

(transforming acidic coiled-coil 3) oncogenic fusions have also been observed in a subset of glioblastomas and other cancers, and early data suggests that such tumors may be sensitive to FGFR inhibition. Additionally, genomic alterations that activate FGFR3 are frequent in bladder cancer, including metastatic bladder urothelial carcinoma.

[0005] FGFR3 has thus been proposed as a potential therapeutic target for cancer. Several small-molecule inhibitors targeting FGFRs have demonstrated cytotoxicity against FGFR3-positive myeloma cells in culture and in mouse models. However, these small molecules are not selective for FGFR3 and exhibit inhibitory activity toward certain other kinases.

[0006] Thus, there remains a need for improved therapeutics (*e.g.*, cancer therapeutics) that can target FGFR3.

SUMMARY

[006a] In one aspect of the present disclosure, there is provided a radioimmunoconjugate comprising the following structure:

A-L-B

Formula I-a

wherein A is a chelating moiety or metal complex thereof,
wherein B is an FGFR3 targeting moiety, and
wherein L is a linker.

[006b] In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the radioimmunoconjugate disclosed herein and a pharmaceutically acceptable carrier.

[006c] In a further aspect of the present disclosure, there is provided a use of a radioimmunoconjugate disclosed herein or a pharmaceutical composition thereof, in the manufacture of a medicament for treating cancer in a subject in need thereof.

[006d] In another aspect of the present disclosure, there is provided a radioimmunoconjugate disclosed herein or a pharmaceutical composition thereof, for use in therapy.

[0007] The present disclosure relates to radioimmunoconjugates that target FGFR3 (*e.g.*, human FGFR3, including wild type and/or mutant FGFR3), pharmaceutical compositions thereof, and methods of treating cancer using such pharmaceutical compositions. In certain examples, provided radioimmunoconjugates exhibit an increased excretion rate (*e.g.*, after being administered to a mammal) compared to some currently

known radiotherapeutics, while still maintaining therapeutic efficacy. In some examples, a faster excretion may limit off-target toxicities by limiting the amount of time that the radioimmunoconjugate stays in a subject. Thus, in some examples, provided immunoconjugates exhibit reduced off-target toxicities.

[0008] In certain examples, provided are radioimmunoconjugate comprising the following structure:



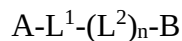
(Formula I-a)

wherein A is a chelating moiety or metal complex thereof, wherein B is an FGFR3 targeting moiety, and wherein L is a linker.

[0009] In some examples, A is a metal complex of a chelating moiety. In some such examples, the metal complex comprises a radionuclide. In some examples, the radionuclide is an alpha emitter, *e.g.*, an alpha emitter selected from the group consisting of Astatine-211 (^{211}At), Bismuth-212 (^{212}Bi), Bismuth-213 (^{213}Bi), Actinium-225 (^{225}Ac),

Radium-223 (^{223}Ra), Lead-212 (^{212}Pb), Thorium-227 (^{227}Th), and Terbium-149 (^{149}Tb), or a progeny thereof. In some examples, the radionuclide is ^{225}Ac or a progeny thereof.

[0010] In some examples, L has the structure $-\text{L}^1-(\text{L}^2)_n-$, as shown within Formula I-b:



Formula I-b

wherein:

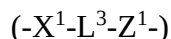
A is a chelating moiety or metal complex thereof;

B is an FGFR3 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 between 1 and 5 (inclusive); and

each L^2 , independently, has the structure:



Formula III

wherein:

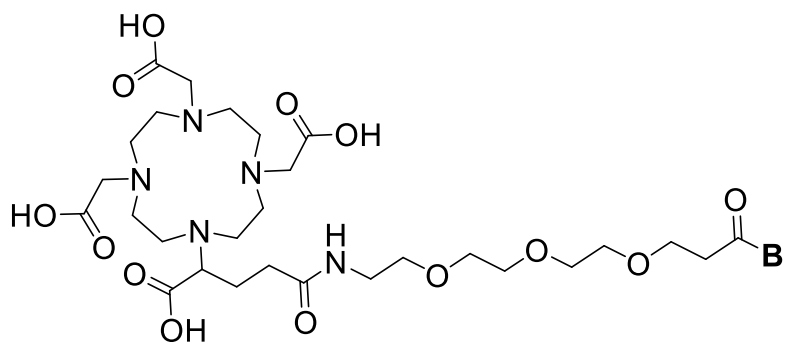
X^1 is $\text{C}=\text{O}(\text{NR}^1)$, $\text{C}=\text{S}(\text{NR}^1)$, $\text{OC}=\text{O}(\text{NR}^1)$, $\text{NR}^1\text{C}=\text{O}(\text{O})$, $\text{NR}^1\text{C}=\text{O}(\text{NR}^1)$, $-\text{CH}_2\text{PhC}=\text{O}(\text{NR}^1)$, $-\text{CH}_2\text{Ph}(\text{NH})\text{C}=\text{S}(\text{NR}^1)$, 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 ($=\text{O}$), 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 , $\text{C}=\text{O}$, $\text{C}=\text{S}$, $\text{OC}=\text{O}$, $\text{NR}^1\text{C}=\text{O}$, or NR^1 , wherein R^1 is a hydrogen or optionally substituted C_1 - C_6 alkyl or pyrrolidine-2,5-dione.

[0011] In some examples, L^3 is C_5 - C_{20} polyethylene glycol.

[0012] In some examples, the radioimmunoconjugate or a pharmaceutically acceptable salt thereof comprises the following structure:



wherein B is an FGFR3 targeting moiety.

[0013] In some examples, the FGFR3 targeting moiety is at least 100 kDa in size, *e.g.*, at least 150 kDa in size, at least 200 kDa in size, at least 250 kDa in size, or at least 300 kDa in size.

[0014] In some examples, the FGFR3 targeting moiety is capable of binding to human FGFR3. In some examples, the FGFR3 targeting moiety is capable of binding to wild type FGFR3. In some examples, the FGFR3 targeting moiety is capable of binding to a mutant FGFR3. In some examples, FGFR3 targeting moiety is capable of binding to both wild type and a mutant FGFR3.

[0015] In some examples, the mutant FGFR3 comprises a point mutation, *e.g.*, a point mutation is associated with cancer. In some examples, the point mutant is selected from the group consisting of FGFR3Y375C, FGFR3R248C, FGFR3S249C, FGFR3G372C, FGFR3K652E, FGFR3K652Q, FGFR3K652M, and combinations thereof.

[0016] In some examples, the mutant FGFR3 comprises an FGFR3 fusion. In some examples, the FGFR3 fusion is selected from the group consisting of FGFR3-TACC3, FGFR3-CAMK2A, FGFR3-JAKMOP1, FGFR3-TNIP2, FGFR3-WHSC1, FGFR3-BAIAP2L1, and combinations thereof.

[0017] In some examples, the FGFR3 targeting moiety comprises an antibody or antigen-binding fragment thereof, *e.g.*, a humanized antibody or antigen-binding fragment thereof.

[0018] In some examples, the antibody or antigen-binding fragment thereof comprises at least one complementarity determining region (CDR) selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4;

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; or

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0019] In some examples, the antibody or antigen-binding fragment thereof comprises at least two CDRs selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4;

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; or

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0020] In some examples, the antibody or antigen-binding fragment thereof comprises at least three CDRs selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4;

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; or

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0021] In some examples, the antibody or antigen-binding fragment thereof comprises at least four CDRs selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4;

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; or

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0022] In some examples, the antibody or antigen-binding fragment thereof comprises at least five CDRs selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4;

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; or

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0023] In some examples, the antibody or antigen-binding fragment thereof comprises:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4;

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; and

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0024] In some examples, the antibody or antigen-binding fragment thereof comprises:

(i) a heavy chain variable domain comprising at least one CDR selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom; and

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4; and

(ii) a light chain variable domain comprising at least one CDR selected from the group consisting of:

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; and

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0025] In some examples, the antibody or antigen-binding fragment thereof comprises:

(i) a heavy chain variable domain comprising at least one CDR selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2; and

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4; and

(ii) a light chain variable domain comprising at least one CDR selected from the group consisting of:

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6; and

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7.

[0026] In some examples, the antibody or antigen-binding fragment thereof comprises:

(i) a heavy chain variable domain comprising at least two CDRs selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom; and

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4; and

(ii) a light chain variable domain comprising at least two CDRs selected from the group consisting of:

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; and

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0027] In some examples, the antibody or antigen-binding fragment thereof comprises:

(i) a heavy chain variable domain comprising at least two CDRs selected from the group consisting of:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2; and

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4; and

(ii) a light chain variable domain comprising at least two CDRs selected from the group consisting of:

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6; and

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7.

[0028] In some examples, the antibody or antigen-binding fragment thereof comprises:

(i) a heavy chain variable domain comprising:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom; and

CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4; and

(ii) a light chain variable domain comprising:

CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;

CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; and

CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

[0029] In some examples, the antibody or antigen-binding fragment thereof comprises:

(i) a heavy chain variable domain comprising:

CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1;

- CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2; and
CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4; and
(ii) a light chain variable domain comprising:
CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5;
CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6; and
CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7.

[0030] In some examples, the antibody or antigen-binding fragment thereof comprises: (i) a heavy chain variable domain having an amino acid sequence with at least 85% identity with the amino acid sequence of SEQ ID NO: 8; and (ii) a light chain variable domain having an amino acid sequence with at least 85% identity with the amino acid sequence of SEQ ID NO: 9.

[0031] In some examples, the antibody or antigen-binding fragment thereof comprises: (i) a heavy chain variable domain having an amino acid sequence with at least 90% identity with the amino acid sequence of SEQ ID NO: 8; and (ii) a light chain variable domain having an amino acid sequence with at least 90% identity with the amino acid sequence of SEQ ID NO: 9.

[0032] In some examples, the antibody or antigen-binding fragment thereof comprises: (i) a heavy chain variable domain having an amino acid sequence with at least 95% identity with the amino acid sequence of SEQ ID NO: 8; and (ii) a light chain variable domain having an amino acid sequence with at least 95% identity with the amino acid sequence of SEQ ID NO: 9.

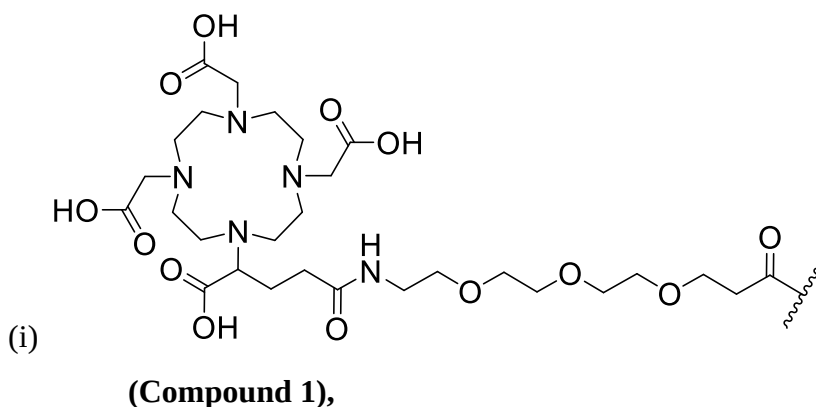
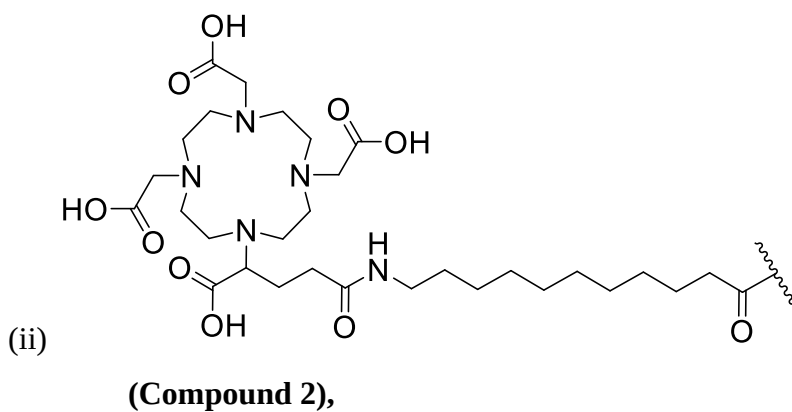
[0033] In some examples, the antibody or antigen-binding fragment thereof comprises: (i) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 8; and
(ii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 9.

