

(Yong and Brechbiel, Dalton Trans. 2001 June 21; 40(23)6068-6076). Failure of the chelator to stably bind a radionuclide creates the risk of reducing delivery of radiation to the tumour while increasing toxicity.

SUMMARY

5 The present disclosure provides antibodies that bind to a metal chelate comprising DOTAM and lead (Pb). DOTAM is able to chelate Pb in a stable manner, to form a Pb[DOTAM] complex.

10 In one aspect, the present disclosure refers to an antibody comprising an antigen binding site specific for DOTAM-lead (Pb) chelate, wherein said antigen binding site comprises at least: a) a heavy chain CDR1; b) heavy chain CDR2 comprising the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO:2), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 2, wherein these substitutions do not include Phe50, Asp56 and Tyr58,
15 and optionally also do not include Gly52 and/or Arg 54; c) heavy chain CDR3 comprising the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:3), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 3, wherein these substitutions do not include Glu95, Arg96, Asp97, Pro98, and optionally also do not include Ala100C, Tyr100D, and/or Pro100E and/or
20 optionally also do not include Tyr99; d) light chain CDR1 comprising the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO:4) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 4, wherein these substitutions do not include Tyr28 and Asp32; e) a light chain CDR2; and f) light chain CDR3 comprising the amino acid sequence LGGYDDESDTYG (SEQ ID NO:6) or a
25 variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 6, wherein these substitutions do not include Gly91, Tyr92, Asp93, Thr95c and Tyr96, wherein numbering is according to Kabat.

30 In another aspect, the present disclosure refers to an antibody as disclosed herein, wherein the antigen binding site comprises a heavy chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7 and SEQ ID NO 9, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 7 or SEQ ID NO: 9.

35 In another aspect, the present disclosure refers to an antibody as disclosed herein, wherein the antigen binding site comprises a light chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 10, or a variant thereof

comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 8 or 10.

5 In another aspect, the present disclosure refers to an antibody as disclosed herein, wherein the antigen binding site comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID No. 7 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO. 8.

10 In another aspect, the present disclosure refers to an antibody as disclosed herein, wherein the antigen binding site comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID No. 9 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO. 10.

15 In another aspect, the present disclosure refers to an antibody as disclosed herein, wherein the antigen binding site binds to the Pb-DOTAM chelate with a K_d value of 100pM, 50pM, 20pM, 10pM, 5pM, 1pM or less.

20 In another aspect, the present disclosure refers to an antibody as disclosed herein, wherein the antigen binding site binds to the Pb-DOTAM chelate and to a Bi-DOTAM chelate, and wherein the ratio of K_d values for the Bi-DOTAM chelate/Pb-DOTAM chelate is in the range of 0.1-10 or 1-10.

25 In another aspect, the present disclosure refers to an antibody as disclosed herein, which is a whole antibody, or which is an antibody fragment selected from the group consisting of an Fv, Fab, Fab', Fab'-SH, F(ab')₂, diabody, linear antibody, or single-chain antibody molecule.

30 In another aspect, the present disclosure refers to an antibody as disclosed herein, wherein the antibody is coupled to a moiety which binds specifically to a target antigen.

In another aspect, the present disclosure refers to an isolated
35 polynucleotide or an isolated set of polynucleotides encoding an antibody as disclosed herein.

In another aspect, the present disclosure refers to an expression vector or set of expression vectors comprising the polynucleotide or the set of
40 polynucleotides as disclosed herein.

In another aspect, the present disclosure refers to a prokaryotic or eukaryotic host cell comprising an isolated polynucleotide or an isolated set of polynucleotides as disclosed, optionally comprising the vector or set of vectors as disclosed herein.

5

In yet another aspect, the present disclosure refers to a method of producing an antibody as disclosed herein, comprising expressing the antibody from the host cell as disclosed herein.

10 The antibodies of the present disclosure bind to a chelate comprising DOTAM and Pb, where the Pb may be either a stable (non-radio) isotope or a radioisotope. Radioisotopes of lead are useful in applications such as radioimmunoimaging and radioimmunotherapy.

15 Preferably, antibodies of the present disclosure have extremely high affinity to the Pb-DOTAM chelate, in the pM to fM range.

The antibodies additionally bind to bismuth (Bi) chelated by DOTAM. ²¹²Pb is the parental radionuclide of ²¹²Bi and can serve as an *in vivo* generator of ²¹²Bi. The ability of the antibodies to bind chelated Bi as well as chelated Pb increases their utility in applications such as radioimmunotherapy, where a Bi isotope is generated as a decay product from a Pb isotope. In some examples, the antibodies may bind to both a Bi-DOTAM chelate and to a Pb-DOTAM chelate with very high affinity, in the pM to fM range.

20

25

Furthermore, the present antibodies are optionally or preferably selective for a Bi-DOTAM chelate and a Pb-DOTAM chelate as compared to other chelator-metal complexes, such as a Cu-DOTAM chelate.

30

In one example, the present disclosure provides an antibody comprising an antigen binding site specific for a Pb-DOTAM chelate, wherein said antigen binding site comprises a heavy chain comprising at least one, two or three heavy chain CDR sequences: wherein:

35 a) heavy chain CDR1 comprises the amino acid sequence GFSLSTYSMS (SEQ ID NO:1);

b) heavy chain CDR2 comprises the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO:2);

c) heavy chain CDR3 comprises the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:3);

40

and/or wherein the antigen binding site comprises a light chain comprising at least one, two or three light chain CDR sequences: wherein:

d) light chain CDR1 comprises the amino acid sequence QSSHSVYSDNDLA
(SEQ ID NO:4)

e) light chain CDR2 comprises the amino acid sequence QASKLAS (SEQ ID NO: 5)

f) light chain CDR3 comprises the amino acid sequence LGGYDDSDTYG
(SEQ ID NO:6).

In some examples, the antigen binding site comprises both a light chain and a heavy chain as defined above.

In another example, the present disclosure provides an antibody comprising an antigen binding site specific for a Pb-DOTAM chelate, wherein said antigen binding site comprises at least:

a) heavy chain CDR2 comprising the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO:2), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 2, wherein these substitutions do not include Phe50, Asp56 and/or Tyr58, and optionally also do not include Gly52 and/or Arg54;

b) heavy chain CDR3 comprising the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:3), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 3, wherein these substitutions do not include Glu95, Arg96, Asp97, Pro98, and optionally also do not include Ala100C, Tyr100D, and/or Pro100E and/or optionally also do not include Tyr99;

c) light chain CDR1 comprising the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO:4) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 4, wherein these substitutions do not include Tyr28 and Asp32;

d) light chain CDR3 comprising the amino acid sequence LGGYDDSDTYG (SEQ ID NO:6) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 6, wherein these substitutions do not include Gly91, Tyr92, Asp93, Thr95c and Tyr96.

Residue numbering is according to Kabat.

