## SUMMARY

**[0005]** Presently disclosed methods can be used to induce infiltration of a $CD8^+$ T cell population into the core of a tumor, even in tumors that are typically not highly responsive to immunotherapies (such as cold tumors). In accordance with presently disclosed methods, a patient in need thereof is administered a radioimmunoconjugate that is capable of binding a target expressed by at least some cells in a tumor. In some ~~embodiment~~example**s**, the CD8+ T cell population that infiltrates into the tumor persists in the patient and may therefore act to prevent metastases from forming and/or reduce the likelihood of recurrences.

**[0006]** In one ~~aspect~~example, provided are methods of inducing CD8+ T cell infiltration into a tumor in a subject in need thereof, wherein the method comprises a step of administering to the subject a radioimmunoconjugate or a pharmaceutical composition thereof, wherein the radioimmunoconjugate comprises the following structure:

A-L-B

**Formula I-a**

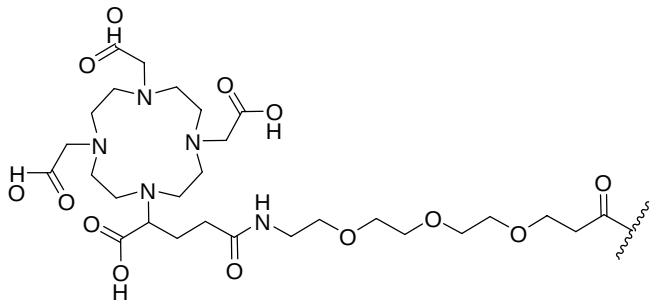
wherein

A is a metal complex of a chelating moiety, wherein the metal complex comprises Actinium-225 ($^{225}Ac$) or a progeny thereof,

L is a linker, and

B is a targeting moiety capable of binding a first tumor-associated antigen expressed by at least some cells in the tumor;

with the proviso that if A-L- is a metal complex of Compound 1 as shown below, then B is not AVE1642



**(Compound 1)**;

wherein said administering of said radioimmunoconjugate results in infiltration of a $CD8^+$ T cell population into the core of the tumor; wherein said $CD8^+$ T cell population comprises CD8+ T cells expressing a T-cell receptor (TCR) specific for a second

tumor-associated antigen expressed by at least some cells in the tumor; and wherein the CD8+ T cell is capable of preferentially killing a cell expressing the second tumor-associated antigen.

**[006a]** In one aspect, the present disclosure refers to a radioimmunoconjugate or a pharmaceutical composition thereof for use in inducing infiltration of a CD8+ T cell population into the core of a tumor in a subject in need thereof, wherein the radioimmunoconjugate comprises the following structure:

A-L-B

Formula I-a

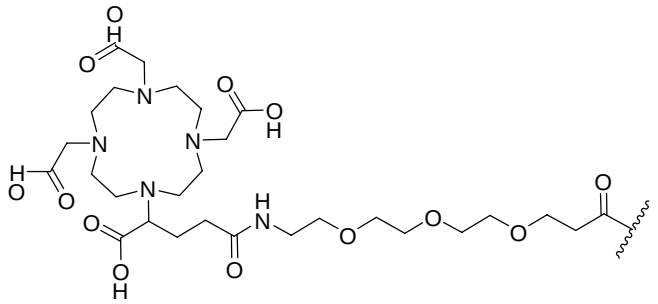
wherein

A is a metal complex of a chelating moiety, wherein the metal complex comprises Actinium-225 ($^{225}$Ac) or a progeny thereof,

L is a linker, and

B is a targeting moiety capable of binding a first tumor-associated antigen expressed by at least some cells in the tumor;

with the proviso that if A-L- is a metal complex of Compound 1 as shown below, then B is not AVE1642



**(Compound 1)**;

wherein said CD8$^+$ T cell population comprises CD8+ T cells expressing a T-cell receptor (TCR) specific for a second tumor-associated antigen expressed by at least some cells in the tumor; and

wherein the CD8+ T cell is capable of preferentially killing a cell expressing the second tumor-associated antigen.