[0034] In some examples, the antibody is MFGR1877S (vofatamab).

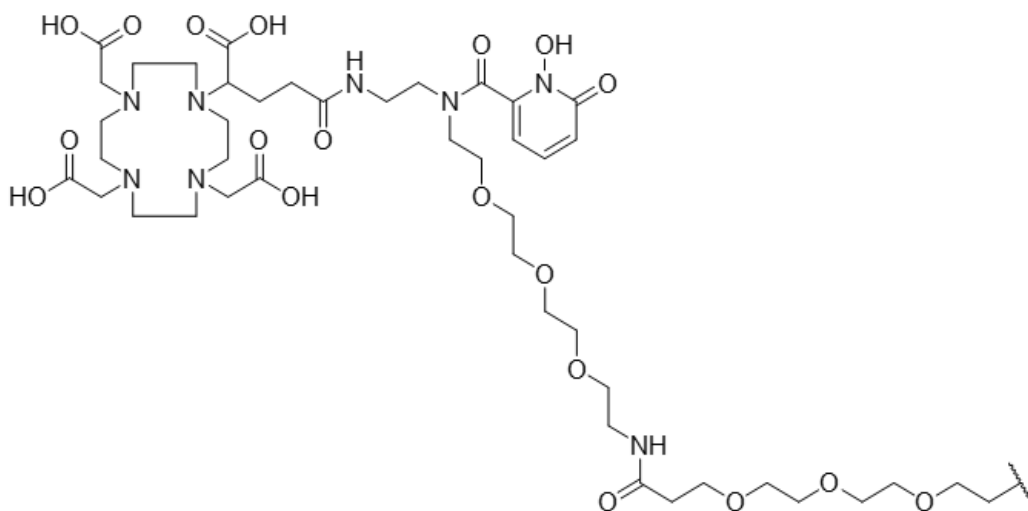
[0035] In some examples, after administration of the radioimmunoconjugate or a composition thereof to a mammal, the proportion of radiation excreted by the intestinal routes, renal route, or both routes is at least 2-fold greater than the proportion of radiation excreted by the same route(s) by a comparable mammal that has been administered a reference radioimmunoconjugate.

[0036] In some examples, after administration of the radioimmunoconjugate or a composition thereof to a mammal, the proportion of radiation excreted by the intestinal routes, renal route, or both routes is at least 3-fold greater than the proportion of radiation excreted by the same route(s) by a comparable mammal that has been administered a reference radioimmunoconjugate.

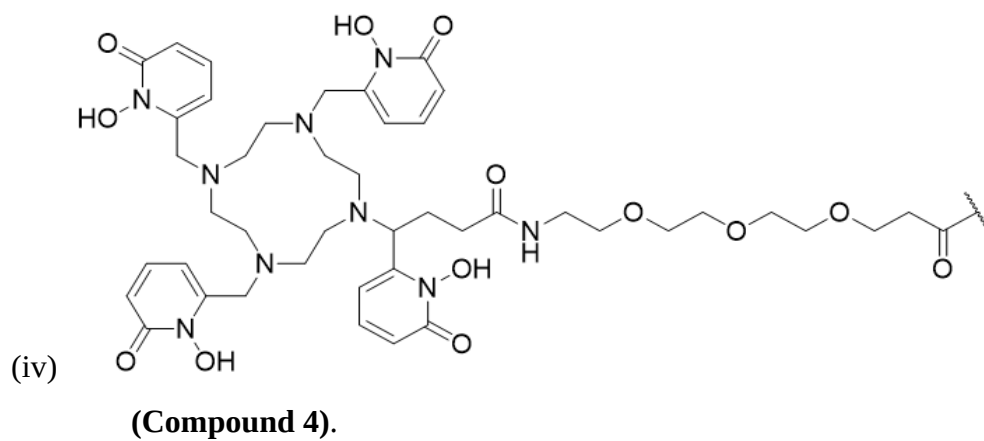
[0037] In some examples, A-L- is a metal complex of a compound selected from the group consisting of:



(iii)

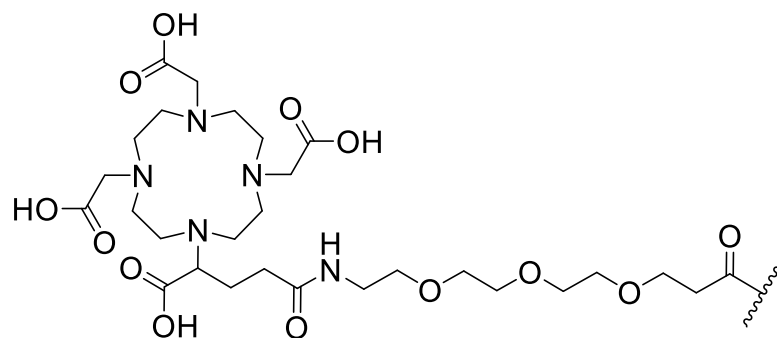


(Compound 3), and



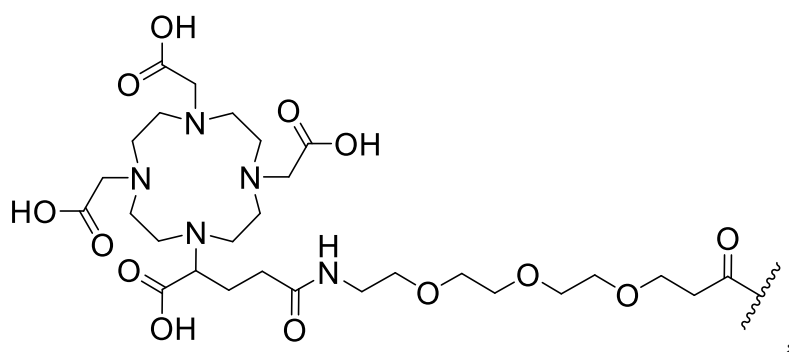
[0038]

In some examples, A-L- is a metal complex of:



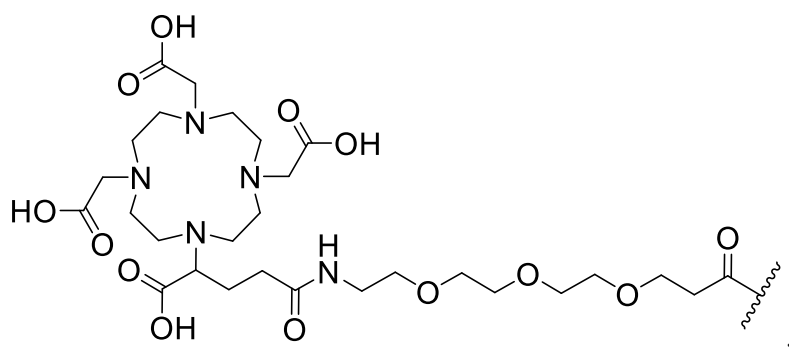
(Compound 1)

[0039] In some examples, A-L- is a metal complex of



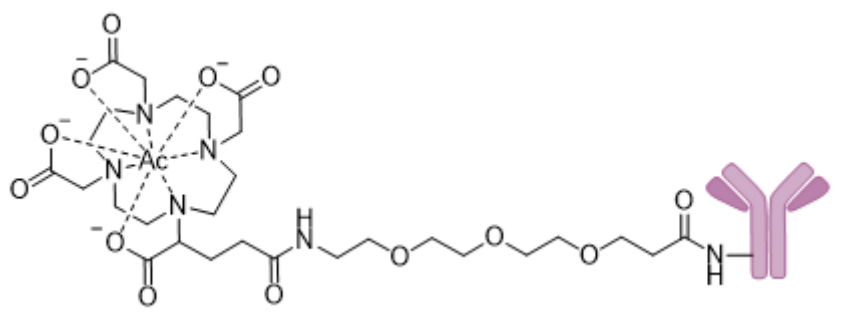
and the metal complex comprises a radionuclide, such as an alpha emitter (*e.g.*, Astatine-211 (^{211}At), Bismuth-212 (^{212}Bi), Bismuth-213 (^{213}Bi), Actinium-225 (^{225}Ac), Radium-223 (^{223}Ra), Lead-212 (^{212}Pb), Thorium-227 (^{227}Th), and Terbium-149 (^{149}Tb), or a progeny thereof). In some examples, the FGFR3 targeting moiety is an antibody or antigen-binding fragment thereof (*e.g.*, a humanized antibody or antigen-binding fragment thereof).

[0040] In some examples, A-L- is a metal complex of



the metal complex comprises ^{225}Ac or a progeny thereof, and the FGFR3 targeting moiety is MFGR1877S (vofatamab) or an antigen-binding fragment thereof. In some examples, the FGFR3 targeting moiety is MFGR1877S (vofatamab).

[0041] In some examples, the radioimmunoconjugate comprising the following structure:





wherein  is MFGR1877S (vofatamab), wherein the amine group NH– attached to the antibody shown above is from a lysine unit that is part of the antibody.

[0042] In certain examples, provided are pharmaceutical compositions comprising a radioimmunoconjugate as described herein and a pharmaceutically acceptable carrier.

[0043] In certain examples, provided are methods of treating cancer, the method comprising administering to a subject in need thereof a pharmaceutical composition comprising an effective amount of a radioimmunoconjugate as described herein.

[0044] In some examples, the subject is a mammal, *e.g.*, a human.

[0045] In some examples, the cancer is a solid tumor cancer. In some examples, the solid tumor cancer is adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial adenocarcinoma, Ewing's sarcoma, gallbladder carcinoma, glioma, head and neck cancer, liver cancer, lung cancer, neuroblastoma, neuroendocrine cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, salivary adenoid cystic cancer, or spermatocytic seminoma. In some examples, the solid tumor cancer is bladder cancer. In some examples, the solid tumor cancer is glioma. In some examples, the solid tumor cancer is neuroblastoma. In some examples, the solid tumor cancer is pancreatic cancer. In some examples, the solid tumor cancer is breast cancer. In some examples, the solid tumor cancer is head and neck cancer. In some examples, the solid tumor cancer is live cancer. In some examples, the solid tumor cancer is lung cancer.

[0046] In some examples, the cancer is a non-solid tumor cancer. In some examples, the cancer is a liquid cancer or hematologic cancer, *e.g.*, a myeloma (*e.g.*, multiple myeloma), a leukemia, or a lymphoma.

[0047] In some examples, the pharmaceutical composition is administered systemically. For example, in some examples, the pharmaceutical composition is administered parenterally, *e.g.*, intravenously, intraarterially, intraperitoneally, subcutaneously, or intradermally. In some examples, the pharmaceutical composition is administered enterically, *e.g.*, trans-gastrointestinally or orally.

[0048] In some examples, the pharmaceutical composition is administered locally, *e.g.*, by peritumoral injection or by intratumoral injection.

[00139] In some embodiments, the antibody or antigen-binding fragment thereof has CDR sequences having amino acid sequences of SEQ ID NOs: 1, 2, 3, 5, 6, and 7 without any variation. For example, in some embodiments, the antibody or antigen-binding fragment thereof comprises heavy chain complementary determining regions CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NOs: 1, 2, and 3, and the chain complementarity determining regions CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs: 5, 6, and 7.

[00140] In some embodiments, the antibody or antigen-binding fragment thereof has CDR sequences having amino acid sequences of SEQ ID NOs: 1, 2, 4, 5, 6, and 7 without any variation. For example, in some embodiments, the antibody or antigen-binding fragment thereof comprises heavy chain complementary determining regions CDR-H1, CDR-H2, and CDR-H3 having the amino acid sequences of SEQ ID NOs: 1, 2, and 4, and the chain complementarity determining regions CDR-L1, CDR-L2, and CDR-L3 having the amino acid sequences of SEQ ID NOs: 5, 6, and 7.

