

[0009] Thus, there is a need for new compositions, and methods of using such compositions, to treat cancers, including in particular late-stage epithelial cancers that have undergone EMT.

SUMMARY OF THE DISCLOSURE

[0010] The present disclosure is based in part on an understanding of epithelial cancer cells and the EMT process, including changes in the immune cell populations in the tumor microenvironment. Central to this transformation process, the epithelial tumor cells gain expression of $\alpha v \beta 3$ integrin on their cell surface, becoming drug resistant and more stem-like in phenotype as well as insensitive to hypoxia or other environmental stress. Expression of $\alpha v \beta 3$ on epithelial cancer cells is triggered by the various forms of cellular stress as well within the microenvironment or the application of a wide range of anti-cancer drugs. Patients that have progressed on standard of care therapeutics and thereby express $\alpha v \beta 3$ are therefore candidates for therapies targeting the $\alpha v \beta 3$ antigen. Given that $\alpha v \beta 3$ is necessary and sufficient for drug resistance it is likely that by selectively targeting $\alpha v \beta 3$ positive tumor cells it would be possible to prevent or reverse cancer acquired drug resistance.

[0011] The present disclosure provides compositions and methods for engaging the appropriate immune effector cells to effectively mediate antibody-dependent cellular cytotoxicity (ADCC) against epithelial cancer cells that have undergone EMT and have gained the cell surface marker $\alpha v \beta 3$.

[0012] The inventors have determined that ADCC is mediated by macrophages, and not NK cells, that leads to the death of cancer cells targeted by the antibody. Further, the cell death does not involve antibody-dependent cellular phagocytosis (ADCP) or direct killing via the antibody alone. It was previously understood that antibody engagement of macrophages as an effector cell typically promoted ADCP. The present inventors have surprisingly determined that the chimeric binding agents of the disclosure do not induce ADCP, but instead exclusively promote macrophage-dependent ADCC of human cell targets. This unexpected finding, among other benefits, advantageously permits treatment of CD47-positive tumor cells that would normally be resistant to phagocytosis or ADCP. A binding agent that promotes ADCC exclusively will kill every cell it encounters while a binding agent that promotes ADCP will not be able to kill CD47-positive cells. Thus, the chimeric binding agents of the present disclosure are expected to be more efficient. Without being bound by theory, it is thought that the advantages of the present disclosure are based on the structure of the chimeric binding agent (e.g., the IgG4 domain) and/or the antigen being recognized (e.g., integrin $\alpha v \beta 3$) that make cells expressing the antigen particularly sensitive to ADCC rather than ADCP.

[0013] Mesenchymal tumors are identified by expression of transcription factors (ZEB, SNAIL, SLUG, and TWIST1) that repress epithelial markers (including E-cadherin, EpCAM, occludins, claudins, and cytokeratins) and promote the expression of mesenchymal markers (including cell adhesion-related proteins N-cadherin, vimentin, fibronectin, $\beta 1$ and $\beta 3$ integrins, and MMPs) (Dongre, 2019). An ideal tumor cell antigen for a mesenchymal-like tumor would be a cell surface marker with high expression on tumor cells but low expression on all other normal cell types. Since EMT has been closely linked with a cancer stem phenotype (Marie-Egyptienne, 2013; Singh, 2010; Ye, 2015), and drug resistance, cancer stem cell markers may represent another type of antigen for targeting mesenchymal tumors, although these often vary between tumor types.

[0014] Among potential cell surface mesenchymal markers, N-cadherin and $\beta 1$ integrin are expressed on many normal cell types and could thus contribute to issues with toxicity or compete with tumor cells for antibody binding. In contrast, integrin $\alpha v \beta 3$ is a more selective candidate for a mesenchymal tumor cell antigen based on its low expression in normal adult tissues and its enrichment on epithelial tumors as they become more aggressive, late-stage, and drug resistant.

[0015] The present disclosure is based on the development of agents that can mediate ADCC by engaging myeloid-derived cells found in mesenchymal tumors and targeting them to antigens that are expressed on epithelial cancer cells that have undergone EMT.