In some examples, the antibody additionally includes a heavy chain CDR1 and a light chain CDR2 which are optionally:

i) a heavy chain CDR1 comprising the amino acid sequence GFSLSTYSMS (SEQ ID NO:1) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 1;

ii) a light chain CDR2 comprising the amino acid sequence QASKLAS (SEQ ID NO: 5) or a variant thereof having at least 1, 2 or 3 substitutions in SEQ ID NO: 5, optionally not including Gln50.

5 In any examples of the present disclosure which relate to variants of a sequence comprising the CDRs as set out above, the protein may be invariant in one or more of the residues as set out above.

10 In some examples, the antibody according to the present disclosure binds to the same epitope, or an overlapping epitope, of a chelated radionuclide as that bound by an antibody disclosed herein.

In some examples, the antibody binds to the same epitope, or an overlapping epitope, as the epitope bound by Fab PRIT-0213 or PRIT-0214. For instance, 15 the antibody may bind to the same epitope, or an overlapping epitope, as:

i) an antibody having a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 7 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 8; or

20 i) an antibody having a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 9 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 10.

In one example, the antigen binding site comprises at least one, two, three four, five, or six CDRs selected from:

25 a) heavy chain CDR1 comprising the amino acid sequence GFSLSTYSMS (SEQ ID NO:1);

b) heavy chain CDR2 comprising the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO:2);

30 c) heavy chain CDR3 comprising the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:3);

d) light chain CDR1 comprising the amino acid sequence QSSHSVYSDNDLA (SEQ ID NO:4);

e) light chain CDR2 comprising the amino acid sequence QASKLAS (SEQ ID NO: 5);

35 f) light chain CDR3 comprising the amino acid sequence LGGYDDESPTYG (SEQ ID NO:6).

Optionally, the antibody in any of the examples described above is human, chimeric or humanized.

40

Optionally, the antigen binding site may comprise a heavy chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7 and SEQ ID NO 9, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 7 or SEQ ID NO: 9.

Optionally, the antigen binding site may comprise a light chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 10, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 8 or 10.

Optionally, the antigen binding site specific for the Pb-DOTAM chelate may comprise a heavy chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 7 or SEQ ID NO: 9, or a variant thereof as defined above, and a light chain variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 8 or SEQ ID NO: 10, or a variant thereof as defined above. For example, the antigen binding site specific for the Pb-DOTAM chelate may comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 7 or a variant thereof, and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 8 or a variant thereof. In another example, it may comprise a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO: 9 or a variant thereof and a light chain variable domain comprising the amino acid sequence of SEQ ID NO: 10 or a variant thereof.

The antibody may be in any format, including whole antibodies and antibody fragments. The antibody can be monospecific. In this form, the antibody finds utility, for instance, in sorting and purification schemes, e.g., to separate successfully radiolabelled moieties.

In some examples, the antibody that specifically binds to the Pb-DOTAM chelate is coupled to a cell binding agent/targeting moiety to produce a targeted agent. Such an agent is useful for instance, in pre-targeted radioimmunotherapy or pre-targeted radioimmunoimaging.

The coupling may preferably be by expression as a fusion polypeptide or protein. Fusion may be direct or via a linker. The fusion polypeptide or protein may be produced recombinantly, avoiding any need for conjugation chemistry.

In some examples, the targeting moiety (comprising the antigen binding site for the target) is an antibody or fragment thereof. That is, in some examples, the antibody described above may be in the form of a multispecific (e.g., bispecific) antibody, as discussed further below.

In another example, the present disclosure further relates to a multispecific antibody/antibody complex suitable for targeting a Pb-DOTAM chelate to a target cell.

Accordingly, in another example, the present disclosure relates to a bispecific or multispecific antibody that specifically binds both to the Pb-DOTAM chelate and to a target antigen, e.g., an antigen expressed on the surface of a target cell. The bispecific antibody comprises at least one antigen binding site specific for DOTAM-chelated lead and at least one antigen binding site for the target antigen.

In some example, the antigen binding site specific for the Pb-DOTAM chelate may be according to any of the examples described above.

The target antigen may be any antigen as discussed further herein, e.g., any tumour-specific antigen. In some examples it may be a protein or polypeptide expressed by a pathogen such as a prokaryote or a virus.

In some examples, the tumour-associated antigen may be CEA (carcinoembryonic antigen). That is, in some examples, the bispecific antibody may comprise at least one antigen binding site specific for the Pb-DOTAM chelate and at least one antigen binding site specific for CEA. CEA is advantageous in the context of the present disclosure because it is relatively slowly internalized, and thus a high percentage of the bispecific antibody will remain available on the surface of the cell after initial treatment, for binding to the radionuclide. Other low internalising targets/tumour associated antigens may also be preferred and are described herein. Other examples of tumour-associated antigen which may be useful in the present disclosure include CD20 or HER2.

In some examples, where the target antigen is CEA, the antigen binding site specific for CEA may comprise a heavy chain comprising at least one, two or three heavy chain CDRs, wherein:

d) heavy chain CDR1 comprises the amino acid sequence of SEQ ID NO: 11;

e) heavy chain CDR2 comprises the amino acid sequence of SEQ ID NO:
12;

f) heavy chain CDR3 comprises the amino acid sequence of SEQ ID NO:
13;

5 and/or the antigen binding site specific for CEA may comprise a light chain comprising at least one, two or three light chain CDRs, wherein:

a) light chain CDR1 comprises the amino acid sequence SEQ ID NO: 14;

b) light chain CDR2 comprises the amino acid sequence SEQ ID NO:15;

c) light chain CDR3 comprises the amino acid sequence SEQ ID NO: 16.

10 In some examples, the antigen binding site for CEA may comprise at least one, two, three, four, five, or six (i.e., all) of the CDRs selected from:

a) heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:
11;

15 b) heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:
12;

c) heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:
13;

d) light chain CDR1 comprising the amino acid sequence of SEQ ID NO:
20 14;

e) light chain CDR2 comprising the amino acid sequence of SEQ ID NO:
15;

f) light chain CDR3 comprising the amino acid sequence of SEQ ID NO:
16.

25 In some examples, the antigen binding site for CEA may comprise a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 17, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 17.

30 Optionally, the antigen binding site may comprise a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 18, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 18.

35 Optionally, the antigen binding site specific for CEA may comprise a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 17 or a variant thereof, and a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 18 or a variant thereof.

Various possible formats for the bispecific antibodies or multispecific antibodies are known in the art, including those described further herein. The antibodies of the disclosure may adopt any of these formats. For example, in some examples the bispecific antibody may be bivalent, trivalent or tetravalent.

It may be preferred that the present antibodies include an Fc region. The presence of an Fc region has benefits in the context of radioimmunotherapy and radioimaging, e.g. prolonging the protein's circulating half-life and/or resulting in higher tumour uptake than may be observed with smaller fragments.