[00141] In some embodiments, the heavy chain variable region of the FGFR3 antibody or antigen-binding fragment thereof comprises an amino acid sequence of SEQ ID NO: 8 or an amino acid sequence differing in 1, 2, 3, or 4 amino acids therefrom, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% identical to SEQ ID NO: 8:

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EVQLVESGGG LVQPGGSLRL SCAASGFTFT STGISWVRQ APGKGLEWVGR
IYPTSGSTNY ADSVKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARTY
GIYDLYVDYT EYVMDYWGGG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA
ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTGPS
SSLGTQTYIC NVNHKPSNTK VDKKVEPKSC DKHTCPPCP APELLGGPSV
FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN
YKTTTPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVME ALHNHYTQKS
LSLSPGK (SEQ ID NO: 8)
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[00142] In some embodiments, the light chain variable region of the FGFR3 antibody or antigen-binding fragment thereof comprises an amino acid sequence of SEQ ID NO: 9 or an amino acid sequence differing in 1, 2, 3, or 4 amino acids therefrom, or an amino acid

sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% identical to SEQ ID NO: 9:

DIQMTQSPSS LSASVGDRVT ITCRASQDVD TSLAWYKQKP GKAPKLLIYS
ASFLYSGVPS RFSGSGSGTD FTLTISSLQP EDFATYYCQQ STGHPQTFGQ
GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNIFY PREAKVQWKV
DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG
LSSPVTKSFN RGEN (SEQ ID NO: 9)

[00143] In some embodiments, the FGFR3 targeting moiety is MFGR1877S (vofatamab) or an antigen-binding fragment thereof.

[00144] In some embodiments, the FGFR3 antibody or antigen-binding fragment thereof is a humanized antibody or antigen-binding fragment thereof. In certain embodiments, the antibody or antigen-binding fragment thereof has a dissociation constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$. In some embodiments, the antibody or antigen-binding fragment thereof has a dissociation constant (K_d) of between 1 nM and 10 nM (inclusive of endpoints) or between 0.1 nM and 1 nM (inclusive of endpoints).

[00145] In one embodiment, K_d is measured by a radio-labeled antigen binding assay (Radioimmunoassay, RIA) performed with the Fab version of an antibody or antigen-binding fragment thereof of interest and its antigen.

[00146] According to another embodiment, K_d is measured using surface plasmon resonance assays with immobilized antigen. In some embodiments, the antibodies or antigen-binding fragments thereof are human monoclonal antibodies directed against an epitope of human FGFR3 as described herein.

[00147] The antibody or antigen-binding fragment thereof may be any antibody or antigen-binding fragment thereof of natural and/or synthetic origin, *e.g.* an antibody of mammalian origin. In some embodiments, the constant domain, if present, is a human constant domain. In some embodiments, the variable domain is a mammalian variable domain, *e.g.*, a humanized or a human variable domain.

[00148] In some embodiments, antibodies used in accordance with this disclosure are monoclonal antibodies. In some embodiments, antibodies are recombinant murine

antibodies, chimeric, humanized or fully human antibodies, multispecific antibodies(*e.g.*, bispecific antibodies), or antigen-binding fragments thereof.

[00149] In some embodiments, are further coupled to other moieties for, *e.g.*, drug targeting and imaging applications.

[00150] In some embodiments, *e.g.*, for diagnostic purposes, the antibody or antigen-binding fragment thereof is labelled, *i.e.* coupled to a labelling group. Non-limiting examples of suitable labels include radioactive labels, fluorescent labels, suitable dye groups, enzyme labels, chromogenes, chemiluminescent groups, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter etc. In some embodiments, one or more labels are covalently bound to the antibody or antigen-binding fragment thereof.

[00151] Those labelled antibodies or antigen-binding fragments thereof (also referred to as "antibody conjugates") may in particular be used in immunohistochemistry assays or for molecular imaging *in vivo*.

[00152] In some embodiments, *e.g.*, for therapeutic purposes, the antibody or antigen-binding fragment thereof is further conjugated with an effector group, in particular, a therapeutic effector group such as a cytotoxic agent or a radioactive group agent.

Polypeptides

[00153] Polypeptides include, for example, any of a variety of hematologic agents (including, for instance, erythropoietin, blood-clotting factors, etc.), interferons, colony stimulating factors, antibodies, enzymes, and hormones. The identity of a particular polypeptide is not intended to limit the present disclosure, and any polypeptide of interest can be a polypeptide in the present methods.

[00154] A reference polypeptide described herein can include a target-binding domain that is capable of binding to a target of interest (*e.g.*, is capable of binding to an antigen, *e.g.*, FGFR3). For example, a polypeptide, such as an antibody, can bind to a transmembrane polypeptide (*e.g.*, receptor) or ligand (*e.g.*, a growth factor).

Modified polypeptides

[00155] Polypeptides suitable for use with compositions and methods of the present disclosure may have a modified amino acid sequence. Modified polypeptides may be substantially identical to the corresponding reference polypeptide (*e.g.*, the amino acid

sequence of the modified polypeptide may have at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the amino acid sequence of the reference polypeptide). In certain embodiments, the modification does not destroy significantly a desired biological activity (*e.g.*, binding to FGFR3). The modification may reduce (*e.g.*, by at least 5%, 10%, 20%, 25%, 35%, 50%, 60%, 70%, 75%, 80%, 90%, or 95%), may have no effect, or may increase (*e.g.*, by at least 5%, 10%, 25%, 50%, 100%, 200%, 500%, or 1000%) the biological activity of the original polypeptide. The modified polypeptide may have or may optimize a characteristic of a polypeptide, such as *in vivo* stability, bioavailability, toxicity, immunological activity, immunological identity, and conjugation properties.

[00156] Modifications include those by natural processes, such as post-translational processing, or by chemical modification techniques known in the art. Modifications may occur anywhere in a polypeptide including the polypeptide backbone, the amino acid side chains and the amino- or carboxy-terminus. The same type of modification may be present in the same or varying degrees at several sites in a given polypeptide, and a polypeptide may contain more than one type of modification. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from post-translational natural processes or may be made synthetically. Other modifications include pegylation, acetylation, acylation, addition of acetamidomethyl (Acm) group, ADP-ribosylation, alkylation, amidation, biotinylation, carbamoylation, carboxyethylation, esterification, covalent attachment to flavin, covalent attachment to a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of drug, covalent attachment of a marker (*e.g.*, fluorescent or radioactive), covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation and ubiquitination.

[00157] A modified polypeptide can also include an amino acid insertion, deletion, or substitution, either conservative or non-conservative (*e.g.*, D-amino acids, desamino acids) in

the polypeptide sequence (*e.g.*, where such changes do not substantially alter the biological activity of the polypeptide). In particular, the addition of one or more cysteine residues to the amino or carboxy-terminus of a polypeptide herein can facilitate conjugation of these polypeptides by, *e.g.*, disulfide bonding. For example, a polypeptide can be modified to include a single cysteine residue at the amino-terminus or a single cysteine residue at the carboxy-terminus. Amino acid substitutions can be conservative (*i.e.*, wherein a residue is replaced by another of the same general type or group) or non-conservative (*i.e.*, wherein a residue is replaced by an amino acid of another type). In addition, a naturally occurring amino acid can be substituted for a non-naturally occurring amino acid (*i.e.*, non-naturally occurring conservative amino acid substitution or a non-naturally occurring non-conservative amino acid substitution).

[00158] Polypeptides made synthetically can include substitutions of amino acids not naturally encoded by DNA (*e.g.*, non-naturally occurring or unnatural amino acid). Examples of non-naturally occurring amino acids include D-amino acids, N-protected amino acids, an amino acid having an acetylaminoethyl group attached to a sulfur atom of a cysteine, a pegylated amino acid, the omega amino acids of the formula $\text{NH}_2(\text{CH}_2)_n\text{COOH}$ wherein n is 2-6, neutral nonpolar amino acids, such as sarcosine, t-butyl alanine, t-butyl glycine, N-methyl isoleucine, and norleucine. Phenylglycine may substitute for Trp, Tyr, or Phe; citrulline and methionine sulfoxide are neutral nonpolar, cysteic acid is acidic, and ornithine is basic. Proline may be substituted with hydroxyproline and retain the conformation conferring properties.

[00159] Analogs may be generated by substitutional mutagenesis and retain the biological activity of the original polypeptide. Examples of substitutions identified as “conservative substitutions” are shown in Table 1. If such substitutions result in a change not desired, then other type of substitutions, denominated “exemplary substitutions” in Table 1, or as further described herein in reference to amino acid classes, are introduced and the products screened.

[00160] Table 1: Amino acid substitutions

Original residue	Exemplary substitution	Conservative substitution
Ala (A)	Val, Leu, Ile	Val

Original residue	Exemplary substitution	Conservative substitution
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln, His, Lys, Arg	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro	Pro
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala	Leu
Pro (P)	Gly	Gly
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Leu, Met, Phe, Ala, norleucine	Leu

[00161] Substantial modifications in function or immunological identity are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, and/or (c) the bulk of the side chain.

Chelating moiety or metal complex thereof

Chelating moieties

[00162] Examples of suitable chelating moieties include, but are not limited to, DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), DOTMA (1R,4R,7R,10R)- α , α' ,

α'' , α''' -tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, DOTAM (1,4,7,10-tetrakis(carbamoylmethyl)-1,4,7,10-tetraazacyclododecane), DOTPA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra propionic acid), DO3AM-acetic acid (2-(4,7,10-tris(2-amino-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetic acid), DOTA-GA anhydride (2,2',2''-(10-(2,6-dioxotetrahydro-2H-pyran-3-yl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid, DOTP (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonic acid)), DOTMP (1,4,6,10-tetraazacyclodecane-1,4,7,10-tetramethylene phosphonic acid, DOTA-4AMP (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(acetamido-methylenephosphonic acid), CB-TE2A (1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-diacetic acid), NOTA (1,4,7-triazacyclononane-1,4,7-triacetic acid), NOTP (1,4,7-triazacyclononane-1,4,7-tri(methylene phosphonic acid), TETPA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetrapropionic acid), TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetra acetic acid), HEHA (1,4,7,10,13,16-hexaazacyclohexadecane-1,4,7,10,13,16-hexaacetic acid), PEPA (1,4,7,10,13-pentaazacyclopentadecane-N,N',N'',N''', N''''-pentaacetic acid), H₄octapa (N,N'-bis(6-carboxy-2-pyridylmethyl)-ethylenediamine-N,N'-diacetic acid), H₂dedpa (1,2-[[6-(carboxy)-pyridin-2-yl]-methylamino]ethane), H₆phospa (N,N'-(methylenephosphonate)-N,N'-[6-(methoxycarbonyl)pyridin-2-yl]-methyl-1,2-diaminoethane), TTHA (triethylenetetramine-N,N,N',N'',N''', N''''-hexaacetic acid), DO2P (tetraazacyclododecane dimethanephosphonic acid), HP-DO3A (hydroxypropyltetraazacyclododecanetriacetic acid), EDTA (ethylenediaminetetraacetic acid), Deferoxamine, DTPA (diethylenetriaminepentaacetic acid), DTPA-BMA (diethylenetriaminepentaacetic acid-bismethylamide), octadentate-HOPO (octadentate hydroxypyridinones), or porphyrins.

[00163] In some embodiments, radioimmunoconjugates comprise a metal complex of a chelating moiety. For example, chelating groups may be used in metal chelate combinations with metals, such as manganese, iron, and gadolinium and isotopes (*e.g.*, isotopes in the general energy range of 60 to 10,000 keV), such as any of the radioisotopes and radionuclides discussed herein.

[00164] In some embodiments, chelating moieties are useful as detection agents, and radioimmunoconjugates comprising such detectable chelating moieties can therefore be used as diagnostic or theranostic agents.

