. The method of claim 18, wherein the cancer is platinum-sensitive ovarian cancer.
21. The method of any one of claims 18-19, wherein the ovarian cancer is epithelial ovarian cancer.
22. The method of any one of claims 18, 19, or 21, wherein the cancer is platinum-resistant, advanced high-grade epithelial ovarian cancer.
23. The method of any one of claims 1-22, wherein the cancer is a recurrent cancer.
24. The method of any one of claims 1-23, wherein the anti-FR α antibody or antigen-binding fragment thereof in the active agent comprises
 - (a) a variable heavy chain (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO:10, a VH-CDR2 comprising the amino acid sequence of SEQ ID NO:11, a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:12, a variable light (VL)-CDR1 comprising the amino acid sequence of SEQ ID NO:15, a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:16, and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:17;
 - (b) a VH comprising the amino acid sequence of SEQ ID NO:38 and a VL comprising the amino acid sequence of SEQ ID NO:44;
 - (c) a heavy chain comprising the amino acid sequence of SEQ ID NO:50 and a light chain comprising the amino acid sequence of SEQ ID NO:56;
 - (d) a variable heavy chain (VH) complementarity determining region (CDR) 1 comprising the amino acid sequence of SEQ ID NO:2, a VH-CDR2 comprising the amino

acid sequence of SEQ ID NO:3, a VH-CDR3 comprising the amino acid sequence of SEQ ID NO:4, a variable light (VL)-CDR1 comprising the amino acid sequence of SEQ ID NO:7, a VL-CDR2 comprising the amino acid sequence of SEQ ID NO:8, and a VL-CDR3 comprising the amino acid sequence of SEQ ID NO:9;

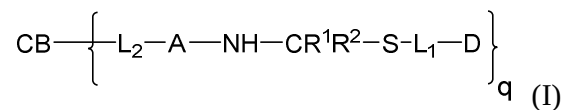
(e) a VH comprising the amino acid sequence of SEQ ID NO:37 and a VL comprising the amino acid sequence of SEQ ID NO:43;

(f) a heavy chain comprising the amino acid sequence of SEQ ID NO:49 and a light chain comprising the amino acid sequence of SEQ ID NO:55;

(g) a single-chain variable fragment (scFv), optionally wherein the scFV comprises the amino acid sequence of SEQ ID NO:60; and/or

(h) a biparatopic antibody or antigen-binding fragment thereof comprising the amino acid sequences of SEQ ID NOs:61, 62, and 56.

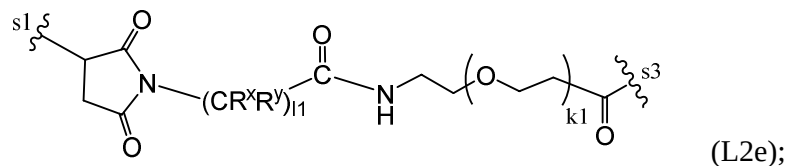
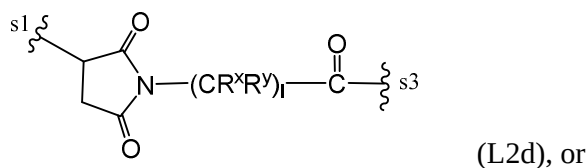
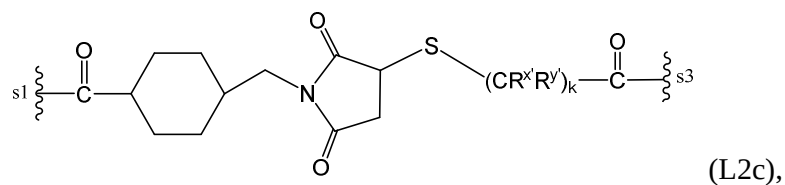
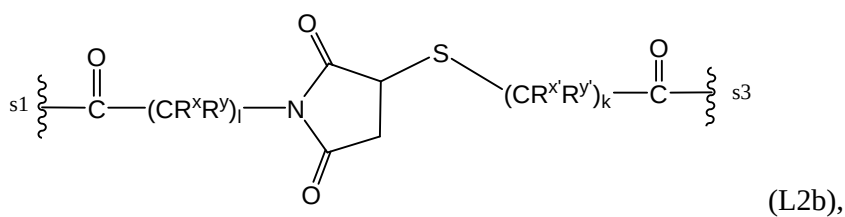
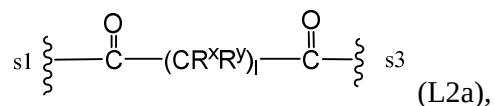
25. The method of any one of claims 1-24, wherein the cytotoxic agent is conjugated to the anti-FR α antibody or antigen-binding fragment thereof by a linker, optionally wherein the linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a hydrophilic linker, and a dicarboxylic acid based linker.
26. The method of any one of claims 1-25, wherein the cytotoxic agent is selected from the group consisting of a maytansinoid, maytansinoid analog, benzodiazepine, taxoid, CC-1065, CC-1065 analog, duocarmycin, duocarmycin analog, calicheamicin, dolastatin, dolastatin analog, auristatin, tomaymycin derivative, and leptomycin derivative or a prodrug of the agent.
27. The method of any one of claims 1-25, wherein the cytotoxic agent is a maytansinoid, optionally wherein the maytansinoid is DM4 or DM24.
28. The method of any one of claims 1-27, wherein the immunoconjugate is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

CB is an anti-FR α antibody or antigen-binding fragment thereof;

L₂ is represented by one of the following formula:



wherein:

R^x, R^y, R^{x'} and R^{y'}, for each occurrence, are independently H, -OH, halogen, -O-(C₁₋₄ alkyl), -SO₃H, -NR₄₀R₄₁R₄₂⁺, or a C₁₋₄ alkyl optionally substituted with -OH, halogen, SO₃H or NR₄₀R₄₁R₄₂⁺, wherein R₄₀, R₄₁ and R₄₂ are each independently H or a