In some examples, where the Fc region is present, it may be preferred that the Fc region is engineered to reduce effector function. This may include substitution of one or more of Fc region residues 234, 235, 238, 265, 269, 270, 297, 327 and/or 329, e.g., one or more of 234, 235 and/or 329. In some examples, the Fc region may be engineered to include the substitution of Pro 329 to Gly, Leu 234 to Ala and/or Leu 235 to Ala (numbering according to EU index).

Various formats of multispecific antibodies which include an Fc domain are known.

In one example, the bispecific or multispecific antibody may comprise i) an Fc domain, ii) at least one Fab, cross-Fab, Fv, scFab, or scFv fragment or a single domain antibody (VHH) comprising an antigen binding site specific for the Pb-DOTAM chelate and iii) at least one Fab, cross-Fab, Fv, scFab or scFv fragment or a single domain antibody (VHH) comprising an antigen binding site specific for the target antigen.

In some examples, it may be preferred that the bispecific or multispecific antibody is multivalent, for example bivalent, for the target antigen (e.g., the tumour-associated antigen). This has the advantage of increasing avidity.

In some examples, it may be preferred that the bispecific or multispecific antibody is monovalent for Pb-DOTAM. This reduces the risk of high molecular weight complex formation when a clearing agent is used (see further discussion below).

Thus, in some examples, the antibody may be trivalent: that is, bivalent for the target antigen and monovalent for Pb-DOTAM.

5 In one exemplary format, the bispecific or multispecific antibody may comprise a full-length antibody (e.g., an IgG) comprising a first and second antibody heavy chain and a first and second antibody light chain, wherein the first heavy chain and the first light chain assemble to form an antigen binding site for the first antigen, and wherein the second heavy chain and second light chain assemble to form an antigen binding site for the second antigen. Option-
10 ally, further antigen binding moieties may be fused e.g., via a polypeptide linker to the N- or C- terminus of the first and/or second heavy chain, to increase the valency for one or both antigens. For instance, a further antigen binding moiety for the first antigen may be fused to the N-terminus of one or both of the heavy chain
15 molecules.

In another exemplary format, the bispecific or multispecific antibody may comprise a full length antibody (e.g., an IgG) comprising an antigen binding site for a first antigen (e.g., which may be divalent for the first
20 antigen), and further comprises at least one antigen binding moiety specific for the second antigen. In various examples, the antigen binding moiety may be a Fab fragment, a crossover-Fab molecule, a scFab, an Fv molecule, an scFv, or a single domain antibody (VHH) or may be part of a second full-length antibody. For instance, the antibody may comprise a
25 full length antibody comprising an antigen binding site for the first antigen, and further comprise at least a second heavy chain variable domain and a second light chain variable domain which together form an antigen binding site for a second antigen. Either the first or the second antigen is the Pb-DOTAM chelate, and the other antigen is the target antigen.

30 In some examples of the formats herein, the second antigen is the Pb-DOTAM chelate and the first antigen is the target, e.g., a tumour-associated antigen (CEA, CD20 or ERBB2 in some examples).

35 In another exemplary format, the bispecific or multispecific antibody may comprise a full length antibody comprising an antigen binding site for the first antigen (e.g., which may be divalent for the first antigen), wherein the N- or C-terminus of one of the heavy chains is linked via a polypeptide linker to a first polypeptide and wherein the first polypeptide associates
40 with a second polypeptide to form a Fab or a cross-Fab comprising a binding site for the second antigen. For instance, this format may comprise:

i) a first polypeptide consisting of a VH domain and a CH1 domain, which is associated with a second polypeptide consisting of a VL and CL domain; or
ii) a first polypeptide consisting of a VL domain and a CH1 domain, which is associated with a second polypeptide consisting of a VH and CL domain;
5 or
iii) a first polypeptide consisting of a VH domain and a CL domain, which is associated with a second polypeptide consisting of a VL and CH1 domain;
such that the first and second polypeptide together form an antigen binding site for a second antigen.

10 In some examples, the fusion may be at the N-terminus of one of the heavy chains of the full length antibody.

In another particular example, the antibody may be a bispecific antibody
15 comprising:

a) a full length antibody specifically binding a first antigen and consisting of two antibody heavy chains and two antibody light chains;

b) a polypeptide consisting of

20 i) an antibody heavy chain variable domain (VH); or

ii) an antibody heavy chain variable domain (VH) and an antibody heavy constant domain (CH1); or

iii) an antibody heavy chain variable domain (VH) and an antibody light chain constant domain (CL);

wherein said polypeptide is fused with the N-terminus of the VH

25 domain via a peptide linker to the C-terminus of one of the two heavy chains of said full-length antibody;

c) a polypeptide consisting of

i) an antibody light chain variable domain (VL); or

30 ii) an antibody light chain variable domain (VL) and an antibody light chain constant domain (CL) or

iii) an antibody light chain variable domain (VL) and an antibody heavy chain constant domain (CH1);

wherein said polypeptide is fused with the N-terminus of the VL

35 domain via a peptide linker to the C-terminus of the other of the two heavy chains of said full-length antibody;

and wherein the antibody heavy chain variable domain of the peptide under (b) and the antibody light chain variable domain of the peptide under (c) together form an antigen-binding site to a second antigen.

In this format, either the first or the second antigen may be the Pb-DOTAM chelate. The other will be the target antigen e.g., a tumour-associated antigen.

- 5 In some examples, the second antigen is the Pb-DOTAM chelate and the first antigen is the target, e.g., a tumour-associated antigen (CEA, CD20 or ERBB2 in some examples).

10 The antibody described above may be trivalent. In another possible example, further antigen binding moieties may be fused to increase the valency for one or both antigens, as discussed further herein.

15 Optionally said linker (and any linker as discussed herein) may be a peptide of at least 5 amino acids, preferably between 25 and 50 amino acids. The linker may be a rigid linker or a flexible linker. In some examples, it is a flexible comprising or consisting of Thr, Ser, Gly and/or Ala residues. For example, it may comprise or consist of Gly and Ser residues. In some examples it may have a repeating motif such as (Gly-Gly-Gly-Gly-Ser)_n, where n is for instance 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In
20 some examples, the linker may be or may comprise the sequence GGGGSGGGSGGGSGGGGS (SEQ ID NO: 26). Other linkers may be used and could be identified by the skilled person.

25 Further details of this antibody format are provided in WO2010/115589 A1 (Roche Glycart AG), which is incorporated by reference herein in its entirety.

Optionally,

- 30 i) in the constant domain CL of the first light chain of the full length antibody under a) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred example independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the first heavy chain under a) the amino acid at position 147 or the amino acid at position 213
35 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index); or
ii) in the constant domain CL of the second light chain under b) the amino acid at position 124 is substituted independently by lysine (K), arginine (R) or histidine (H) (numbering according to Kabat) (in one preferred
40 example independently by lysine (K) or arginine (R)), and in the constant domain CH1 of the second heavy chain under b) the amino acid at position

147 or the amino acid at position 213 is substituted independently by glutamic acid (E) or aspartic acid (D) (numbering according to Kabat EU index).