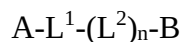
Radioisotopes and Radionuclides

[00165] In some embodiments, the metal complex comprises a radionuclide. Examples of suitable radioisotopes and radionuclides include, but are not limited to, ^3H , ^{14}C , ^{15}N , ^{18}F , ^{35}S , ^{47}Sc , ^{55}Co , ^{60}Cu , ^{61}Cu , ^{62}Cu , ^{64}Cu , ^{66}Ga , ^{67}Ga , ^{67}Cu , ^{68}Ga , ^{75}Br , ^{76}Br , ^{77}Br , ^{82}Rb , ^{89}Zr , ^{86}Y , ^{87}Y , ^{90}Y , ^{97}Ru , ^{99}Tc , $^{99\text{m}}\text{Tc}$, ^{105}Rh , ^{109}Pd , ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{149}Pm , ^{149}Tb , ^{153}Sm , ^{166}Ho , ^{177}Lu , $^{117\text{m}}\text{Sn}$, ^{186}Re , ^{188}Re , ^{198}Au , ^{199}Au , ^{201}Tl , ^{203}Pb , ^{211}At , ^{212}Pb , ^{212}Bi , ^{213}Bi , ^{223}Ra , ^{225}Ac , ^{227}Th , and ^{229}Th .

[00166] In some embodiments, the radionuclide is an alpha emitter, *e.g.*, Astatine-211 (^{211}At), Bismuth-212 (^{212}Bi), Bismuth-213 (^{213}Bi), Actinium-225 (^{225}Ac), Radium-223 (^{223}Ra), Lead-212 (^{212}Pb), Thorium-227 (^{227}Th), or Terbium-149 (^{149}Tb), or a progeny thereof. In some embodiments, the alpha-emitter is Actinium-225 (^{225}Ac), or a progeny thereof.

Linker

[00167] In some embodiments, the linker is as shown within the structure of Formula I-b, as that part of Formula I-b absent A and B:



Formula I-b

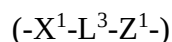
(A and B are as defined in Formula I-a.)

[00168] Thus, in some embodiments, the linker is $\text{-L}^1\text{-(L}^2\text{)}_n\text{-}$, wherein:

L^1 is optionally substituted $\text{C}_1\text{-C}_6$ alkyl, optionally substituted $\text{C}_1\text{-C}_6$ heteroalkyl, or optionally substituted aryl or heteroaryl;

n is between 1 and 5 (inclusive); and

each L^2 , independently, has the structure:



Formula III

wherein:

X^1 is $\text{C=O(NR}^1\text{)}$, $\text{C=S(NR}^1\text{)}$, $\text{OC=O(NR}^1\text{)}$, $\text{NR}^1\text{C=O(O)}$, $\text{NR}^1\text{C=O(NR}^1\text{)}$, $\text{-CH}_2\text{PhC=O(NR}^1\text{)}$, $\text{-CH}_2\text{Ph(NH)C=S(NR}^1\text{)}$, O , or NR^1 ; and each R^1 independently is H , optionally substituted $\text{C}_1\text{-C}_6$ alkyl, optionally substituted $\text{C}_1\text{-C}_6$ heteroalkyl, or optionally substituted aryl or heteroaryl, in which $\text{C}_1\text{-C}_6$ alkyl can be substituted by oxo ($=\text{O}$), heteroaryl, or a combination thereof;

L^3 is optionally substituted C_1 - C_{50} alkyl or optionally substituted C_1 - C_{50} heteroalkyl (*e.g.*, C_5 - C_{20} polyethylene glycol);

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.

[00169] In some embodiments, L^1 is substituted C_1 - C_6 alkyl or substituted C_1 - C_6 heteroalkyl, the substituent comprising a heteroaryl group (*e.g.*, six-membered nitrogen-containing heteroaryl).

[00170] In some embodiments, L^3 is substituted C_1 - C_{50} alkyl or substituted C_1 - C_{50} heteroalkyl, the substituent comprising a heteroaryl group (*e.g.*, six-membered nitrogen-containing heteroaryl).

[00171] In some embodiments, A is a macrocyclic chelating moiety comprising one or more heteroaryl groups (*e.g.*, six-membered nitrogen-containing heteroaryl).

Cross-linking groups

[00172] In some embodiments, radioimmunoconjugates comprise a cross-linking group instead of or in addition to the targeting moiety (*e.g.*, B in Formula I comprises a cross-linking group).

[00173] A cross-linking group is a reactive group that is able to join two or more molecules by a covalent bond. Cross-linking groups may be used to attach the linker and chelating moiety to a therapeutic or targeting moiety. Cross-linking groups may also be used to attach the linker and chelating moiety to a target *in vivo*. In some embodiments, the cross-linking group is an amino-reactive, methionine reactive or thiol-reactive cross-linking group, or a sortase-mediated coupling. In some embodiments, the amino-reactive or thiol-reactive cross-linking group comprises an activated ester such as a hydroxysuccinimide ester, 2,3,5,6-tetrafluorophenol ester, 4-nitrophenol ester or an imidate, anhydride, thiol, disulfide, maleimide, azide, alkyne, strained alkyne, strained alkene, halogen, sulfonate, haloacetyl, amine, hydrazide, diazirine, phosphine, tetrazine, isothiocyanate, or oxaziridine. In some embodiments, the sortase recognition sequence may comprise of a terminal glycine-glycine-glycine (GGG) and/or LPTXG amino acid sequence, where X is any amino acid. A person having ordinary skill in the art will understand that the use of cross-linking groups is not limited to the specific constructs disclosed herein, but rather may include other known cross-linking groups.

Pharmaceutical compositions

[00174] In one aspect, the present disclosure provides pharmaceutical compositions comprising radioimmunoconjugates disclosed herein. Such pharmaceutical compositions can be formulated for use in a variety of drug delivery systems. One or more physiologically acceptable excipients or carriers can also be included in a pharmaceutical composition for proper formulation. Non-limiting examples of suitable formulations compatible for use with the present disclosure include those described in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed., 1985. For a brief review of methods for drug delivery, See, e.g., Langer (*Science*. 249:1527-1533, 1990).

[00175] Pharmaceutical compositions may be formulated for any of a variety of routes of administration discussed herein (See, e.g., the "Administration and Dosage" subsection herein), Sustained release administration is contemplated, by such means as depot injections or erodible implants or components. Thus, the present disclosure provides pharmaceutical compositions that include agents disclosed herein (e.g., radioimmunoconjugates) dissolved or suspended in an acceptable carrier, preferably an aqueous carrier, e.g., water, buffered water, saline, or PBS, among others. In some embodiments, pharmaceutical compositions contain pharmaceutically acceptable auxiliary substances to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, or detergents, among others. In some embodiments, pharmaceutical compositions are formulated for oral delivery and may optionally contain inert ingredients such as binders or fillers for the formulation of a unit dosage form, such as a tablet or a capsule. In some embodiments, pharmaceutical compositions are formulated for local administration and may optionally contain inert ingredients such as solvents or emulsifiers for the formulation of a cream, an ointment, a gel, a paste, or an eye drop.

[00176] In some embodiments, provided pharmaceutical compositions are sterilized by conventional sterilization techniques, e.g., may be sterile filtered. Resulting aqueous solutions may be packaged for use as is, or lyophilized. Lyophilized preparations can be, for example, combined with a sterile aqueous carrier prior to administration. The pH of preparations typically will be between 3 and 11, more preferably between 5 and 9 or between 6 and 8, and most preferably between 6 and 7, such as 6 to 6.5. Resulting compositions in solid form may be packaged, for example, in multiple single dose units, each containing a fixed amount of

the above-mentioned agent or agents, such as in a sealed package of tablets or capsules. Pharmaceutical compositions in solid form can also be packaged in a container for a flexible quantity, such as in a squeezable tube designed for a topically applicable cream or ointment.

Methods of treatment

[00177] In one aspect, the present disclosure provides methods of treatment comprising a subject a radioimmunoconjugate as disclosed herein.

Subjects

[00178] In some disclosed methods, a therapy (*e.g.*, comprising a therapeutic agent) is administered to a subject. In some embodiments, the subject is a mammal, *e.g.*, a human.

[00179] In some embodiments, the subject has cancer or is at risk of developing cancer. For example, the subject may have been diagnosed with cancer. For example, the cancer may be a primary cancer or a metastatic cancer. Subjects may have any stage of cancer, *e.g.*, stage I, stage II, stage III, or stage IV with or without lymph node involvement and with or without metastases. Provided radioimmunoconjugates and compositions may prevent or reduce further growth of the cancer and/or otherwise ameliorate the cancer (*e.g.*, prevent or reduce metastases). In some embodiments, the subject does not have cancer but has been determined to be at risk of developing cancer, *e.g.*, because of the presence of one or more risk factors such as environmental exposure, presence of one or more genetic mutations or variants, family history, etc. In some embodiments, the subject has not been diagnosed with cancer.

[00180] In some embodiments, the cancer is a solid tumor cancer, *e.g.*, a sarcoma or carcinoma.

[00181] In some embodiments, the solid tumor cancer is adrenocortical carcinoma, bladder cancer (*e.g.*, urothelial carcinoma), breast cancer, cervical cancer, colorectal cancer, endometrial adenocarcinoma, Ewing's sarcoma, gallbladder carcinoma, glioma (*e.g.*, glioblastoma multiforme), head and neck cancer, liver cancer, lung cancer (*e.g.*, small cell lung cancer or non-small cell lung cancer, or adenocarcinoma of the lung), neuroblastoma, neuroendocrine cancer, pancreatic cancer (*e.g.*, pancreatic exocrine carcinoma), prostate cancer, renal cell carcinoma, salivary adenoid cystic cancer, or spermatocytic seminoma.

[00182] In some embodiments, the cancer is selected from the group consisting of bladder cancer, breast cancer, head and neck cancer, liver cancer, and lung cancer. In some

embodiments, the cancer is bladder cancer. In some embodiments, the cancer is head and neck cancer. In some embodiments, the cancer is liver cancer.

[00183] In some embodiments, the cancer is a non-solid tumor cancer, *e.g.*, a liquid cancer or hematologic cancer. In some embodiments, the cancer is a myeloma, *e.g.*, multiple myeloma. In some embodiments, the cancer is a leukemia, *e.g.*, acute myeloid leukemia. In some embodiments, the cancer is a lymphoma.

Administration and dosage

[00184] Radioimmunoconjugates and pharmaceutical compositions thereof disclosed herein may be administered by any of a variety of routes of administration, including systemic and local routes of administration

[00185] Systemic routes of administration include parenteral routes and enteral routes. In some embodiments, radioimmunoconjugates or pharmaceutical compositions thereof are administered by a parenteral route, for example, intravenously, intraarterially, intraperitoneally, subcutaneously, or intradermally. In some embodiments, radioimmunoconjugates or pharmaceutical compositions thereof are administered intravenously. In some embodiments, radioimmunoconjugates or pharmaceutical compositions thereof are administered by an enteral route of administration, for example, trans-gastrointestinal, or orally.

[00186] Local routes of administration include, but are not limited to, peritumoral injections and intratumoral injections.

[00187] Pharmaceutical compositions can be administered for radiation treatment planning, diagnostic, and/or therapeutic treatments. When administered for radiation treatment planning or diagnostic purposes, the radioimmunoconjugate may be administered to a subject in a diagnostically effective dose and/or an amount effective to determine the therapeutically effective dose. In therapeutic applications, pharmaceutical compositions may be administered to a subject (*e.g.*, a human) already suffering from a condition (*e.g.*, cancer) in an amount sufficient to cure or at least partially arrest the symptoms of the disorder and its complications. An amount adequate to accomplish this purpose is defined as a “therapeutically effective amount,” an amount of a compound sufficient to substantially improve at least one symptom associated with the disease or a medical condition. For example, in the treatment of cancer, an agent or compound that decreases, prevents, delays,

suppresses, or arrests any symptom of the disease or condition would be therapeutically effective. A therapeutically effective amount of an agent or compound is not required to cure a disease or condition but may, for example, provide a treatment for a disease or condition such that the onset of the disease or condition is delayed, hindered, or prevented, such that the disease or condition symptoms are ameliorated, or such that the term of the disease or condition is changed. For example, the disease or condition may become less severe and/or recovery is accelerated in an individual. In some embodiments, a subject is administered a first dose of a radioimmunoconjugate or composition in an amount effective for radiation treatment planning, then administered a second dose or set of doses of the radioimmunoconjugate or composition in a therapeutically effective amount.