[0015a] In one aspect, the disclosure provides a chimeric binding agent comprising a first domain that specifically binds to integrin $\alpha v \beta 3$ 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; wherein the first domain is a humanized or human antibody Fab domain comprising the amino acid sequence of a light chain of hLM609-hIgG4-S228P (SEQ ID NO:2) and a Fab portion of a heavy chain of hLM609-hIgG4-S228P (SEQ ID NO:3); and wherein the second domain is a humanized or human Fc domain of an IgG4 antibody comprising the amino acid sequence of a heavy chain Fc domain and hinge domain of hLM609-hIgG4-S228P (SEQ ID NO:4); and wherein the second domain comprises a S228P mutation, according to the EU numbering system, in the hinge domain.

[0015b] In another aspect, the disclosure provides a polynucleotide encoding the chimeric binding agent as disclosed herein.

[0015c] In another aspect, the disclosure provides a vector comprising the polynucleotide as disclosed herein.

[0015d] In another aspect, the disclosure provides a host cell comprising the polynucleotide or the vector as disclosed herein.

[0015e] In another aspect, the disclosure provides a pharmaceutical composition comprising the chimeric binding agent as disclosed herein and a pharmaceutically acceptable carrier.

[0015f] In another aspect, the disclosure provides a *ex vivo* method of targeting a macrophage to a cancer cell expressing integrin $\alpha\text{v}\beta 3$, comprising contacting the cancer cell and the macrophage with an effective amount of the chimeric binding agent as disclosed herein.

[0015g] In another aspect, the disclosure provides a use of the chimeric binding agent or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein: a) a subject having cancer cells that are enriched for integrin $\alpha\text{v}\beta 3$ and enriched for macrophages is to be selected; and b) said subject is to be administered a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition, optionally wherein the cancer is an epithelial cell cancer.

[0016] Thus, one example of the disclosure relates to a chimeric binding agent comprising a first domain that specifically binds to an antigen on an epithelial cancer cell expressing at least one mesenchymal cell marker and a second domain that mediates ADCC by engaging a myeloid-derived cell that accumulates in mesenchymal tumors, and compositions or pharmaceutical compositions comprising the chimeric binding agents.

[0017] Another example of the disclosure relates to a polynucleotide encoding the chimeric binding agent of the disclosure and vectors and host cells comprising the polynucleotide.

[0018] An additional example of the disclosure relates to a method of targeting a myeloid-derived cell that accumulates in mesenchymal tumors to an epithelial cancer cell expressing at least one mesenchymal cell marker, comprising contacting the cancer cell and the myeloid-derived cell with an effective amount of the chimeric binding agent of the disclosure.

[0019] A further example of the disclosure relates to 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 or the pharmaceutical composition of the disclosure to the subject,

thereby treating the epithelial cell cancer. In particular, $\alpha v \beta 3$ is expressed in increased amounts on drug resistant cancers making it possible to prevent or reverse drug resistance.

[0020] Another example of the disclosure relates to 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 an antigen specifically bound by the chimeric binding agent of the disclosure and enriched for myeloid-derived cells that accumulate in mesenchymal tumors; and

b) administering a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition of the disclosure to the subject, thereby treating the epithelial cell cancer. Cancer patients that become drug resistant gain the expression of $\alpha v \beta 3$ and thereby become candidates for such therapies targeting this marker.

[0021] Another example of this disclosure relates to antigen-effector cell matching of tumors such that the antigen is specifically present on the tumor cell (e.g., a tumor cell antigen) and a therapeutic antibody contains effector cell binding regions that are specific to those effector cells found in the tumor (e.g., neutrophils, dendritic cells, NK cells etc.).