**[006b]** In one aspect, the present disclosure refers to use of a radioimmunoconjugate or a pharmaceutical composition thereof in the manufacture of a medicament for inducing infiltration of a CD8+ T cell population into the core of a tumor in a subject in need thereof, wherein the radioimmunoconjugate comprises the following structure:

A-L-B

Formula I-a

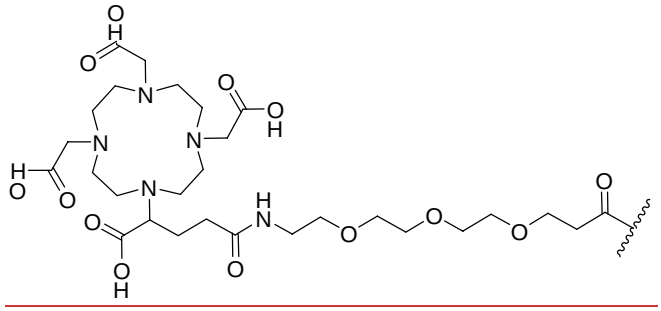
wherein

A is a metal complex of a chelating moiety, wherein the metal complex comprises Actinium-225 ($^{225}$Ac) or a progeny thereof,

L is a linker, and

B is a targeting moiety capable of binding a first tumor-associated antigen expressed by at least some cells in the tumor;

with the proviso that if A-L- is a metal complex of Compound 1 as shown below, then B is not AVE1642



**(Compound 1);**

wherein said $CD8^+$ T cell population comprises CD8+ T cells expressing a T-cell receptor (TCR) specific for a second tumor-associated antigen expressed by at least some cells in the tumor; and

wherein the CD8+ T cell is capable of preferentially killing a cell expressing the second tumor-associated antigen.

**[0007]** In some ~~embodiment~~examples, the CD8+ T cell population is detectable in the core of the tumor at a level greater than a reference level, e.g., at least two-fold, at least three-fold, at least four-fold, or at least five-fold greater than the reference level.

**[0008]** In some ~~embodiment~~examples, the CD8+ T cell population represents at least 5%, at least 7.5%, at least 10%, at least 12.5%, or at least 15% of cells (e.g., of viable cells) in the core of the tumor.

**[0009]** In some ~~embodiment~~examples, the CD8+ T cells represent at least 15%, at least 20%, at least 25%, at least 30%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, or at least 70% of said CD8+ T cell population.

**[0010]** In some ~~embodiment~~examples, the CD8+ T cells are detectable in the subject at least 10 days, at least 15 days, at least 20 days, at least 25 days, at least 30 days, at least 35 days, or at least 40 days after the step of administering.

**[0011]** In some ~~embodiment~~examples, the first tumor-associated antigen is different than the second tumor-associated antigen. In some ~~embodiment~~examples, the second tumor-associated antigen is a neoantigen.

**[0012]** In some ~~embodiment~~examples, the tumor is a primary tumor. In some ~~embodiment~~examples, the tumor is a secondary tumor.

**[0013]** In some ~~embodiment~~examples, the tumor is not highly immunogenic. For example, the tumor may be moderately immunogenic or immunologically cold.

**[0014]** In some ~~embodiment~~examples, the tumor is at least 100 $mm^3$, at least 150 $mm^3$, or at least or about 175 $mm^3$ in volume at the time of administering.

**[0015]** In some ~~embodiment~~examples, the tumor is a solid tumor.

**[0016]** For example, the solid tumor may be a sarcoma, e.g., a sarcoma selected from the group consisting of angiosarcoma or hemangioendothelioma, astrocytoma, chondrosarcoma, Ewing's sarcoma, fibrosarcoma, glioma, leiomyosarcoma, liposarcoma, malignant fibrous histiocytoma (MFH), mesenchymous or mixed mesodermal tumor, mesothelial sarcoma or mesothelioma, myxosarcoma, osteosarcoma, rhabdomyosarcoma, and synovial sarcoma. In some ~~embodiment~~examples, the sarcoma is osteosarcoma.