[00188] Effective amounts may depend on the severity of the disease or condition and other characteristics of the subject (*e.g.*, weight). Therapeutically effective amounts of disclosed radioimmunoconjugates and compositions for subjects (*e.g.*, mammals such as humans) can be determined by the ordinarily-skilled artisan with consideration of individual differences (*e.g.*, differences in age, weight, and the condition of the subject).

[00189] In some embodiments, disclosed radioimmunoconjugates exhibit an enhanced ability to target cancer cells. In some embodiments, effective amount of disclosed radioimmunoconjugates are lower than (*e.g.*, less than or equal to about 90%, 75%, 50%, 40%, 30%, 20%, 15%, 12%, 10%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of) the equivalent dose for a therapeutic effect of the unconjugated, and/or non-radiolabeled targeting moiety.

[00190] Single or multiple administrations of pharmaceutical compositions disclosed herein including an effective amount can be carried out with dose levels and pattern being selected by the treating physician. Dose and administration schedule can be determined and adjusted based on the severity of the disease or condition in the subject, which may be monitored throughout the course of treatment according to the methods commonly practiced by clinicians or those described herein.

[00191] The following specific examples are to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

Examples

Example 1. General materials and methods

[00192] Lutetium-177 can be obtained from Perkin Elmer as lutetium trichloride in a 0.05 N hydrochloric acid solution; indium-111, as a trichloride salt, can be obtained from Nordion; and actinium-225 can be obtained as actinium-225 trinitrate from Oak Ridge National Laboratories.

[00193] Analytical HPLC-MS can be performed using a Waters Acquity HPLC-MS system comprised of a Waters Acquity Binary Solvent Manager, a Waters Acquity Sample Manager (samples cooled to 10°C), a Water Acquity Column Manager (column temperature 30°C), a Waters Acquity Photodiode Array Detector (monitoring at 254 nm and 214 nm), a Waters Acquity TQD with electrospray ionization and a Waters Acquity BEH C18, 2.1×50 (1.7 µm) column. Preparative HPLC can be performed using a Waters HPLC system comprised of a Waters 1525 Binary HPLC pump, a Waters 2489 UV/Visible Detector (monitoring at 254 nm and 214 nm) and a Waters XBridge Prep phenyl or C18 19×100 mm (5 µm) column.

[00194] HPLC elution method 1: Waters Acquity BEH C18 2.1×50 mm (1.7 µm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate = 0.3 mL/min; initial = 90% A, 3-3.5 min = 0% A, 4 min = 90% A, 5 min = 90% A.

[00195] HPLC elution method 2: Waters XBridge Prep Phenyl 19×100 mm (5 µm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 10 mL/min; initial = 80% A, 13 min = 0% A.

[00196] HPLC elution method 3: Waters Acquity BEH C18 2.1×50 mm (1.7 µm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate = 0.3 mL/min; initial = 90% A, 8 min = 0% A, 10 min = 0% A, 11 min = 90% A, 12 min = 90% A.

[00197] HPLC elution method 4: Waters XBridge Prep C18 OBD 19×100 mm (5 µm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 10 mL/min; initial = 80% A, 3 min = 80% A, 13 min = 20% A, 18 min = 0% A.

[00198] HPLC elution method 5: Waters XBridge Prep C18 OBD 19×100 mm (5 µm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 10 mL/min; initial = 90% A, 3 min = 90% A, 13 min = 0% A, 20 min = 0% A.

[00199] HPLC elution method 6: Waters XBridge Prep C18 OBD 19×100 mm (5 μm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 10 mL/min; initial = 75% A, 13 min = 0% A, 15 min = 0% A.

[00200] HPLC elution method 7: Waters XBridge Prep C18 OBD 19×100 mm (5 μm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 10 mL/min; initial = 80% A, 12 min = 0% A, 15 min = 0% A.

[00201] HPLC elution method 8: Waters XBridge Prep C18 OBD 19×100 mm (5 μm) column; mobile phase A: H₂O (0.1% v/v TFA); mobile phase B: acetonitrile (0.1% v/v TFA); flow rate: 10 mL/min; initial = 90% A, 12 min = 0% A, 15 min = 0% A.

[00202] Analytical Size Exclusion Chromatography (SEC) can be performed using a Waters system comprised of a Waters 1525 Binary HPLC pump, a Waters 2489 UV/Visible Detector (monitoring at 280 nm), a Bioscan Flow Count radiodetector (FC-3300) and TOSOH TSKgel G3000SWxl, 7.8×300 mm column. The isocratic SEC method can have a flow rate of, *e.g.*, mL/min, with a mobile phase of 0.1 M phosphate, 0.6 M NaCl, 0.025% sodium azide, pH = 7.

[00203] MALDI-MS (positive ion) can be performed using a MALDI Bruker Ultraflexreme Spectrometer.

[00204] Radio thin-layer chromatography (radioTLC) can be performed with Bioscan AR-2000 Imaging Scanner, and can be carried out on iTLC-SG glass microfiber chromatography paper (Agilent Technologies, SGI0001) plates using citrate buffer (0.1 M, pH 5.5).

Example 2. Synthesis of 4-{{11-oxo-11-(2,3,5,6-tetrafluorophenoxy)undecyl}carbamoyl}-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]butanoic acid (Compound B)

[00205] A bifunctional chelate, 4-{{11-oxo-11-(2,3,5,6-tetrafluorophenoxy)undecyl}carbamoyl}-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]butanoic acid (Compound B), can be synthesized according to the scheme provided in **FIG. 2**. To a solution of 5-(tert-butoxy)-5-oxo-4-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)pentanoic acid (DOTA-GA-(tBu)₄, 50 mg, 0.07 mmol) in ACN (2.0 mL), DSC (50 mg, 0.21 mmol) is added, followed by pyridine, (0.20 mL, 2.48 mmol). The reaction is stirred at room temperature for 1 hour. To the reaction mixture is added 11-aminoundecanoic acid, (70 mg, 0.36 mmol) followed by

PBS solution (1.0 mL) at room temperature. The reaction is stirred for 72 hours at room temperature. The reaction mixture is filtered with a syringe filter and purified directly by Prep-HPLC using method 6 to yield Intermediate 2-A.

[00206] To a solution of Intermediate 2-A (40 mg, 0.03 mmol), TFP (90 mg, 0.54 mmol) and EDC (40 mg, 0.27 mmol) in ACN (1.0 mL) is added pyridine (0.05 mL, 50 mg, 0.62 mmol) at room temperature. The solution is stirred at room temperature for 24 hours. The reaction is purified directly by Prep-HPLC using method 7 to provide Intermediate 2-B as a wax after concentration using a Biotage V10 Rapid Evaporator.

[00207] Intermediate 2-B is dissolved in DCM / TFA (1.0 mL / 2.0 mL) and allowed to stir at room temperature for 24 hours. The reaction is concentrated by air stream and purified directly by Prep-HPLC using method 8 to yield Compound B as a clear wax after concentration. An aliquot is analyzed by HPLC-MS elution method 3.

[00208] ^1H NMR (600 MHz, DMSO- d_6) δ 7.99 – 7.88 (m, 1H), 7.82 (t, J = 5.5 Hz, 1H), 3.78 (broad s, 4H), 3.43 (broad s, 12H), 3.08 (broad s, 4H), 3.00 (m, 3H), 2.93 (broad s, 3H), 2.77 (t, J = 7.2 Hz, 2H), 2.30 (broad s, 2H), 1.88 (broad s, 2H), 1.66 (p, J = 7.3 Hz, 2H), 1.36 (m, 4H), 1.32 – 1.20 (m, 9H).

Example 3. Synthesis of [^{225}Ac]-Compound B-anti-FGFR3 conjugate

[00209] Compound B (1 μmole) is dissolved in a hydrochloric acid solution (0.001 M). An aliquot of Compound B solution (5 μL , 70 nmole) is added to a solution containing an anti-FGFR3 antibody (1.8 nmoles) in a phosphate buffer (pH 8). After 3 hours at ambient temperature, the resulting immunoconjugate is purified via a Sephadex G-50 resin packed column. The immunoconjugate Compound B-anti-FGFR3 is eluted from the column with acetate buffer (pH 6.5).

[00210] Ac-225 (15 μCi , 10 μL) is added to a solution of Compound B-anti-FGFR3 (300 μg in acetate buffer (pH 6.5)). The radiolabeling reaction is incubated at 30° C for 1 hour. The crude product, [^{225}Ac]-Compound B-anti-FGFR3, is purified via a Sephadex G-50 resin packed column eluted with acetate buffer.

Example 4. Synthesis of 4-([2-(2-{2-[3-oxo-3-(2,3,5,6-tetrafluorophenoxy)propoxy]ethoxy}ethoxy)ethyl]carbamoyl)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]butanoic acid (Compound C)

[00211] A bifunctional chelate, 4-{[2-(2-{2-[3-oxo-3-(2,3,5,6-tetrafluorophenoxy)propoxy]ethoxy}ethoxy)ethyl]carbamoyl}-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]butanoic acid (Compound C), is synthesized according to the scheme provided in **FIG. 3**.

[00212] To a solution of 5-(tert-butoxy)-5-oxo-4-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)pentanoic acid (DOTA-GA(tBu)₄, 100 mg, 0.143 mmol) in ACN (8.0 mL) is added DSC (73 mg, 0.285 mmol) and pyridine (0.80 mL, 9.89 mmol). The reaction mixture is stirred for 90 min at ambient temperature. This solution is added to a semi-solution of amino-PEG3-acid (63 mg, 0.285 mmol in 1.2 mL of DMF) in a 100 mL round bottom flask. After 4 hours at ambient temperature, the reaction is worked up by concentrating to dryness under a stream of air. The crude material is purified by HPLC elution method 2 (dissolved the crude in 6 mL of 20% ACN/H₂O). The fractions containing product are pooled and concentrated under vacuum and then co-evaporated with ACN (3 x 2 mL).

[00213] To a vial containing Intermediate 1-A (82 mg, 60 µmol) is added ACN (2 mL), NEt₃ (50 µL, 360 µmol, 6 equiv.), HBTU (23 mg, 60 µmol, 1 equiv) and a TFP solution (50 mg, 300 µmol, 5 equiv., dissolved in 250 µL of ACN). The resulting clear solution is stirred at ambient temperature for 3 hours. The reaction is worked up by concentrating the solution to dryness under an air stream and is then diluted with ACN/H₂O (1:1, 3 mL total) and purified on preparative HPLC using elution method 4. Fractions containing product are pooled and concentrated under vacuum and then co-evaporated with ACN (3 x 2 mL). Intermediate 1-B is obtained as a clear residue.

[00214] To a vial containing Intermediate 1-B (67 mg, 64 µmol) is added DCM (2 mL) and TFA (2 mL). The resulting solution is stirred at ambient temperature for 16 hour. Additional, TFA (2 mL) is added, and the reaction is stirred at ambient temperature for 6 hours. The reaction is concentrated to dryness under an air stream, with the crude product being finally dissolved in ACN/H₂O (1 mL of 10% ACN/H₂O). The crude reaction solution is then purified by preparative HPLC using elution method 5. The fractions containing product are pooled, frozen and lyophilized. Compound C is obtained as a white solid. An aliquot is analyzed by HPLC-MS elution method 3.

[00215] ^1H NMR ($\text{DMSO-}d_6$, 600 MHz) δ 7.97-7.91 (m, 2H), 3.77 (t, 2H, $J = 6.0$ Hz), 3.58-3.55 (m, 2H), 3.53-3.48 (m, 8H), 3.44-3.38 (m, 10H), 3.23-3.08 (m, 11H), 3.02 (t, 2H, $J = 6.0$ Hz), 2.93 (broad s, 4H), 2.30 (broad s, 2H), 1.87 (broad s, 2H).

Example 5. Synthesis of [^{225}Ac]-Compound C-anti-FGFR3 conjugate

[00216] Compound C (1 μmole) is dissolved in a hydrochloric acid solution (0.001 M). An aliquot of Compound C solution (5 μL , 70 nmole) is added to a solution containing anti-FGFR3 antibody (1.8 nmoles) in a phosphate buffer (pH 8). After 3 hours at ambient temperature, the resulting immunoconjugate is purified via a Sephadex G-50 resin packed column. The immunoconjugate Compound C-anti-FGFR3 is eluted from the column with acetate buffer (pH 6.5). Identities of eluates can be confirmed by, *e.g.*, MALDI-TOF.