[0022] These and other examples of the disclosure are set forth in more detail in the description of the disclosure below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **FIG. 1** shows anti- $\alpha v \beta 3$ mouse monoclonal antibody LM609 sensitizes tumor xenografts to erlotinib. LM609 re-sensitizes resistant tumors to erlotinib. HCC827-R18 and PC9-R4L erlotinib-resistant tumor cells generated as reported in Wettersten *et al.*, *Cancer Res.* 79:5048 (2019), incorporated by reference herein in its entirety, were injected to form subcutaneous flank tumors in nu/nu recipient mice. Once tumors reached a volume of 100 mm³, mice were randomized to receive erlotinib alone (6.25 mg/kg) or the combination of erlotinib and LM609 (10 mg/kg). Tumor dimensions were measured biweekly and volume calculated as $V = \frac{1}{2} (\text{length} \times \text{width}^2)$. Graph shows mean $\pm$ SE. *, $P < 0.05$ for erlotinib vs. erlotinib/LM609 using ANOVA.

[0024] **FIG. 2** shows the amino acid sequence for mAb LM609-mIgG1-kappa heavy chain (SEQ ID NO:11) and light chain (SEQ ID NO:12).

[0025] **FIG. 3** shows the amino acid sequence for hLM609-hIgG1-WT (humanized LM609) heavy chain (SEQ ID NO:9) and light chain (SEQ ID NO:10).

[0026] **FIG. 4** shows the amino acid sequence for two distinct forms of shLM609-hIgG1-WT (super-humanized LM609): LM609_7 Fab domain of heavy chain (SEQ ID NO:5) and light

CLAIMS

What is claimed is:

1. A chimeric binding agent comprising a first domain that specifically binds to integrin $\alpha v\beta 3$ 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;

wherein the first domain is a humanized or human antibody Fab domain comprising the amino acid sequence of a light chain of hLM609-hIgG4-S228P (SEQ ID NO:2) and a Fab portion of a heavy chain of hLM609-hIgG4-S228P (SEQ ID NO:3); and

wherein the second domain is a humanized or human Fc domain of an IgG4 antibody comprising the amino acid sequence of a heavy chain Fc domain and hinge domain of hLM609-hIgG4-S228P (SEQ ID NO:4); and

wherein the second domain comprises a S228P mutation, according to the EU numbering system, in the hinge domain.

2. The chimeric binding agent of claim 1, wherein the first domain further specifically binds a second antigen, optionally wherein the first domain is a bispecific antibody domain, optionally wherein the second antigen is an immune checkpoint molecule, or PD-1, PD-L1, or CTLA-4; a cancer stem cell marker, or CD133, CD44, CD90, CD117, CD166, or CD105, or an effector cell antigen; or an effector cell antigen.

3. The chimeric binding agent of claim 1, wherein the second domain 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/K334/A;
- 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) 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) F234A/L235A; or
ba) any combination of a) to az) ,
according to the Eu numbering system, in the hinge domain.

4. A polynucleotide encoding the chimeric binding agent of any one of claims 1-3.
5. A vector comprising the polynucleotide of claim 4.
6. A host cell comprising the polynucleotide of claim 4 or the vector of claim 5.
7. A pharmaceutical composition comprising the chimeric binding agent of any one of claims 1-3 and a pharmaceutically acceptable carrier.
8. An *ex vivo* method of targeting a macrophage to a cancer cell expressing integrin $\alpha\text{v}\beta 3$, comprising contacting the cancer cell and the macrophage with an effective amount of the chimeric binding agent of any one of claims 1-3.
9. The method of claim 8, wherein the cancer cell expresses integrin $\alpha\text{v}\beta 3$ due to cellular stress.
10. The method of claim 8, wherein the cancer cell expresses integrin $\alpha\text{v}\beta 3$ due to undergoing an epithelial to mesenchymal transition.
11. Use of the chimeric binding agent of any one of claims 1-3 or the pharmaceutical composition of claim 7 in the manufacture of a medicament for treating a cancer in a subject in need thereof, wherein:
 - a) a subject having cancer cells that are enriched for integrin $\alpha\text{v}\beta 3$ and enriched for macrophages is to be selected; and

b) said subject is to be administered a therapeutically effective amount of the chimeric binding agent or the pharmaceutical composition,
optionally wherein the cancer is an epithelial cell cancer.

12. The use of claim 11, wherein the cancer is or has become chemotherapy resistant or refractory.

13. The use of any one of claims 11-12, wherein the subject is a human.