5 In one example, the bispecific antibody of the present disclosure may have the trivalent structure as set out above, and may comprise:

a) a full length antibody specifically binding CEA and consisting of two antibody heavy chains and two antibody light chains;

10 wherein the heavy chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 450 (inclusive, using sequential numbering) of SEQ ID NO: 22 or 23 (i.e., to the portion of the sequence preceding the linker); and wherein the light chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO 21; 15 and/or

b) a polypeptide consisting of

i) an antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable domain of SEQ ID NO: 7; or 20 ii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable of SEQ ID NO: 7 and an antibody heavy chain constant domain (CH1); or 25 iii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable of SEQ ID NO: 7 and an antibody light chain constant domain; wherein said polypeptide is fused with the N-terminus of the VH domain via a peptide linker to the C-terminus of one of the two heavy 30 chains of said full-length antibody; and/or

c) a polypeptide consisting of

i) an antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable domain of SEQ ID NO: 8; or 35 ii) said antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable of SEQ ID NO: 8 and an antibody light chain constant domain (CL); or iii) said antibody light chain variable domain (VL) having at least 40 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light

chain variable of SEQ ID NO: 8 and an antibody heavy chain constant domain (CH1);

wherein said polypeptide is fused with the N-terminus of the VL

domain via a peptide linker to the C-terminus of the other of the two

5 heavy chains of said full-length antibody;

wherein the antibody heavy chain variable domain of the peptide under (b)

and the antibody light chain variable domain of the peptide under (c)

together form an antigen-binding site to the Pb-DOTAM chelate.

10 In one example, one of the heavy chains of the full length antibody comprises the so-called "knob mutations" (T366W and optionally one of S354C or Y349C, preferably S354C) and the other comprises the so-called "hole mutations" (T366S, L368A and Y407V and optionally Y349C or S354C, preferably Y349C) (see, e.g., Carter, P. et al., Immunotechnol. 2 (1996)
15 73) according to EU index numbering.

In some examples, the two antibody heavy chains under (a) comprise i) a first antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 450

20 (inclusive, using sequential numbering) of SEQ ID NO: 23 (i.e., the sequence preceding the linker), and which has C at position 349, S at position 366, A at position 368 and V at position 407 (EU numbering); and ii) a second antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1-
25 450 (inclusive, using sequential numbering) of SEQ ID NO: 22 which has C at position 354 and W at position 366 (EU numbering).

The herein mentioned "fixed" residues are "knob-into-hole" mutations in CH3 or are other residues such as disulphide bridge forming residues, which

30 pair with corresponding residues on CH3 of the other heavy chain to favour the formation of the desired molecule. Possible residues which may be present on the other heavy chain can be derived from the sequences of table 2 (e.g., sequences 19 and 20 or 22 and 23). For instance, in one example if the first antibody heavy chain has C at position 349, then the second
35 antibody heavy chain has C at 354.

Optionally, the linker is as described above.

In another example, the bispecific antibody of the present disclosure may
40 have the trivalent structure as set out above, and may comprise:

a) a full length antibody specifically binding CEA and consisting of two antibody heavy chains and two antibody light chains;

wherein the heavy chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1 to 450 of SEQ ID NO: 19 or 20 (inclusive and based on sequential numbering: i.e., the sequence preceding the linker) and wherein the light chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO: 21;

b) a polypeptide consisting of

i) an antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable domain of SEQ ID NO: 9; or
ii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable of SEQ ID NO: 9 and an antibody heavy chain constant domain (CH1); or
iii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable of SEQ ID NO: 9 and an antibody light chain constant domain;

wherein said polypeptide is fused with the N-terminus of the VH domain via a peptide linker to the C-terminus of one of the two heavy chains of said full-length antibody;

c) a polypeptide consisting of

i) an antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable domain of SEQ ID NO: 10; or
ii) said antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable of SEQ ID NO: 10 and an antibody light chain constant domain (CL), or

iii) said antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable of SEQ ID NO: 10 and an antibody heavy chain constant domain (CH1),

wherein said polypeptide is fused with the N-terminus of the VL domain via a peptide linker to the C-terminus of the other of the two heavy chains of said full-length antibody;

and wherein the antibody heavy chain variable domain of the peptide

under (b) and the antibody light chain variable domain of the peptide under (c) together form an antigen-binding site to the Pb-DOTAM chelate.

As described above, in any of the above examples, one of the heavy chains of the full length antibody may comprise the so-called "knob mutations" (T366W and optionally one of S354C or Y349C, preferably S354C) and the other comprises the so-called "hole mutations" (T366S, L368A and Y407V and optionally Y349C or S354C, preferably Y349C) (see, e.g., Carter, P. et al., Immunotechnol. 2 (1996) 73) according to EU index numbering.

In one example, the two antibody heavy chains under (a) may comprise i) a first antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 450 of SEQ ID NO: 22, which has C at position 354 and W at position 366 (EU numbering); and

ii) a second antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 450 of SEQ ID NO: 23 and which has C at position 349, S at position 366, A at position 368 and V at position 407 (EU numbering).

Optionally, the linker is as described above.

In another example, the bispecific antibody comprises:

i) a first heavy chain having an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 22,

ii) a second heavy chain having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 23,

iii) two antibody light chains having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO: 21.

In yet another example, the bispecific antibody is the molecule referred to herein as PRIT-0213, comprising

i) a first heavy chain having the amino acid sequence of SEQ ID NO: 22;

ii) a second heavy chain having the amino acid sequence of SEQ ID NO: 23; and

iii) two antibody light chains having the amino acid sequence of SEQ ID NO: 21.

In another example, the bispecific antibody comprises:

i) a first heavy chain having an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 19,

5 ii) a second heavy chain having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 20,

iii) two antibody light chains having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO: 21.

10 In yet another example, the bispecific antibody is the molecule referred to herein as PRIT-0214, comprising

i) a first heavy chain having the amino acid sequence of SEQ ID NO: 19;

ii) a second heavy chain having the amino acid sequence of SEQ ID NO: 20; and

15 iii) two antibody light chains having the amino acid sequence of SEQ ID NO: 21.

