

CLAIMS

What is claimed is:

1. A chimeric binding agent comprising a first domain that specifically binds to integrin $\alpha\text{v}\beta 3$ on an epithelial cancer cell expressing at least one mesenchymal cell marker and a second domain that mediates antibody-directed cellular cytotoxicity (ADCC) by engaging a macrophage that accumulates in mesenchymal tumors.
2. The chimeric binding agent of claim 1, wherein the epithelial cancer cell is a late stage epithelial cancer cell.
3. The chimeric binding agent of claim 2, wherein the epithelial cancer cell has at least partially transitioned to a mesenchymal cell.
4. The chimeric binding agent of any one of claims 1-3, wherein the epithelial cancer cell is chemotherapy resistant or refractory.
5. The chimeric binding agent of any one of claims 1-4, wherein the first domain is an antibody domain.
6. The chimeric binding agent of any one of claims 1-5, wherein the second domain is an antibody domain.
7. The chimeric binding agent of any one of claims 1-6, wherein the first domain is a humanized or human antibody domain.
8. The chimeric binding agent of any one of claims 1-7, wherein the second domain is a humanized or human antibody domain.
9. The chimeric binding agent of any one of claims 1-8, which is a chimeric antibody or an antigen-binding fragment thereof.

10. The chimeric binding agent of any one of claims 1-9, wherein the first domain comprises a Fab domain of an antibody.
11. The chimeric binding agent of claim 10, wherein the first domain comprises a Fab domain of an IgG antibody.
12. The chimeric binding agent of claim 11, wherein the first domain comprises a Fab domain of an IgG4 antibody.
13. The chimeric binding agent of claim 12, wherein the first domain comprises the amino acid sequence of the light chain of hLM609-hIgG4-S228P (SEQ ID NO:2) or a sequence at least 90% identical thereto and the Fab portion of the heavy chain of hLM609-hIgG4-S228P (SEQ ID NO:3) or a sequence at least 90% identical thereto.
14. The chimeric binding agent of claim 13, wherein the first domain comprises the amino acid sequence of the Fab portion of the heavy chain of LM609_7 (SEQ ID NO:5) or a sequence at least 90% identical thereto and the light chain of LM609_7 (SEQ ID NO:6) or a sequence at least 90% identical thereto, the Fab portion of the heavy chain of JC7U (SEQ ID NO:7) or a sequence at least 90% identical thereto and the light chain of JC7U (SEQ ID NO:8), or a sequence at least 90% identical thereto.
15. The chimeric binding agent of any one of claims 1-12, wherein the first domain further specifically binds a second antigen.
16. The chimeric binding agent of claim 15, wherein the first domain is a bispecific antibody domain.
17. The chimeric binding agent of claim 15 or 16, wherein the second antigen is an immune checkpoint molecule, such as PD-1, PD-L1, or CTLA-4.
18. The chimeric binding agent of claim 15 or 16, wherein the second antigen is a cancer stem cell marker, such as CD133, CD44, CD90, CD117, CD166, or CD105, or an effector cell antigen.

19. The chimeric binding agent of claim 15 or 16, wherein the second antigen is an effector cell antigen.
20. The chimeric binding agent of any one of claims 1-19, wherein the second domain does not significantly engage natural killer cells.
21. The chimeric binding agent of any one of claims 1-20, wherein the second domain does not significantly engage lymphocytes.
22. The chimeric binding agent of any one of claims 1-21, wherein the second domain specifically binds a protein on the surface of the myeloid-derived cell.
23. The chimeric binding agent of claim 22, wherein the second domain specifically binds an Fc-gamma receptor.
24. The chimeric binding agent of claim 22, wherein the second domain specifically binds Fc-gamma receptor I (Fc γ RI, CD64).
25. The chimeric binding agent of any one of claims 1-24, wherein the second domain comprises an Fc domain of an antibody.
26. The chimeric binding agent of claim 25, wherein the second domain comprises an Fc domain of an IgG antibody.
27. The chimeric binding agent of claim 26, wherein the second domain comprises an Fc domain of an IgG4 antibody.
28. The chimeric binding agent of claim 26, wherein the second domain comprises an Fc domain of an IgA or IgE antibody.
29. The chimeric binding agent of any one of claims 25-28, wherein the second domain further comprises a hinge domain of an antibody.

30. The chimeric binding agent of claim 29, wherein the second domain comprises the amino acid sequence of the heavy chain Fc domain and hinge domain of hLM609-hIgG4-S228P (SEQ ID NO:4) or a sequence at least 90% identical thereto.

31. The chimeric binding agent of any one of claims 1-30, wherein the amino acid sequence comprises a S228P mutation (Eu numbering system) in the hinge region.

32. The chimeric binding agent of claim 31, comprising the amino acid sequence of the hLM609-hIgG4-S228P heavy chain (SEQ ID NO:1) and light chain (SEQ ID NO:2) or a sequence at least 90% identical thereto.