[00217] Ac-225 (15 μCi , 10 μL) is added to a solution of Compound C-anti-FGFR3 (300 μg in acetate buffer (pH 6.5)). The radiolabeling reaction is incubated at 30° C for 1 hour. The crude product, [^{225}Ac]-Compound C-anti-FGFR3, is purified via a Sephadex G-50 resin packed column eluted with acetate buffer.

Example 6. Effects of [^{225}Ac]-anti-FGFR3 conjugates on tumor growth and survival in a bladder cancer xenograft model

[00218] [^{225}Ac]-anti-FGFR3 conjugates are tested using the human UM-UC-1 bladder cell line, which expresses wild type FGFR3. UM-UC-1 cells are injected into immunocompromised mice. After the establishment of tumors, mice are administered an [^{225}Ac]-anti-FGFR3 conjugate, control (*e.g.*, PBS buffer or other vehicle alone), or optionally unconjugated anti-FGFR3.

[00219] Tumor volume is monitored twice weekly using caliper measurements, and the results are compared across treatment groups. Survival is recorded. Greater inhibition of tumor growth and/or greater survival in [^{225}Ac]-anti-FGFR3 conjugate treatment groups indicates increased efficacy.

Example 7. Effects of [^{225}Ac]-anti-FGFR3 conjugates on tumor growth and survival in WT and mutant FGFR3 bladder cancer xenograft models

[00220] While wild-type FGFR3 is overexpressed in certain cancers, some tumors are associated with mutant FGFR3. In this Example, [²²⁵Ac]-anti-FGFR3 conjugates are tested using various human bladder cell lines that express either wild type or mutant FGFR3.

[00221] RT112 bladder cancer cells, which express WT FGFR3, are injected into nude (nu/nu) mice, and tumors are allowed to grow to a mean volume of ~100-150 mm³. Animals are dosed twice weekly with vehicle or with an [²²⁵Ac]-anti-FGFR3 conjugate. Optionally, a third set of animals are dosed with unconjugated anti-FGFR3.

[00222] Tumors are measured twice weekly using a caliper, and tumor volume is calculated using the formula:

$$V = 0.5 \times a \times b^2$$

wherein a and b are the length and width of the tumor, respectively.

[00223] Tumor growth is compared across groups.

[00224] To assess the effects of [²²⁵Ac]-anti-FGFR3 conjugates on FGFR3 signaling, tumor lysates are collected at 48 and 72 hour after treatment. Phosphorylation and total protein levels of FRS2α, AKT, and p44/42 MAPK (downstream mediators of FGFR3 signaling) in tumor lysates are examined.

[00225] Additionally, effects of [²²⁵Ac]-anti-FGFR3 conjugates are studied in a Ba/F3-FGFR3^{S249C} allograft model. *See, e.g.*, Qing et al., “Antibody-based targeting of FGFR3 in bladder carcinoma and t(4;14)-positive multiple myeloma in mice.” J Clin Invest. 2009 May 1; 119(5): 1216–1229. (S249C is the most frequent FGFR3 mutation found in bladder cancer.) Tumor growth and tumor lysates are assessed as mentioned above for the RT112 xenograft model.

Example 8. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on tumor growth and survival in multiple myeloma xenograft models

[00226] OPM2 and KMS11 are t(4;14)+ multiple myeloma cell lines harboring K650E and Y373C FGFR3 mutations, respectively. [²²⁵Ac]-anti-FGFR3 conjugates are tested in OPM2 and KMS11 xenograft models. Cells are expanded, and 15 x 10⁶ OPM2 or 20 x 10⁶ KMS11 cells are implanted subcutaneously into the flanks of mice in a volume of 0.2 ml in Hank’s Balanced Salt Solution (HBSS)/Matrigel (1:1 v/v: BD Biosciences). Tumors are measured twice weekly as a caliper, and tumor volume is calculated as described in Example 7.

[00227] When tumors reach an average size of 150-200 mm³, animals are randomly assigned to a treatment or control group. Each [²²⁵Ac]-anti-FGFR3 conjugate may be tested in a separate treatment group. A control group may include mice administered HBSS or other vehicle. Optionally, for comparison, one or more treatment groups are included in which mice are administered unconjugated anti-FGFR3 (cold antibody). Mice in all groups are administered the relevant agents for their group twice weekly intraperitoneally.

[00228] Tumor volume is monitored twice weekly using caliper measurements, and the results are compared across treatment groups. Survival is recorded. Greater inhibition of tumor growth and/or greater survival in [²²⁵Ac]-anti-FGFR3 conjugate treatment groups indicates increased efficacy.

Example 9. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on tumor growth and survival in a liver cancer xenograft model

[00229] [²²⁵Ac]-anti-FGFR3 conjugates are tested in a tumor xenograft model based on a liver cancer cell line (Huh7) essentially as described in Example 8.

Example 10. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on tumor growth and survival in a breast cancer xenograft model

[00230] [²²⁵Ac]-anti-FGFR3 conjugates are tested in a tumor xenograft model based on a breast cancer cell line (Cal-51) essentially as described in Example 8.

Example 11. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on tumor growth and survival in a colon adenocarcinoma xenograft model

[00231] [²²⁵Ac]-anti-FGFR3 conjugates are tested in the MC38 mouse colon adenocarcinoma xenograft model. FGFR3-positive MC38 cells are expanded, and 1x10⁶ MC38 cells are implanted subcutaneously into the flanks of female C57BL/6 mice that are 8 to 12 weeks of age. When tumors reach an average size of 80-120 mm³, animals are pair matched and assigned to a treatment or control group. Each [²²⁵Ac]-anti-FGFR3 conjugate may be tested in a separate treatment group. A control group may include mice administered phosphate-buffered saline (PBS). Optionally, for comparison, one or more treatment groups are included in which mice are administered unconjugated anti-FGFR3 (cold antibody). Mice in all groups may be administered the relevant agents for their group according to a regular

schedule, *e.g.*, weekly, twice a week, or thrice per week, for one or more (*e.g.*, 1, 2, or 3) weeks intravenously or intraperitoneally.

[00232] Tumor volume is monitored twice weekly using caliper measurements, and the results are compared across treatment groups. Survival is recorded. Greater inhibition of tumor growth and/or greater survival in [²²⁵Ac]-anti-FGFR3 conjugate treatment groups indicates increased efficacy.

Example 12. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on immune cell infiltration using an adenocarcinoma cell line

[00233] MC38 (adenocarcinoma) cells are implanted subcutaneously into the flanks of female C57BL/6 mice that are 8 to 12 weeks of age. When tumors reach an average size of 80-120 mm³, animals are pair matched and divided into treatment and control group. A control group of mice receive PBS, immunoconjugate treatment group(s) receive [²²⁵Ac]-anti-FGFR3 conjugates, and optional antibody treatment group(s) receive unconjugated anti-FGFR3. All groups are administered according to the same route and dosing schedule: twice weekly intravenously.

[00234] After 7 days of treatment, half of the animals from each group are sacrificed, and tumors are collected. After 14 days of treatment, the remaining half of animals in each group are sacrificed, and tumors are collected. Half of each tumor is processed for paraffin embedding, while the other half is used to prepare a single cell suspension for flow cytometry analyses. Samples for flow cytometry analyses are stained for CD8 and for markers of T-regulatory cells. Higher ratios of CD8+ to regulatory T cells may indicate enhanced efficacy via immune cell infiltration into tumors.

Example 13. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on lung tumor development

[00235] [²²⁵Ac]-anti-FGFR3 conjugates are tested in two mouse lung cancer xenograft models: Madison 109 (M109) and Lewis Lung Carcinoma cells, both of which are FGFR3-positive. 1x10⁶ Lewis Lung Carcinoma tumor cells are implanted subcutaneously into flanks of female C57BL/6 mice that are 8 to 12 weeks of age. Additionally, 1x10⁶ Madison 109 tumor cells are implanted subcutaneously into the flanks of CR female BALB/c mice that are 8 to 12 weeks of age.

[00236] When tumors reach an average size of 100-200 mm³, animals are pair matched and treatment is initiated. Each [²²⁵Ac]-anti-FGFR3 conjugate may be tested in a separate treatment group. A control group may include mice administered phosphate-buffered saline (PBS). Optionally, for comparison, one or more treatment groups are included in which mice are administered unconjugated anti-FGFR3 (cold antibody). Mice in all groups may be administered (intravenously or intraperitoneally) the relevant agents for their group according to a regular schedule, *e.g.*, weekly, twice a week, or thrice per week. In this example, mice are treated for one, two, or three weeks (see below).

[00237] Tumors are measured using calipers twice weekly, and the results are compared across treatment groups. Greater inhibition of tumor growth in [²²⁵Ac]-anti-FGFR3 conjugate treatment groups indicates increased efficacy.

[00238] After 7 days of treatment, some of the animals from each group are sacrificed, and tumors are collected. After 14 days of treatment, some of the remaining animals in each group are sacrificed, and tumors are collected. The remaining animals continue to be dosed until day 21, at which time they are sacrificed and their tumors are collected. Half of each tumor is processed for paraffin embedding, while the other half is frozen in Optimal Cutting Temperature (O.C.T.) compound.

Example 14. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on survival

[00239] Example 12 and/or 13 is performed, except that mice are not sacrificed and are instead monitored for tumor growth and survival over a period of at least months. Enhanced survival in [²²⁵Ac]-anti-FGFR3 conjugate treatment groups indicates enhanced therapeutic efficacy.

Example 15. Effects of [²²⁵Ac]-anti-FGFR3 conjugates on tumor growth and survival in a bladder cancer cell lines involving FGFR3 fusions

[00240] [²²⁵Ac]-anti-FGFR3 conjugates are tested in a tumor xenograft models based on one or more of the RT4, RT112, SW780, and UMUC-14 bladder cell lines, essentially as described in Example 8. RT4 and RT112 cells contain an FGFR3-TACC3 fusion, SW780 cells contain an FGFR- BAIAP2L1 fusion, and UMUC-14 harbors an FGFR3^{S249C}.

Example 16. Binding of DOTA-anti-FGFR3 conjugate to cancer cells expressing FGFR3

[00241] The present Example demonstrates binding of conjugated anti-FGFR3 to FGFR3-positive cancer cells at subnanomolar/picomolar K_d ranges.

[00242] An unlabeled DOTA-anti-FGFR3 conjugate was synthesized using 1) a pure R enantiomer of Compound C (see Example 4) (that is, an R-enantiomer of a (2R)-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]pentanedioic acid (R-DOTA-GA), connected through a PEG3 acid linker to a 2,3,5,6-tetrafluorophenol active ester) and 2) MFGR1877S (vofatamab), an anti-FGFR3 antibody. Binding of DOTA-anti-FGFR3 to FGFR3-positive cancer cell lines RT4 (bladder), RT112 (bladder), and HepG2 (liver) was assessed by flow cytometry.

[00243] **FIGs. 4A, 4B, and 4C** show the binding curves for RT4, RT112, and HepG2, respectively, and the corresponding binding affinities (K_d) are summarized in **Table 2**.

[00244] **Table 2. Binding affinities of anti-FGFR3 conjugate to FGFR3+ cancer cells**

	K _d [nM] RT4	K _d [nM] RT112	K _d [nM] HepG2
Anti-FGFR3 conjugate	0.448	0.248	0.279

Example 17. *In vivo* biodistribution of [¹⁷⁷Lu]-DOTA-anti-FGFR3 conjugate

[00245] A Balb/c nude / RT4 cell line xenograft mouse model was used to assess the *in vivo* biodistribution of a radiolabeled anti-FGFR3 conjugate. A [¹⁷⁷Lu]-DOTA-anti-FGFR3 conjugate was synthesized using a pure R enantiomer of Compound C (see Example 4), MFGR1877S (vofatamab), and lutetium-177.

