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 examples, 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 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

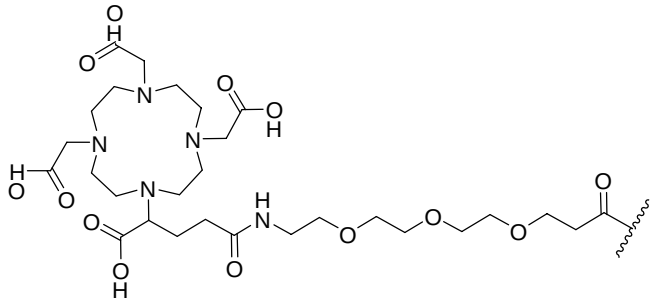
wherein

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

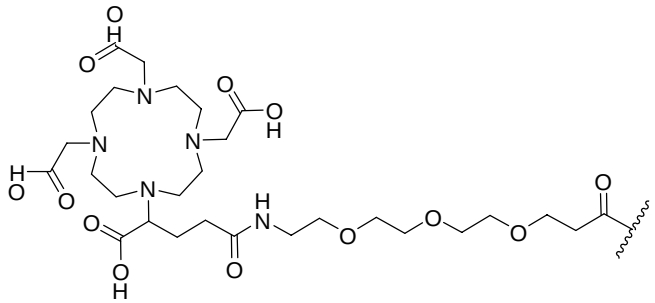
wherein

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

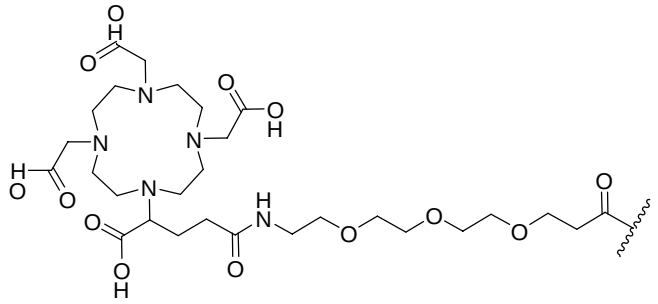
wherein

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 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 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 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 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 examples, the first tumor-associated antigen is different than the second tumor-associated antigen. In some examples, the second tumor-associated antigen is a neoantigen.

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

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

[0014] In some 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 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 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

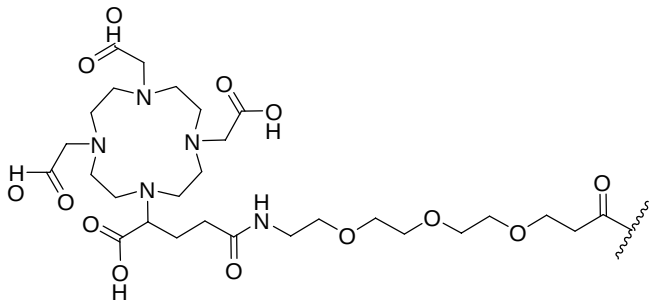
cancer, breast cancer, cervical cancer, colorectal cancer, endometrial cancer, gallbladder carcinoma, gastric cancer, head and neck cancer, lung cancer (e.g., small cell lung cancer or non-small cell lung cancer, or adenocarcinoma of the lung), neuroblastoma, neuroendocrine cancer, ovarian cancer, pancreatic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

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

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

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

(i)



(Compound 1),

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

$$A-L^1-(L^2)_n-B$$

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:

$$(-X^1-L^3-Z^1-)$$

Formula III

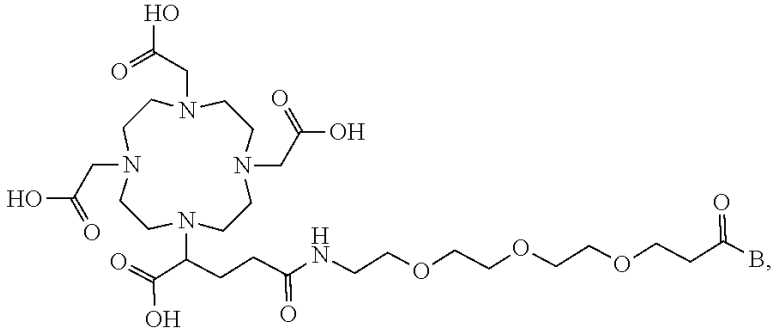
wherein

X^1 is $C=O(NR^1)$, $C=S(NR^1)$, $OC=O(NR^1)$, $NR^1C=O(O)$, $NR^1C=O(NR^1)$, $-CH_2PhC=O(NR^1)$, $-CH_2Ph(NH)C=S(NR^1)$, 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, $NR^1C=O$, 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 examples, the radioimmunoconjugate comprises the following structure:



wherein B is the targeting moiety.

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

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

[0025] In some 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 examples, the targeting moiety is a small molecule.

[0027] In some 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 examples, the subject is a mammal, e.g., a human. In some examples, the subject is in need of treatment or prevention of cancer. In some examples, the subject is diagnosed as having cancer. In some examples, the subject is in need of treatment of a refractory cancer.

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

[0030] In some 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 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 examples, the radioimmunoconjugate is not administered in combination with another cytotoxic agent.

[0033] In some 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 examples, the radioimmunoconjugate is administered in a lower