33. The chimeric binding agent of claim 31 or 32, wherein the amino acid sequence comprises a mutation selected from:

- a) S239D/A330L/I332E;
- b) I332E;
- c) G236A/S239D/I332E;
- d) G236A;
- e) N297A/E382V/M428I;
- f) M252Y/S254T/T256E;
- g) Q295R/L328W/A330V/P331A/I332Y/E382V/M428I;
- h) L234A/L235A/P329G;
- i) M428L/N434S;
- j) L234A/L235A/P331S;
- k) L234A/L235A/P329G/M252Y/S254T/T256E;
- l) S298A/E333A/K334A;
- m) S239D/I332E;
- n) G236A/S239D/A330L/I332E;
- o) S239D/I332E/G236A;
- p) L234Y/G236W/S298A;
- q) F243L/R292P/Y300L/V305I/P396L;
- r) K326W/E333S;
- s) K326A/E333A;
- t) K326M/E333S;
- u) C221D/D222C;

- v) S267E/H268F/S324W;
- w) H268F/S324W;
- x) E345R
- y) R435H;
- z) N434A;
- aa) M252Y/S254T/T256E;
- ab) M428L/N434S;
- ac) T252L/T/253S/T254F;
- ad) E294delta/T307P/N434Y;
- ae) T256N/A378V/S383N/N434Y;
- af) E294delta
- ag) L235E;
- ah) L234A/L235A;
- ai) S228P/L235E;
- aj) P331S/L234E/L225F;
- ak) D265A;
- al) G237A;
- am) E318A;
- an) E233P;
- ao) G236R/L328R;
- ap) H268Q/V309L/A330S/P331S;
- aq) L234A/L235A/G237A/P238S/H268A/A330S/P331S;
- ar) A330L;
- as) D270A;
- at) K322A;
- au) P329A;
- av) P331A;
- aw) V264A;
- ax) F241A;
- ay) N297A or G or N
- az) S228P/F234A/L235A; or
- ba) any combination of a) to az).

34. A polynucleotide encoding the chimeric binding agent of any one of claims 1-33.

35. A vector comprising the polynucleotide of claim 34.
36. A host cell comprising the polynucleotide of claim 34 or the vector of claim 35.
37. A composition comprising the chimeric binding agent of any one of claims 1-33 and a carrier.
38. A pharmaceutical composition comprising the chimeric binding agent of any one of claims 1-33 and a pharmaceutically acceptable carrier.
39. The pharmaceutical composition of claim 38, further comprising an additional therapeutic agent.
40. The pharmaceutical composition of claim 39, wherein the additional therapeutic agent is a chemotherapeutic agent.
41. A kit comprising the chimeric binding agent of any one of claims 1-33.
42. A method of targeting a macrophage to a cancer cell expressing integrin $\alpha v \beta 3$, comprising contacting the cancer cell and the macrophage with an effective amount of the chimeric binding agent of any one of claims 1-33.
43. The method of claim 42, wherein the cancer cell expresses integrin $\alpha v \beta 3$ due to cellular stress.
44. The method of claim 42, wherein the cancer cell expresses integrin $\alpha v \beta 3$ due to undergoing an epithelial to mesenchymal transition.
45. A method of targeting a macrophage that accumulates in mesenchymal tumors to an epithelial cancer cell expressing at least one mesenchymal cell marker, comprising contacting the cancer cell and the macrophage with an effective amount of the chimeric binding agent of any one of claims 1-33.

46. A method of treating a cancer expressing integrin $\alpha v \beta 3$ in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the cancer.

47. A method of treating an epithelial cell cancer in a subject in need thereof, comprising administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the epithelial cell cancer.

48. A method of treating a cancer in a subject in need thereof, comprising the steps of:

- a) selecting a subject having cancer cells that are enriched for integrin $\alpha v \beta 3$ and enriched for macrophages; and
- b) administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the cancer.

49. A method of treating an epithelial cell cancer in a subject in need thereof, comprising the steps of:

- a) selecting a subject having epithelial cancer cells that are enriched for integrin $\alpha v \beta 3$ and enriched for macrophages that accumulate in mesenchymal tumors; and
- b) administering a therapeutically effective amount of the chimeric binding agent of any one of claims 1-33 or the pharmaceutical composition of any one of claims 38-40 to the subject, thereby treating the epithelial cell cancer.

50. The method of claim 48 or 49, wherein step a) comprises obtaining a sample of the cancer from the subject and measuring the level of integrin $\alpha v \beta 3$ and macrophages in the sample.

51. The method of claim 49, wherein the epithelial cell cancer is a late stage epithelial cell cancer.

52. The method of claim 49, wherein one or more of the epithelial cells in the cancer have at least partially transitioned to mesenchymal cells.

53. The method of any one of claims 49-52, wherein the epithelial cell cancer is or has become chemotherapy resistant or refractory.
54. The method of any one of claims 46-53, wherein the cancer is a carcinoma such as a cancer of the gastrointestinal tract, breast, lungs (e.g., non-small cell lung cancer), colon, prostate, or bladder.
55. The method of any one of claims 42-54, further comprising administering to the subject a CD47 blocking agent and/or an immune checkpoint inhibitor and/or an EGFR inhibitor.
56. The method of any one of claims 42-54, wherein the method does not comprise administering to the subject a CD47 blocking agent.
57. The method of any one of claims 46-56, wherein the epithelial cell cancer expresses CD47.
58. The method of any one of claims 46-56, wherein the epithelial cell cancer does not express CD47.
59. The method of any one of claims 42-58, further comprising administering to the subject an additional cancer therapeutic agent or treatment.
60. The method of any one of claims 42-59, wherein the chimeric binding agent or pharmaceutical composition is administered to the subject intravenously, subcutaneously, or intramuscularly or is injected in situ into or near the cancer.
61. The method of any one of claims 42-60, further comprising the step of isolating macrophages from the subject, contacting the macrophages with the chimeric binding agent or pharmaceutical composition, and administering the contacted macrophages to the subject.
62. The method of any one of claims 42-61, wherein the subject is a human.