In one example, the bispecific antibody of the present disclosure may have the trivalent structure as set out above, and may comprise:

20 a) a full length antibody specifically binding ERBB2 and consisting of two antibody heavy chains and two antibody light chains;

wherein the heavy chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 449 (inclusive, using sequential numbering) of SEQ ID NO: 36 or 37 (i.e., to the portion of the sequence preceding the linker);

25 and wherein the light chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO 38; and/or

b) a polypeptide consisting of

30 i) an antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable domain of SEQ ID NO: 7 or 9 (in some examples, preferably 7); or

ii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable of SEQ ID NO: 7 or 9 (in some examples, preferably 7) and an antibody heavy chain constant domain (CH1); or

35 iii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable of SEQ ID NO: 7 or 9 (in some examples, preferably 7) and an antibody light chain constant domain (CL),

wherein said polypeptide is fused with the N-terminus of the VH domain via a peptide linker to the C-terminus of one of the two heavy chains of said full-length antibody; and/or

c) a polypeptide consisting of

- 5 i) an antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable domain of SEQ ID NO: 8 or 10 (in some examples, preferably 8); or
- 10 ii) said antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable of SEQ ID NO: 8 or 10 (in some examples, preferably 8) and an antibody light chain constant domain (CL); or
- 15 iii) said antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable of SEQ ID NO: 8 or 10 (in some examples, preferably 8) and an antibody heavy chain constant domain (CH1);

wherein said polypeptide is fused with the N-terminus of the VL domain via a peptide linker to the C-terminus of the other of the two heavy chains of said full-length antibody;

- 20 wherein the antibody heavy chain variable domain of the peptide under (b) and the antibody light chain variable domain of the peptide under (c) together form an antigen-binding site to the Pb-DOTAM chelate.

In one example, one of the heavy chains of the full length antibody comprises the so-called "knob mutations" (T366W and optionally one of S354C or Y349C, preferably S354C) and the other comprises the so-called "hole mutations" (T366S, L368A and Y407V and optionally S354C or Y349C, preferably Y349C) (see, e.g., Carter, P. et al., Immunotechnol. 2 (1996) 73) according to EU index numbering.

- 30 In some examples, the two antibody heavy chains under (a) comprise i) a first antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 449 (inclusive, using sequential numbering) of SEQ ID NO: 37 (i.e., the sequence preceding the linker), and which has C at position 349, S at
- 35 position 366, A at position 368 and V at position 407 (EU numbering); and
- ii) a second antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 449 (inclusive, using sequential numbering) of SEQ ID NO: 36 which has C at position 354 and W at position 366 (EU numbering).

40

Optionally, the linker is as described above.

In another example, the bispecific antibody comprises:

i) a first heavy chain having an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 36,

ii) a second heavy chain having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 37,

iii) two antibody light chains having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO: 38.

In yet another example, the bispecific antibody is the molecule referred to herein as P1AD9827, comprising

i) a first heavy chain having the amino acid sequence of SEQ ID NO: 36;

ii) a second heavy chain having the amino acid sequence of SEQ ID NO: 37; and

iii) two antibody light chains having the amino acid sequence of SEQ ID NO: 38.

In another example, the bispecific antibody of the present disclosure may have the trivalent structure as set out above, and may comprise:

a) a full length antibody specifically binding CD20 and consisting of two antibody heavy chains and two antibody light chains;

wherein the heavy chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98,

99 or 100% identity to the heavy chain of amino acids 1- 448 (inclusive, using sequential numbering) of SEQ ID NO: 47 or 48 (i.e., to the portion of the sequence preceding the linker);

and wherein the light chains have at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO 49; and/or

b) a polypeptide consisting of

i) an antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable domain of SEQ ID NO: 7 or 9 (in some examples, preferably 7); or

ii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable domain of SEQ ID NO: 7 or 9 (in some examples, preferably 7) and an antibody heavy chain constant domain (CH1),

iii) said antibody heavy chain variable domain (VH) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain variable

domain of SEQ ID NO: 7 or 9 (in some examples, preferably 7) and an antibody light chain constant domain (CL),

wherein said polypeptide is fused with the N-terminus of the VH domain via a peptide linker to the C-terminus of one of the two heavy chains of said full-length antibody; and/or

c) a polypeptide consisting of

- 5 i) an antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable domain of SEQ ID NO: 8 or 10 (in some examples, preferably 8); or
ii) said antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable
10 domain of SEQ ID NO: 8 or 10 (in some examples, preferably 8) and an antibody light chain constant domain; or iii) said antibody light chain variable domain (VL) having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain variable domain of SEQ ID NO: 8 or 10 (in some examples, preferably 8) and an antibody heavy chain constant
15 domain; ,

wherein said polypeptide is fused with the N-terminus of the VL domain via a peptide linker to the C-terminus of the other of the two heavy chains of said full-length antibody;

- wherein the antibody heavy chain variable domain of the peptide under (b)
20 and the antibody light chain variable domain of the peptide under (c) together form an antigen-binding site to the Pb-DOTAM chelate.

In one example, one of the heavy chains of the full length antibody comprises the so-called "knob mutations" (T366W and optionally one of S354C
25 or Y349C, preferably S354C) and the other comprises the so-called "hole mutations" (T366S, L368A and Y407V and optionally Y349C or S354C, preferably Y349C) (see, e.g., Carter, P. et al., Immunotechnol. 2 (1996) 73) according to EU index numbering.

- 30 In some examples, the two antibody heavy chains under (a) comprise i) a first antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 448 (inclusive, using sequential numbering) of SEQ ID NO: 48 (i.e., the sequence preceding the linker), and which has C at position 349, S at
35 position 366, A at position 368 and V at position 407 (EU numbering); and ii) a second antibody heavy chain which has at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of amino acids 1- 448 (inclusive, using sequential numbering) of SEQ ID NO: 47 which has C at position 354 and W at position 366 (EU numbering).

40

Optionally, the linker is as described above.

In another example, the bispecific antibody comprises:

i) a first heavy chain having an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 47,

ii) a second heavy chain having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the heavy chain of SEQ ID NO: 48,

iii) two antibody light chains having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to the light chain of SEQ ID NO: 49.

In yet another example, the bispecific antibody is the molecule referred to herein as P1AD9826, comprising

i) a first heavy chain having the amino acid sequence of SEQ ID NO: 47;

ii) a second heavy chain having the amino acid sequence of SEQ ID NO: 48; and

iii) two antibody light chains having the amino acid sequence of SEQ ID NO: 49.

In a further example, the present disclosure relates to a polynucleotide or set of polynucleotides encoding any of the antibodies described herein. In further examples, the present disclosure relates to a vector or set of expression vectors comprising said polynucleotide or polynucleotides, optionally an expression vector. In further objects the present disclosure relates to a prokaryotic or eukaryotic host cell comprising a vector of the present disclosure. In addition a method of producing an antibody comprising culturing the host cell so that the antibody is produced is provided.

Bispecific or multispecific antibodies as described herein may find use in a variety of applications, including therapeutic and diagnostic applications, such as pre-targeted radioimmunotherapy and pre-targeted radioimmunoimaging.

Thus, in a further example the present disclosure relates to any bispecific or multispecific antibody as described herein for use in pre-targeted radioimaging. In such examples, the chelated Pb is preferably ²⁰³Pb.

A method of targeting a radioisotope to a tissue or organ for imaging may comprise:

i) administering to the subject a multispecific or bispecific antibody as described herein, wherein the antibody binds to the target antigen and localises to the surface of a cell expressing the target antigen; and

5 ii) subsequently administering a Pb radionuclide chelated with DOTAM or a functional variant thereof to the individual, wherein the Pb radionuclide chelated with DOTAM or said functional variant thereof binds to the antibody localised to the surface of the target cell.