[00246] Groups of tumor-bearing animals were injected intravenously with [¹⁷⁷Lu]-DOTA-anti-FGFR3. Doses contained about 23 microcuries (μCi) of activity on 2 μg (0.1 mg/kg) of antibody. Animals were euthanized at 4 h, 24 h, 48 h, 96 h, and 168 h after injection to determine levels of radioactivity in the blood, kidney, liver, lungs, spleen, skin, tumor, and tail (n = 3 per time point).

[00247] Results were expressed as the percentage injected dose per gram of tissue (% ID/g) and are depicted in **FIG. 5**. [¹⁷⁷Lu]-DOTA-anti-FGFR3 cleared rapidly from the blood and demonstrated transient uptake in the liver, lungs, and spleen. Tumor uptake was about 5% ID/g at all time points. Without wishing to be bound by any particular theory, the

observed level of tumor uptake could be attributable to the small size of the RT4 tumors (about 50 mm³).

Example 18. *In vivo* biodistribution of [¹⁷⁷Lu]-DOTA-anti-FGFR3 conjugate after pre-dosing with cold anti-FGFR3

[00248] The present Example demonstrates uptake of presently disclosed radioimmunoconjugates in tumor cells with lower levels of uptake in normal tissues.

[00249] A Balb/c nude / RT112 cell line xenograft mouse model was used to assess the *in vivo* biodistribution of [¹⁷⁷Lu]-DOTA-anti-FGFR3 after pre-dosing with cold (non-radiolabeled, unconjugated) anti-FGFR3 antibody.

[00250] Groups of tumor-bearing mice were injected intravenously with [¹⁷⁷Lu]-DOTA-anti-FGFR3. Doses contained about 23 microcuries (μCi) of activity on 2 μg (0.1 mg/kg) of antibody. Approximately three hours before administration of [¹⁷⁷Lu]-DOTA-anti-FGFR3, half of the mice were administered 100 μg cold anti-FGFR3 (vofatamab) by intraperitoneal injection. Animals were euthanized at 4 h, 24 h, 48 h, and 96 h after injection to determine levels of radioactivity in the blood, intestine (small and large), kidney and adrenal glands, liver and gall bladder, lungs, spleen, skin, bladder, urine, and tumor (n = 3 per time point).

[00251] Results were expressed as the % ID/g and are depicted in **FIGs. 6A** and **6B**. Pre-dosing with cold anti-FGFR3 reduced clearance of radioactivity from the blood, reduced uptake of [¹⁷⁷Lu]-DOTA-anti-FGFR3 in normal tissues, and increased uptake of [¹⁷⁷Lu]-DOTA-anti-FGFR3 in tumors.

Example 19. *In vivo* biodistribution of radiolabeled anti-FGFR3 conjugates co-dosed with cold anti-FGFR3

[00252] The present Example demonstrates uptake of presently disclosed radioimmunoconjugates in tumor cells with lower levels of uptake in normal tissues. Moreover, the present Example demonstrates that DOTA-anti-FGFR3 conjugates labeled with different radionuclides exhibit similar biodistribution profiles.

[00253] [¹¹¹In]-DOTA-anti-FGFR3 conjugate was synthesized using a pure R enantiomer of Compound C (see Example 4), MFGR1877S (vofatamab), and indium-111.

[00254] A Balb/c nude / RT112 cell line xenograft mouse model was used to assess the *in vivo* biodistribution of [¹⁷⁷Lu]-DOTA-anti-FGFR3 conjugate and [¹¹¹In]-DOTA-anti-FGFR3 conjugates when co-dosed with cold anti-FGFR3.

[00255] Groups of tumor-bearing mice were injected intravenously with [¹⁷⁷Lu]-DOTA-anti-FGFR3 at about 22 microcuries (μCi) of activity on 2 μg (0.1 mg/kg) of antibody. Mice were also co-administered 50, 100, or 200 μg of cold anti-FGFR3 via the same intravenous injection. Animals were euthanized at 24 h and 96 h after injection to determine levels of radioactivity in the blood, intestine, kidney, liver, lungs, spleen, skin, bladder, urine, and tumor (n = 3 per time point).

[00256] Results were expressed as the % ID/g and depicted in **FIGs. 7A-7C**. Co-dosing with 100 μg or 200 μg cold anti-FGFR3 reduced clearance of radioactivity from the blood, reduced uptake of [¹⁷⁷Lu]-DOTA-anti-FGFR3 in normal tissues, and increased uptake of [¹⁷⁷Lu]-DOTA-anti-FGFR3 in tumors.

[00257] A biodistribution study was also performed using [¹¹¹In]-DOTA-anti-FGFR3 co-dosed with 100 μg of cold anti-FGFR3, similarly as described for the [¹⁷⁷Lu]-DOTA-anti-FGFR3 co-dosing experiment in this Example. **FIGs. 8A** and **8B** show the results %ID/g in mice administered [¹⁷⁷Lu]-DOTA-anti-FGFR3 (**FIG. 8A**) or [¹⁷⁷Lu]-DOTA-anti-FGFR3 (**FIG. 8B**), each co-dosed with cold anti-FGFR3. Both [¹⁷⁷Lu]-DOTA-anti-FGFR3 and [¹¹¹In]-DOTA-anti-FGFR3 showed good tumor intake with about 34% - 37% ID/g at 96 h after dosing.

Example 20. Effects of [²²⁵Ac]-DOTA-anti-FGFR3 conjugate on tumor growth and survival in a bladder cancer xenograft model

[00258] The present Example demonstrates therapeutic efficacy of an [²²⁵Ac]-DOTA-anti-FGFR3 conjugate in a bladder cancer model.

[00259] A [²²⁵Ac]-DOTA-anti-FGFR3 conjugate was synthesized using a pure R enantiomer of Compound C (see Example 4), MFGR1877S (vofatamab), and actinium-225.

[00260] A Balb/c nude / RT112 cell line xenograft mouse model was used to assess the *in vivo* activity of [²²⁵Ac]-DOTA-anti-FGFR3 conjugate after pre-dosing with cold anti-FGFR3. Tumors were grown subcutaneously to about 150 mm³ in volume. Groups of tumor-bearing mice were injected intravenously with [²²⁵Ac]-DOTA-anti-FGFR3 (50 nCi, 100 nCi, 200 nCi, or 400 nCi doses), cold anti-FGFR3, or vehicle controls (n = 5 per group). Except

for mice in a control group, 3 hours before administration of [^{225}Ac]-DOTA-anti-FGFR3, mice were injected intraperitoneally with 100 μg cold anti-FGFR3. Relative tumor volume (**FIG. 9A**) and relative body weights (**FIG. 9B**) were evaluated up to 28 days after administration.

[00261] As shown in **FIG. 9A**, treatment with 200 nCi or 400 nCi [^{225}Ac]-DOTA-anti-FGFR3 significantly inhibited tumor growth. One mouse in the 400 nCi group lost 30% of its body weight and was sacrificed on Day 11. However, as shown in **FIG. 9B**, on average, mice in treatment groups did not demonstrate significant weight loss relative to mice in control groups, suggesting that the treatment was tolerated and that toxicity was limited.

Example 21. Effects of [^{225}Ac]-DOTA-anti-FGFR3 conjugates on tumor growth and survival in a bladder cancer xenograft model

[00262] The present Example demonstrates therapeutic efficacy of an [^{225}Ac]-DOTA-anti-FGFR3 conjugate in a bladder cancer model.

[00263] A Balb/c nude / RT112 cell line xenograft mouse model was used to assess the *in vivo* activity of [^{225}Ac]-DOTA-anti-FGFR3 conjugate with co-dosing of cold anti-FGFR3. Tumors were allowed to grow subcutaneously to about 150 mm^3 in volume. Groups of tumor bearing mice were injected intravenously with [^{225}Ac]-DOTA-anti-FGFR3 (50 nCi, 100 nCi, 200 nCi, or 400 nCi) co-dosed with 100 μg anti-FGFR3. Control groups received cold anti-FGFR3 only or a vehicle control. $n = 5$ per group. Relative tumor volume (**FIG. 10A**) and relative body weights (**FIG. 10B**) were evaluated up to 28 days after administration.

[00264] As shown in **FIG. 10A**, treatment with 200 nCi or 400 nCi [^{225}Ac]-DOTA-anti-FGFR3 significantly inhibited tumor growth, and treatment with lower doses (50-100 nCi) of [^{225}Ac]-DOTA-anti-FGFR3 resulted in some inhibition of tumor growth.

[00265] In the 400 nCi treatment group, two mice lost significant weight and were sacrificed, and the other three mice were not affected. However, mice in the other treatment groups did not demonstrate significant weight loss relative to mice in control groups. (See **FIG. 10B**.)

OTHER EMBODIMENTS

[00266] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features herein before set forth.

WHAT IS CLAIMED IS:

1. A radioimmunoconjugate comprising the following structure:



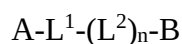
Formula I-a

wherein A is a chelating moiety or metal complex thereof,

wherein B is an FGFR3 targeting moiety, and

wherein L is a linker.

2. The radioimmunoconjugate of claim 1, wherein A is a metal complex of a chelating moiety.
3. The radioimmunoconjugate of claim 2, wherein the metal complex comprises a radionuclide.
4. The radioimmunoconjugate of claim 3, wherein the radionuclide is an alpha emitter.
5. The radioimmunoconjugate of claim 4, wherein the radionuclide is an alpha emitter selected from the group consisting of Astatine-211 (^{211}At), Bismuth-212 (^{212}Bi), Bismuth-213 (^{213}Bi), Actinium-225 (^{225}Ac), Radium-223 (^{223}Ra), Lead-212 (^{212}Pb), Thorium-227 (^{227}Th), and Terbium-149 (^{149}Tb), or a progeny thereof.
6. The radioimmunoconjugate of claim 5, wherein the radionuclide is ^{225}Ac or a progeny thereof.
7. The radioimmunoconjugate of claim 1, wherein L is has the structure $-L^1-(L^2)_n-$, as shown within Formula I-b:



Formula I-b

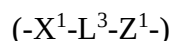
wherein

A is a chelating moiety or metal complex thereof;

B is an FGFR3 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 between 1 and 5 (inclusive); and
each L², independently, has the structure:



Formula III

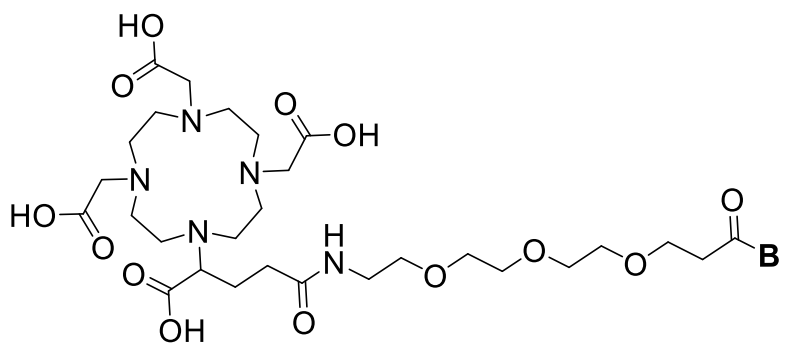
wherein

X¹ is C=O(NR¹), C=S(NR¹), OC=O(NR¹), NR¹C=O(O), NR¹C=O(NR¹), -CH₂PhC=O(NR¹), -CH₂Ph(NH)C=S(NR¹), O, or NR¹; and each R¹ independently is H, optionally substituted C₁-C₆ alkyl, optionally substituted C₁-C₆ heteroalkyl, or optionally substituted aryl or heteroaryl, in which C₁-C₆ alkyl can be substituted by oxo (=O), heteroaryl, or a combination thereof;

L³ is optionally substituted C₁-C₅₀ alkyl or optionally substituted C₁-C₅₀ heteroalkyl;
and

Z¹ is CH₂, C=O, C=S, OC=O, NR¹C=O, or NR¹, wherein R¹ is a hydrogen or optionally substituted C₁-C₆ alkyl or pyrrolidine-2,5-dione.