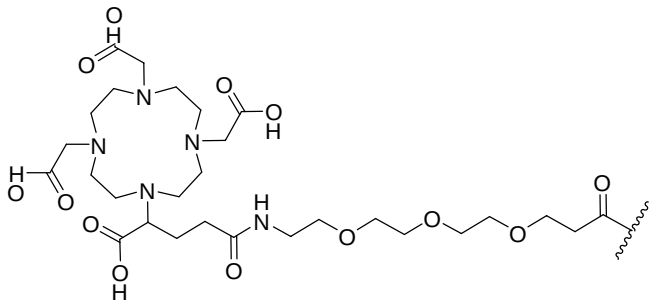
**[0017]** For example, the solid tumor may be a carcinoma, e.g., a carcinoma selected from the group consisting of adenoid cystic carcinoma, adrenocortical carcinoma, bladder

cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, gallbladder carcinoma, gastric cancer, head and neck cancer, lung cancer (e.g., small cell lung cancer or non-small cell lung cancer, or adenocarcinoma of the lung), neuroblastoma, neuroendocrine cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, testicular cancer. In some ~~embodiment~~examples, the carcinoma is bladder cancer. In some ~~embodiment~~examples, the carcinoma is pancreatic cancer. In some ~~embodiment~~examples, the carcinoma is breast cancer. In some ~~embodiment~~examples, the carcinoma is head and neck cancer. In some ~~embodiment~~examples, the carcinoma is liver cancer. In some ~~embodiment~~examples, the carcinoma is lung cancer. In some ~~embodiment~~examples, the carcinoma is a brain cancer. In some ~~embodiment~~examples, the carcinoma is neuroblastoma. In some ~~embodiment~~examples, the carcinoma is melanoma.

**[0018]** In some ~~embodiment~~examples, the tumor is a liquid tumor.

**[0019]** In some ~~embodiment~~examples, the step of administering results in inhibition of cell proliferation in the core of the tumor. In some ~~embodiment~~examples, the step of administering results in slowing or inhibiting progression of the tumor. In some ~~embodiment~~examples, the step of administering results in regression of the tumor. In some ~~embodiment~~examples, the step of administering results in complete regression of the tumor. In some ~~embodiment~~examples, the step of administering prevents or inhibits metastasis of tumor cells.

**[0020]** In some ~~embodiment~~examples, the A-L- is a metal complex of a compound selected from the group consisting of

(i) 

**(Compound 1),**

**[0021]** In some ~~embodiment~~examples, the L has the structure -$L^1$-($L^2$)$_n$-, as shown within Formula I-b:

$$A\text{-}L^1\text{-}(L^2)_n\text{-}B$$

**Formula I-b**

wherein

A is a metal complex of chelating moiety, wherein the metal complex comprises a Actinium-225 ($^{225}Ac$) or a progeny thereof;

B is a targeting moiety;

$L^1$ is optionally substituted $C_1$-$C_6$ alkyl, optionally substituted $C_1$-$C_6$ heteroalkyl, or optionally substituted aryl or heteroaryl;

n is 1-5; and

each $L^2$, independently, has the structure:

$$(\text{-}X^1\text{-}L^3\text{-}Z^1\text{-})$$

**Formula III**

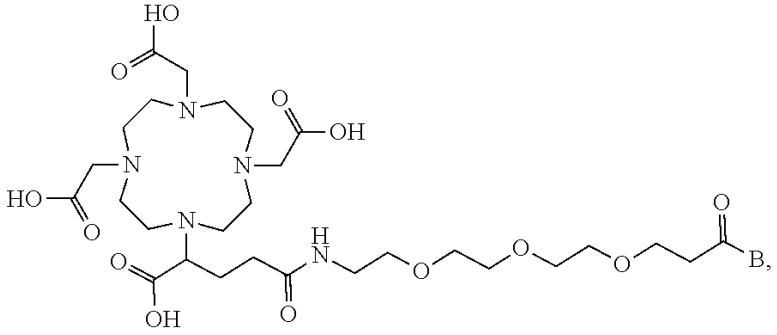
wherein

$X^1$ is C=O($NR^1$), C=S($NR^1$), OC=O($NR^1$), $NR^1$C=O(O), $NR^1$C=O($NR^1$), -$CH_2$PhC=O($NR^1$), -$CH_2$Ph(NH)C=S($NR^1$), O, or $NR^1$; and each $R^1$ independently is H, optionally substituted $C_1$-$C_6$ alkyl, optionally substituted $C_1$-$C_6$ heteroalkyl, or optionally substituted aryl or heteroaryl, in which $C_1$-$C_6$ alkyl can be substituted by oxo (=O), heteroaryl, or a combination thereof;

$L^3$ is optionally substituted $C_1$-$C_{50}$ alkyl or optionally substituted $C_1$-$C_{50}$ heteroalkyl; and

$Z^1$ is $CH_2$, C=O, C=S, OC=O, $NR^1$C=O, or $NR^1$, wherein $R^1$ is a hydrogen or optionally substituted $C_1$-$C_6$ alkyl or pyrrolidine-2,5-dione.

**[0022]** In some ~~embodiment~~examples, the radioimmunoconjugate comprises the following structure:



wherein B is the targeting moiety.

**[0023]** In some ~~embodiment~~examples, the targeting moiety comprises a polypeptide.

**[0024]** In some ~~embodiment~~examples, the targeting moiety comprises an antibody or an antigen-binding fragment thereof.

**[0025]** In some ~~embodiment~~examples, the targeting moiety has a molecular weight of at least 100 kDa, at least 125 kDa, or at least 150 kDa.

**[0026]** In some ~~embodiment~~examples, the targeting moiety is a small molecule.

**[0027]** In some ~~embodiment~~examples, the first tumor-associated antigen is selected from the group consisting of Insulin-like Growth Factor 1 Receptor (IGF-1R), tumor epithelial marker-1 (TEM-1), and Fibroblast Growth Factor Receptor 3 (FGFR3).

**[0028]** In some ~~embodiment~~examples, the subject is a mammal, e.g., a human. In some ~~embodiment~~examples, the subject is in need of treatment or prevention of cancer. In some ~~embodiment~~examples, the subject is diagnosed as having cancer. In some ~~embodiment~~examples, the subject is in need of treatment of a refractory cancer.

**[0029]** In some ~~embodiment~~examples, the step of administering comprises systemic administration of the radioimmunoconjugate. In some ~~embodiment~~examples, systemic administration comprises parenteral administration, e.g., intravenous administration, intraarterial administration, intraperitoneal administration, subcutaneous administration, or intradermal administration. In some ~~embodiment~~examples, systemic administration comprises enteric administration, e.g., trans-gastroenteric administration or oral administration.

**[0030]** In some ~~embodiment~~examples, the step of administering comprises local administration of the radioimmunoconjugate. For example, the local administration may comprise peritumoral injection and/or intratumoral injection.

**[0031]** In some ~~embodiment~~examples, the step of administering comprises contacting, *ex vivo*, the radioimmunoconjugate with a body fluid of said subject, wherein said body fluid contains at least one cancer cell.

**[0032]** In some ~~embodiment~~examples, the radioimmunoconjugate is not administered in combination with another cytotoxic agent.

**[0033]** In some ~~embodiment~~examples, the method further comprises administering to the subject an additional therapeutic agent after the step of administering the radioimmunoconjugate. For example, the additional therapeutic agent may be a non-cytotoxic agent. In some such ~~embodiment~~examples, the radioimmunoconjugate is administered in a lower