10 Optionally, between steps (i) and (ii) a clearing agent is administered, wherein the clearing agent binds to the antigen binding site specific for the Pb-DOTAM chelate. The clearing agent blocks the antigen binding site for Pb-DOTAM, preventing circulating antibody from binding to the chelated Pb radionuclide. Alternatively or additionally, the clearing agent may
15 increase the rate of clearance of antibody from the body. The "clearing agent" may alternatively be referred to as a "blocking agent": these terms can be substituted for each other in the discussion that follows.

The clearing agent may comprise a complex of a metal ion with DOTAM or a
20 functional variant thereof, where said complex is recognised by the antigen binding site for Pb-DOTAM. Preferably, the metal ion is a stable isotope or essentially stable isotope. By "stable isotope" we mean an isotope that does not undergo radioactive decay. By "essentially stable isotope" we mean an isotope that undergoes radioactive decay with a very long half-
25 life, making it safe for use. Preferably, the metal ion is selected from ions of Pb, Ca and Bi. For example, the clearing agent may comprise a stable isotope of Pb complexed with DOTAM or a functional variant thereof, Ca complexed with DOTAM or a functional variant thereof, or ²⁰⁹Bi (an essentially stable isotope with a half-life of 1.9×10^{19} years) complexed
30 with DOTAM or a functional variant thereof. The Pb may be naturally occurring lead, which is a mixture of the stable (non-radioactive) isotopes ²⁰⁴Pb, ²⁰⁶Pb, ²⁰⁷Pb and ²⁰⁸Pb.

The DOTAM or functional variant thereof is conjugated to a clearing moiety.
35 This moiety provides the clearing agent with low uptake into the tumour, e.g., by virtue of its size and/or high hydrodynamic radius. Suitable clearing moieties are discussed further below. In some examples, the clearing agent may comprise DOTAM or a functional variant thereof conjugated to dextran or a derivative thereof.

In another example of the imaging methods, the multispecific or bispecific antibody may be bound to the chelated Pb radionuclide at the time of administration.

5 Optionally, in any example, the method may further comprise:

 iii) imaging the tissue or organ where the Pb radionuclide chelated with DOTAM or the functional variant thereof has localized.

10 In some examples, the target antigen may be a tumour-specific antigen and the imaging may be a method of imaging a tumour or tumours.

15 In a still further example, the present disclosure relates to antibody as described herein for use in a method of pre-targeted radioimmunotherapy. In such examples, the chelated Pb is preferably ²¹²Pb.

 A method of targeting a radioisotope to a tissue or organ for therapy may comprise:

20 i) administering to the subject a multispecific or bispecific antibody as described herein, wherein the antibody binds to the target antigen and localizes to the surface of a cell expressing the target antigen; and

25 ii) subsequently administering a Pb radionuclide chelated with DOTAM or with a functional variant thereof, wherein the Pb radionuclide chelated with DOTAM or a functional variant thereof binds to the antibody localised to the surface of the cell. Optionally, between steps (i) and (ii) a clearing agent is administered as described above.

30 In some examples, the target antigen is a tumour-associated antigen and the method is a method of treating cancer. In other examples the target antigen may be an antigen associated with infection, e.g., a protein expressed by a prokaryote or by a virus-infected cell.

In some examples, the antibodies described herein may be administered as part of a combination therapy. For example, they may be administered in combination with one or more radiosensitizers and/or chemotherapeutic agents: the radiosensitizer or chemotherapeutic agent and the antibody may be administered simultaneously or sequentially, in either order.

The methods of radioimaging and radioimmunotherapy described herein may optionally be combined, e.g., by administering the antibody and both ^{203}Pb -DOTAM and ^{212}Pb -DOTAM, e.g., as a mixture.

The present disclosure also further relates to pharmaceutical compositions comprising an antibody of the present disclosure and a pharmaceutically acceptable excipient.

In further examples, the present disclosure relates to a kit comprising an antibody of the present disclosure and one, two, three, four or all of:

- i) a pharmaceutically acceptable excipient;
- ii) a Pb radionuclide chelated by DOTAM or a functional variant thereof;
- iii) a clearing agent as described herein;
- iv) one or more additional chemotherapeutic agents; and/or
- v) one or more radiosensitizers.

In a still further example of the disclosure, the present inventors have developed a novel clearing agent. Such a clearing agent may be used in any of the methods of diagnosis, imaging or treatment as described herein.

In one example the present disclosure relates to a clearing agent comprising dextran or a derivative thereof conjugated to a chelator selected from DOTAM and a functional variant of DOTAM, wherein said chelator forms a complex with Pb, Zn, Ca or Bi. The clearing agent typically includes a chelated metal ion, such as a Pb, Zn, Ca or Bi ion.

The clearing agent may comprise aminodextran coupled to DOTAM or a functional variant of DOTAM. For example, the clearing agent may comprise DOTAM coupled to aminodextran with isothiocyanate coupling (for example, a compound obtainable by reacting aminodextran with *p*-SCN-Bn-TCMC).

One potential difficulty with the use of clearing agents is the possibility that they may enter tumours, negatively affecting subsequent binding of radioligands.

The present inventors have further found that good clearance from the blood can be achieved together with low clearing agent penetration into tumours, when a dextran-based clearing agent is used which has i) a high average molecular weight and ii) has been subject to a molecular weight cut-off, such that fragments below a certain size have been removed.

Thus, a preferred clearing agent may be one in which i) the average molecular weight of the dextran or derivative thereof in the clearing agent is 200-800kDa, optionally greater than 300, 350, 400 or 450 kDa, and optionally less than 700, 650, 600 or 550kDa, optionally about 500kDa, and ii) dextran, dextran derivatives or clearing agents of less than a specified molecular weight cut-off have been removed, wherein the molecular weight cut-off is 50kDa or above, 100kDa or above or 200kDa or above, optionally in the range 50kDa-250kDa or 50kDa-200kDa, optionally 100kDa-200kDa and optionally about 100kDa, 150kDa or 200kDa.

In a further example, the present disclosure relates to methods of preparing the clearing agent, and to use of the clearing agents in methods of radioimmunotherapy or radioimmunoimaging. These and other examples of the disclosure are discussed further below.

FIGURES

Figure 1 shows a schematic representation of a possible bispecific antibody format. This format comprises two antigen-binding sites for one target (A) and one antigen-binding site for a second target (B) (2:1 format).

Figure 2 shows the structure of PRIT-0213 in complex with Pb-DOTAM.

Figure 3 shows the view on the interaction site, of PRIT-0213 in complex with Pb-DOTAM, numbered according to Kabat.

Figure 4 shows distribution of ^{212}Pb 24 h after injection of radiolabeled DOTAM (%ID/g $\pm$ SD, n = 3). PRIT-0206, -0207, -0208, and -0165 target T84.66, whereas PRIT-0186, -0187, and -0156 target CH1A1A. PRIT-0175 is a non-CEA-binding control.