C₁₋₄ alkyl;

l and k are each independently an integer from 1 to 10;

l1 is an integer from 2 to 5;

k1 is an integer from 1 to 5; and

s1 indicates the site connected to the cell-binding agent CB and s3 indicates the site connected to the A group;

A is an amino acid residue or a peptide comprising 2 to 20 amino acid residues;

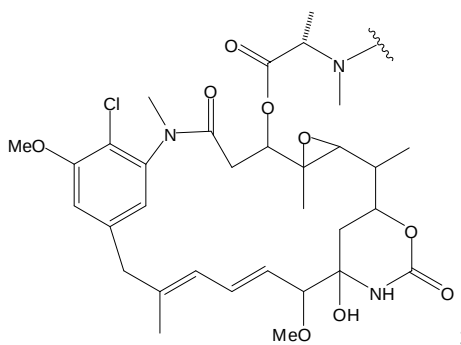
R¹ and R² are each independently H or a C₁₋₃alkyl;

L₁ is represented by the following formula:



wherein R³ and R⁴ are each independently H or Me, and the $-\text{C}(=\text{O})-$ moiety in L₁ is connected to D;

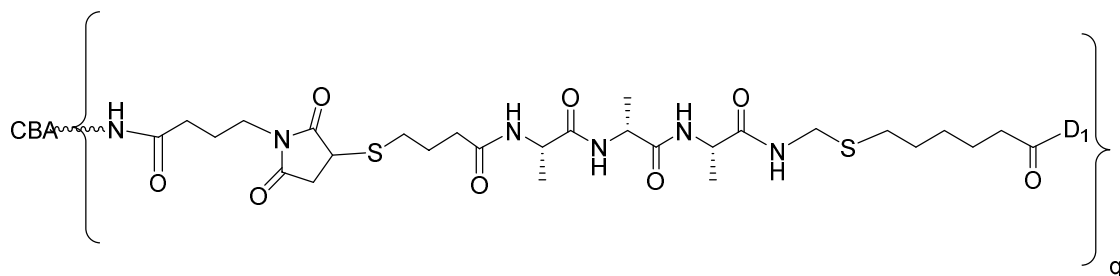
D is represented by the following formula:



q is an integer from 1 to 20.

29. The method of any one of claims 1-27, wherein the immunoconjugate comprises an anti-FR α antibody or antigen-binding fragment thereof comprising a VH comprising the amino acid sequence of SEQ ID NO:38 and a VL comprising the amino acid sequence of SEQ ID NO:44, wherein the antibody or antigen-binding fragment thereof is conjugated to DM4 via a sulfo-SPDB linker.

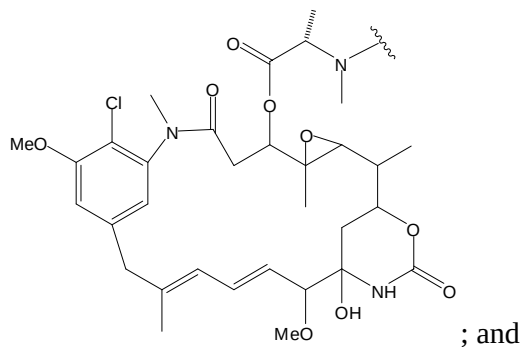
30. The method of claim 29, wherein the immunoconjugate is administered at a dose of about 6 mg/kg adjusted ideal body weight (AIBW).
31. The method of claim 29, wherein the immunoconjugate is administered at a dose of about 5 mg/kg adjusted ideal body weight (AIBW).
32. The method of any one of claims 1-27, wherein the immunoconjugate is represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

CBA is an antibody or an antigen-binding fragment comprising the amino acid sequences of SEQ ID NOs:61, 62, and 56;

D₁ is represented by the following formula:



q is an integer from 1 to 10.

33. The method of any one of claims 1-32, wherein the immunoconjugate comprises an average of 2 to 5 cytotoxic agents per antibody or antigen-binding fragment thereof,

optionally wherein the immunoconjugate comprises an average of 3 to 4 cytotoxic agents per antibody or antigen-binding fragment thereof.

34. The method of any one of claims 1-33, wherein soluble FR α is detected using a detection antibody or antigen-binding fragment thereof that specifically binds to FR α and comprises:

- (a) the VH-CDR1, VH-CDR2, VH-CDR3, VL-CDR1, VL-CDR2 and VL-CDR3 amino acid sequences of SEQ ID NOs:18-20 and SEQ ID NOs:21-23, respectively; or
- (b) the VH-CDR1, VH-CDR2, VH-CDR3, VL-CDR1, VL-CDR2 and VL-CDR3 amino acid sequences of SEQ ID NOs:24-26 and SEQ ID NOs:27-29, respectively.
- (c) the VH and VL amino acid sequences of SEQ ID NO:39 and SEQ ID NO:45, respectively; and/or
- (d) the VH and VL amino acid sequences of SEQ ID NO:40 and SEQ ID NO:46, respectively.

35. The method of claim any one of claims 1-34, wherein soluble FR α is detected by (i) capturing FR α with an immunocapture reagent bound to a solid support (ii) eluting FR α from the solid support, (iii) digesting the eluted FR α , and (iv) performing LC/MS analysis on the digested FR α , wherein said FR α is detected by monitoring the chromatographic separation and mass spectrometric response of at least one signature FR α peptide.

36. The method of any one of claims 1-35, wherein the soluble FR α is detected in a body fluid sample, optionally wherein the body fluid is plasma, serum, or ascites fluid, or a peripheral blood sample.