8. The radioimmunoconjugate of claim 7, wherein L³ is C₅-C₂₀ polyethylene glycol.
9. The radioimmunoconjugate of claim 7, or a pharmaceutically acceptable salt thereof wherein the radioimmunoconjugate comprises the following structure:



wherein B is an FGFR3 targeting moiety.

10. The radioimmunoconjugate of any one of claims 1-9, wherein the FGFR3 targeting moiety is at least 100 kDa in size.
11. The radioimmunoconjugate of any one of claims 1-10, wherein the FGFR3 targeting moiety is capable of binding to human FGFR3.

12. The radioimmunoconjugate of any one of claims 1-11, wherein the FGFR3 targeting moiety is capable of binding to wild type FGFR3, a mutant FGFR3, or both.
13. The radioimmunoconjugate of claim 12, wherein the mutant FGFR3 comprises a point mutation.
14. The radioimmunoconjugate of 12 or 13, wherein the mutant FGFR3 comprises an FGFR3 fusion.
15. The radioimmunoconjugate of any one of claims 1-14, wherein the FGFR3 targeting moiety comprises an antibody or antigen-binding fragment thereof.
16. The radioimmunoconjugate of claim 15, wherein the antibody or antigen-binding fragment thereof is humanized.
17. The radioimmunoconjugate of claim 15 or 16, wherein the antibody or antigen-binding fragment thereof comprises at least one complementarity determining region (CDR) selected from the group consisting of:
- CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence differing in 1 or 2 amino acids therefrom;
 - CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or an amino acid sequence differing in 1 or 2 amino acids therefrom;
 - CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4, or an amino acid sequence differing in 1 or 2 amino acids from SEQ ID NO: 3 or 4;
 - CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence differing in 1 or 2 amino acids therefrom;
 - CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence differing in 1 or 2 amino acids therefrom; or
 - CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7, or an amino acid sequence differing in 1 or 2 amino acids therefrom.

18. The radioimmunoconjugate of claim 17, wherein the antibody or antigen-binding fragment thereof comprises

- (i) a heavy chain variable domain comprising:
 - CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1;
 - CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2; and
 - CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3 or 4;
- and
- (ii) a light chain variable domain comprising:
 - CDR-L1 comprising the amino acid sequence of SEQ ID NO: 5;
 - CDR-L2 comprising the amino acid sequence of SEQ ID NO: 6; and
 - CDR-L3 comprising the amino acid sequence of SEQ ID NO: 7.

19. The radioimmunoconjugate of claim 17 or 18, wherein the antibody or antigen-binding fragment thereof comprises

- (i) a heavy chain variable domain having an amino acid sequence with at least 85% identity with the amino acid sequence of SEQ ID NO: 8; and
- (ii) a light chain variable domain having an amino acid sequence with at least 85% identity with the amino acid sequence of SEQ ID NO: 9.

20. The radioimmunoconjugate of claim 19, wherein the antibody or antigen-binding fragment thereof comprises

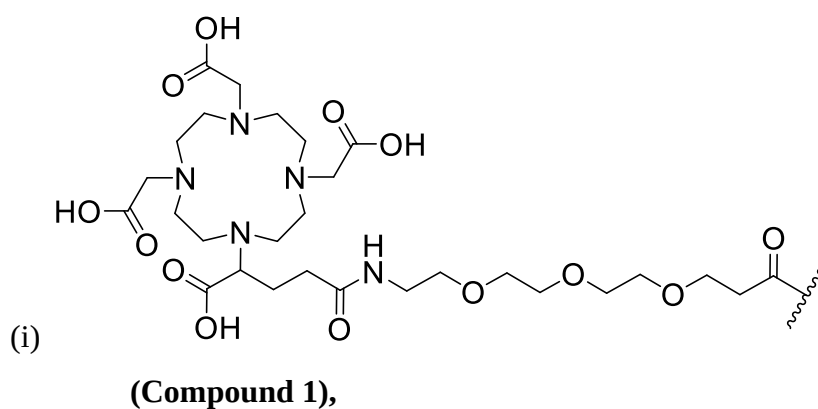
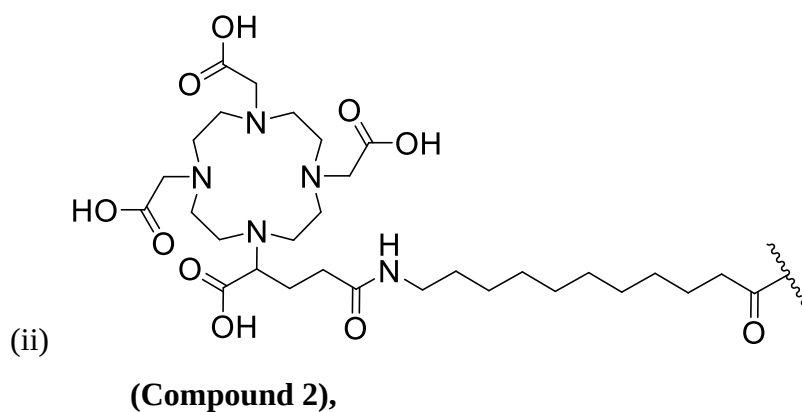
- (i) a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 8; and
- (ii) a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 9.

21. The radioimmunoconjugate of claim 20, wherein the antibody is MFGR1877S (vofatamab).

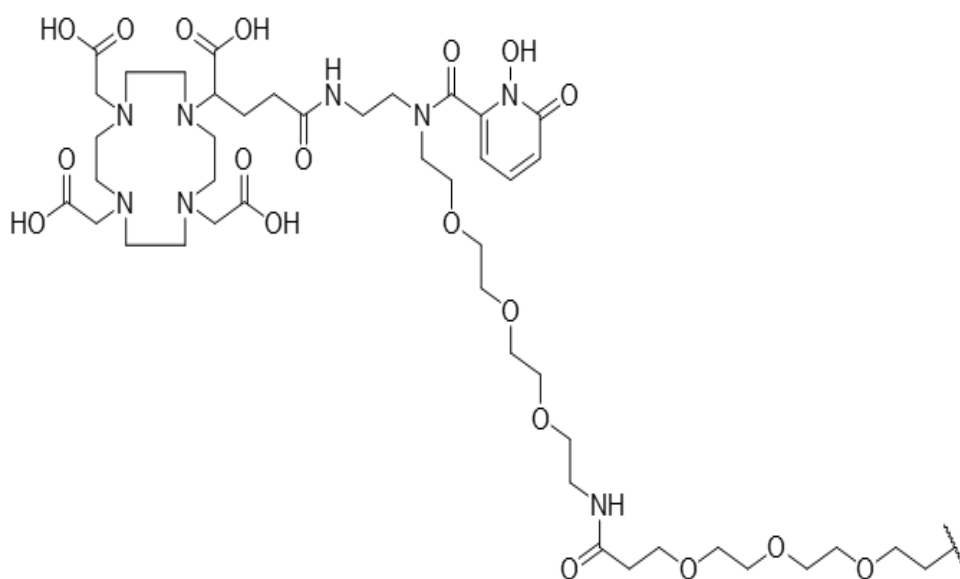
22. The radioimmunoconjugate of any one of claims 1-21, wherein, after administration of the radioimmunoconjugate or a composition thereof to a mammal, the proportion of radiation excreted by the intestinal routes, renal route, or both routes is at least 2-fold greater

than the proportion of radiation excreted by the same route(s) by a comparable mammal that has been administered a reference radioimmunoconjugate.

23. The radioimmunoconjugate of any one of claims 1-22, wherein A-L- is a metal complex of a compound selected from the group consisting of:

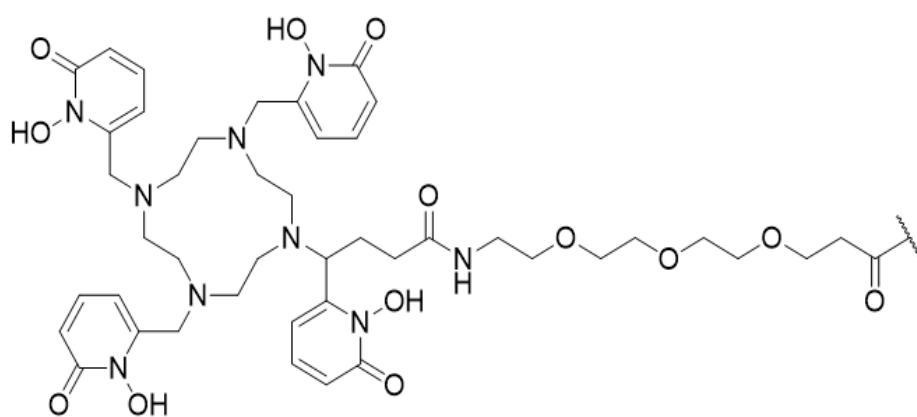


(iii)



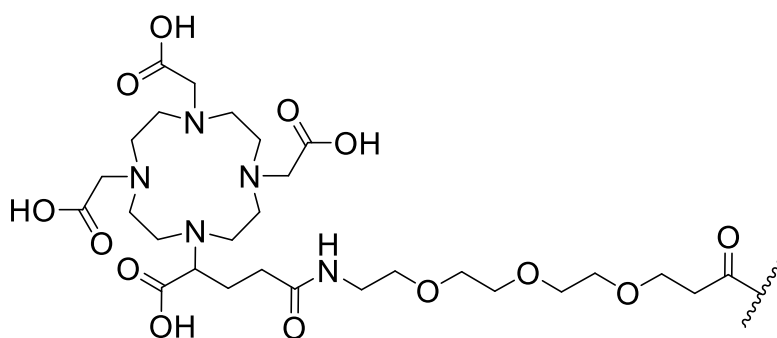
(Compound 3), and

(iv)



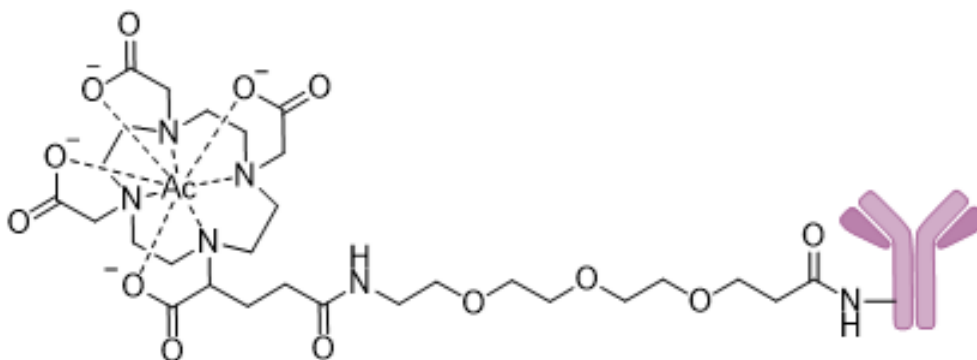
(Compound 4).

24. The radioimmunoconjugate of claim 23, wherein A-L- is a metal complex of:



(Compound 1)

25. The radioimmunoconjugate of claim 24, wherein said radioimmunoconjugate comprising the following structure:



wherein  is MFGR1877S (vofatamab), wherein the amine group NH- attached to the antibody shown above is from a lysine unit that is part of the antibody.

26. A pharmaceutical composition comprising the radioimmunoconjugate of any one of claims 1-25 and a pharmaceutically acceptable carrier.

27. Use of a radioimmunoconjugate of any one of claims 1-25 or a pharmaceutical composition thereof, in the manufacture of a medicament for treating cancer in a subject in need thereof.

28. The use of claim 27, wherein the subject is a human.

29. The use of claim 27 or 28, wherein the cancer is a solid tumor cancer.
30. The use of claim 29, wherein the solid tumor cancer is adrenocortical carcinoma, bladder cancer, breast cancer, cervical cancer, colorectal cancer, endometrial adenocarcinoma, Ewing's sarcoma, gallbladder carcinoma, glioma, head and neck cancer, liver cancer, lung cancer, neuroblastoma, neuroendocrine cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, salivary adenoid cystic cancer, or spermatocytic seminoma.
31. The use of claim 27 or 28, wherein the cancer is a liquid cancer or hematologic cancer.
32. The radioimmunoconjugate of any one of claims 1-25 or a pharmaceutical composition thereof, for use in therapy.