5 Figure 5 shows distribution of ^{203}Pb expressed as counts per minute (CPM) 96 h after injection of PRIT antibodies pre-bound with radiolabeled DOTAM (CPM $\pm$ SD, n = 3). PRIT-0205, -0206, -0207, -0208, and -0209 are fully humanized constructs, whereas PRIT-0165 and -0175 are positive and negative controls, respectively.

10 Figure 6 shows accumulation/clearance of ^{203}Pb -DOTAM-bsAb in BxPC3 tumors and blood expressed as CPM $\pm$ SD (n = 3) at various time points after injection of PRIT antibodies pre-bound with radiolabeled DOTAM. PRIT-0206 is a fully humanized version of PRIT-0165; PRIT-0175 is a non-CEA-binding control.

CLAIMS

1. An antibody comprising an antigen binding site specific for DOTAM-lead (Pb) chelate, wherein said antigen binding site comprises at least:
- a) a heavy chain CDR1;
 - 5 b) heavy chain CDR2 comprising the amino acid sequence FIGSRGDTYYASWAKG (SEQ ID NO:2), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 2, wherein these substitutions do not include Phe50, Asp56 and Tyr58, and optionally also do not include Gly52 and/or Arg 54;
 - 10 c) heavy chain CDR3 comprising the amino acid sequence ERDPYGGGAYPPHL (SEQ ID NO:3), or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 3, wherein these substitutions do not include Glu95, Arg96, Asp97, Pro98, and optionally also do not include Ala100C, Tyr100D, and/or Pro100E and/or optionally also do not include Tyr99;
 - 15 d) light chain CDR1 comprising the amino acid sequence QSSHSVYSNDLA (SEQ ID NO:4) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 4, wherein these substitutions do not include Tyr28 and Asp32;
 - e) a light chain CDR2; and
 - f) light chain CDR3 comprising the amino acid sequence LGGYDDSDTYG
20 (SEQ ID NO:6) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 6, wherein these substitutions do not include Gly91, Tyr92, Asp93, Thr95c and Tyr96,
wherein numbering is according to Kabat.
- 25 2. The antibody of claim 1, wherein:
- i) the heavy chain CDR1 comprises the amino acid sequence GFSLSTYSMS (SEQ ID NO:1) or a variant thereof having up to 1, 2, or 3 substitutions in SEQ ID NO: 1, optionally conservative substitutions; and/or
 - ii) the light chain CDR2 comprises the amino acid sequence QASKLAS
30 (SEQ ID NO: 5) or a variant thereof having at least 1, 2 or 3 substitutions in SEQ ID NO: 5, optionally conservative substitutions.
3. The antibody according any preceding claim, wherein the antigen binding site comprises at least one, two, three, four, five, or six CDRs
35 selected from:
- a) heavy chain CDR1 comprising the amino acid sequence GFSLSTYSMS (SEQ ID NO:1)
 - b) heavy chain CDR2 comprising the amino acid sequence FIGSRGDTYYASWAKWG (SEQ ID NO:2)

c) heavy chain CDR3 comprising the amino acid sequence
ERDPYGGGAYPPHL (SEQ ID NO:3)

d) light chain CDR1 comprising the amino acid sequence QSSHSVYSDNDLA
(SEQ ID NO:4)

5 e) light chain CDR2 comprising the amino acid sequence QASKLAS (SEQ
ID NO: 5)

f) light chain CDR3 comprising the amino acid sequence LGGYDDESPTYG
(SEQ ID NO:6).

10 4. An antibody according to claim 1, which binds to the same epitope, or
an overlapping epitope, of the Pb-DOTAM chelate as:

i) an antibody having a heavy chain variable domain comprising an
amino acid sequence of SEQ ID NO: 7 and a light chain variable domain
comprising an amino acid sequence of SEQ ID NO: 8; or

15 i) an antibody having a heavy chain variable domain comprising an
amino acid sequence of SEQ ID NO: 9 and a light chain variable domain
comprising an amino acid sequence of SEQ ID NO: 10.

20 5. The antibody according to claim 1, which is human, chimeric or
humanized.

6. An antibody according to claim 1, wherein the antigen binding site
comprises a heavy chain variable domain comprising an amino acid sequence
selected from the group consisting of SEQ ID NO: 7 and SEQ ID NO 9, or a
25 variant thereof comprising an amino acid sequence having at least 90, 91,
92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 7 or SEQ ID NO:
9.

7. An antibody according to claim 1, wherein the antigen binding site
30 comprises a light chain variable domain comprising an amino acid sequence
selected from the group consisting of SEQ ID NO: 8 and SEQ ID NO: 10, or a
variant thereof comprising an amino acid sequence having at least 90, 91,
92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 8 or 10.

35 8. An antibody according to claim 1, wherein the antigen binding site
comprises a heavy chain variable domain comprising an amino acid sequence
of SEQ ID No. 7 and a light chain variable domain comprising an amino acid
sequence of SEQ ID NO. 8.

40 9. An antibody according to claim 1, wherein the antigen binding site
comprises a heavy chain variable domain comprising an amino acid sequence

of SEQ ID No. 9 and a light chain variable domain comprising an amino acid sequence of SEQ ID NO. 10.

10. An antibody according to claim 1, wherein the antigen binding site binds to the Pb-DOTAM chelate with a K_d value of 100pM, 50pM, 20pM, 10pM, 5pM, 1pM or less.

11. An antibody according to claim 1, wherein the antigen binding site binds to the Pb-DOTAM chelate and to a Bi-DOTAM chelate, and wherein the ratio of K_d values for the Bi-DOTAM chelate/Pb-DOTAM chelate is in the range of 0.1-10 or 1-10.

12. An antibody according to claim 1, which is a whole antibody, or which is an antibody fragment selected from the group consisting of an Fv, Fab, Fab', Fab'-SH, F(ab')₂, diabody, linear antibody, or single-chain antibody molecule.

13. An antibody according to claim 1, wherein the antibody is coupled to a moiety which binds specifically to a target antigen.

14. The antibody according to claim 13, wherein the target antigen is a tumour specific antigen.

15. The antibody according to claim 13, which is a multispecific or bispecific antibody.

16. The multispecific or bispecific antibody of claim 14, wherein the tumour specific antigen is selected from the group consisting of CEA, HER2 and CD20.

17. The multispecific or bispecific antibody of claim 16, wherein the tumour specific antigen is carcinoembryonic antigen (CEA).

18. The multispecific or bispecific antibody of claim 15, wherein the multispecific or bispecific antibody comprises at least one antigen binding site specific for the Pb-DOTAM chelate and at least one antigen binding site specific for CEA, and wherein the antigen binding site specific for CEA comprises a heavy chain comprising at least one, two or three heavy chain CDRs: wherein:

a) heavy chain CDR1 comprises the amino acid sequence of SEQ ID NO:

11

b) heavy chain CDR2 comprises the amino acid sequence of SEQ ID NO:
12

c) heavy chain CDR3 comprises the amino acid sequence of SEQ ID NO:
13;

5 and/or the antigen binding site specific for CEA comprises a light
chain comprising at least one, two or three light chain CDRs: wherein:

d) light chain CDR1 comprises the amino acid sequence SEQ ID NO: 14;

e) light chain CDR2 comprises the amino acid sequence SEQ ID NO:15;

f) light chain CDR3 comprises the amino acid sequence SEQ ID NO: 16.

10

19. The multispecific or bispecific antibody of claim 18, wherein the
antigen binding site for CEA comprises at least one, two, three four, five,
or six (i.e., all) of the CDRs selected from:

a) heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:

15 11;

b) heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO:

12;

c) heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO:

13;

20 d) light chain CDR1 comprising the amino acid sequence of SEQ ID NO:

14;

e) light chain CDR2 comprising the amino acid sequence of SEQ ID NO:

15;

f) light chain CDR3 comprising the amino acid sequence of SEQ ID NO:

25 16.

20. The multispecific or bispecific antibody of claim 15, wherein the
multispecific or bispecific antibody comprises at least one antigen binding
site specific for the Pb-DOTAM chelate and at least one antigen binding
30 site specific for CEA, and wherein the antigen binding site specific for
CEA comprise

i) a heavy chain variable domain comprising an amino acid sequence of
SEQ ID NO: 17, or a variant thereof comprising an amino acid sequence
having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ
35 ID NO: 17; and/or

ii) a light chain variable domain comprising an amino acid sequence
of SEQ ID NO: 18, or a variant thereof comprising an amino acid sequence
having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ
ID NO: 18.

40

21. The multispecific or bispecific antibody of claim 20, wherein the antigen binding site specific for CEA comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 17 and/or a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 18.

5

22. The multispecific or bispecific antibody according to claim 15, wherein the multispecific or bispecific antibody comprises at least one antigen binding site specific for a Pb-DOTAM chelate and at least one antigen binding site specific for ERBB2, and wherein the antigen binding site specific for ERBB2 comprises at least one, two, three, four, five, or six CDRs selected from (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:28; (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:29; (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:30; (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:31; (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:32; and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:33.

10

15

23. The multispecific or bispecific antibody according to any one of claim 15, wherein the multispecific or bispecific antibody comprises at least one antigen binding site specific for the Pb-DOTAM chelate and at least one antigen binding site specific for ERBB2, and wherein the antigen binding site specific for ERBB2 comprises

20

i) a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 34, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 34; and/or

25

ii) a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 35, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 35.

30

24. The multispecific or bispecific antibody according to claim 23, wherein the antigen binding site specific for ERBB2 comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 34 and/or a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 35.

35

25. The multispecific or bispecific antibody according to claim 15, wherein the multispecific or bispecific antibody comprises at least one antigen binding site specific for a Pb-DOTAM chelate and at least one antigen binding site specific for CD20, and wherein the antigen binding site specific for CD20 comprises at least one, two, three, four, five, or

40

six CDRs selected from (a) CDR-H1 comprising the amino acid sequence of SEQ ID NO:39; (b) CDR-H2 comprising the amino acid sequence of SEQ ID NO:40; (c) CDR-H3 comprising the amino acid sequence of SEQ ID NO:41; (d) CDR-L1 comprising the amino acid sequence of SEQ ID NO:42; (e) CDR-L2 comprising the amino acid sequence of SEQ ID NO:43; and (f) CDR-L3 comprising the amino acid sequence of SEQ ID NO:44.

26. The multispecific or bispecific antibody according to claim 15, wherein the multispecific or bispecific antibody comprises at least one antigen binding site specific for the Pb-DOTAM chelate and at least one antigen binding site specific for CD20, and wherein the antigen binding site specific for CD20 comprises

i) a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 45, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 45; and/or

ii) a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 46, or a variant thereof comprising an amino acid sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identity to SEQ ID NO: 46.

27. The multispecific or bispecific antibody according to paragraph 26, wherein the antigen binding site specific for CD20 comprises a heavy chain variable domain comprising an amino acid sequence of SEQ ID NO: 45 and/or a light chain variable domain comprising an amino acid sequence of SEQ ID NO: 46.

28. The multispecific or bispecific antibody of claim 15, which comprises an Fc region.

29. The multispecific or bispecific antibody of claim 28, wherein the Fc region is engineered to reduce effector function.

30. The multispecific or bispecific antibody of claim 29, wherein the Fc region by substitution of one or more of Fc region residues 234, 235, 238, 265, 269, 270, 297, 327 and/or 329.

31. The multispecific or bispecific antibody of claim 28, comprising:

i) a full length antibody comprising an antigen binding site for a first antigen; and

ii) at least a second heavy chain variable domain and second light chain variable domain which together form an antigen binding site for a second antigen,

wherein either the first or the second antigen is the Pb-DOTAM chelate, and the other is the target antigen.

32. The multispecific or bispecific antibody of claim 31, comprising a full length antibody comprising an antigen binding site for the first antigen, wherein the N- or C-terminus of one of the heavy chains is linked via a polypeptide linker to a first polypeptide and wherein the first polypeptide associates with a second polypeptide to form a Fab or a cross-Fab comprising a binding site for the second antigen.

33. The multispecific or bispecific antibody of claim 32, which comprises:

i) a first polypeptide consisting of a VH domain and a CH1 domain, which is associated with a second polypeptide consisting of a VL and CL domain; or

ii) a first polypeptide consisting of a VL domain and a CH1 domain, which is associated with a second polypeptide consisting of a VH and CL domain; or

iii) a first polypeptide consisting of a VH domain and a CL domain, which is associated with a second polypeptide consisting of a VL and CH1 domain; such that the first and second polypeptide together form an antigen binding site for the second antigen.

34. The multispecific or bispecific antibody of claim 33, which comprises a full length antibody comprising an antigen binding site for the first antigen, wherein the C-terminus of one of the heavy chains is linked via a polypeptide linker to a first polypeptide consisting of a VL domain and a CH1 domain, which is associated with a second polypeptide consisting of a VH and CL domain.

35. The multispecific or bispecific antibody according to claim 31, wherein the first antigen is the target antigen and the second antigen is the Pb-DOTAM chelate.

36. The multispecific or bispecific antibody according to claim 35, wherein the first antigen is CEA.

37. An isolated polynucleotide or an isolated set of polynucleotides encoding an antibody according to claim 1.

5 38. An expression vector or set of expression vectors comprising the polynucleotide or the set of polynucleotides according to claim 37.

39. A prokaryotic or eukaryotic host cell comprising an isolated polynucleotide or an isolated set of polynucleotides according to claim 37, optionally comprising the vector or set of vectors of claim 38.

10

40. A method of producing an antibody according to claims 1, comprising expressing the antibody from the host cell of claim 39.

15