



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2020/07/02
(87) Date publication PCT/PCT Publication Date: 2021/01/07
(85) Entrée phase nationale/National Entry: 2021/12/13
(86) N° demande PCT/PCT Application No.: AU 2020/050690
(87) N° publication PCT/PCT Publication No.: 2021/000018
(30) Priorité/Priority: 2019/07/02 (AU2019902344)

(51) Cl.Int./Int.Cl. *C07K 16/30* (2006.01),
A61K 51/10 (2006.01), *A61P 13/08* (2006.01)
(71) Demandeur/Applicant:
TELEX INTERNATIONAL PTY LTD, AU
(72) Inventeurs/Inventors:
WHEATCROFT, MICHAEL PAUL, AU;
BEHRENBRUCH, CHRISTIAN PETER, AU
(74) Agent: CPST INTELLECTUAL PROPERTY INC.

(54) Titre : ANTICORPS DE LIAISON A PSMA AVEC UNE AFFINITE REDUITE POUR LE RECEPTEUR FC NEONATAL
(54) Title: ANTIBODIES FOR BINDING PSMA WITH REDUCED AFFINITY FOR THE NEONATAL FC RECEPTOR

(57) **Abrégé/Abstract:**

The invention relates to anti-PSMA antibodies comprising a heavy chain constant region comprising one or more amino acid substitutions compared to a wild-type IgG, wherein the one or more amino acid substitutions reduce the affinity of the antibody for the neonatal Fc receptor (FcRn), thereby reducing the serum half-life of the modified antibody compared to a wild-type antibody of class IgG. The one or more amino acid modification having the effect of reducing FcRn binding is selected from positions His310, His433, His435, His436, Ile253. Antibodies of the present invention are particularly suited for use in radioimmunotherapy.

Date Submitted: 2021/12/13

CA App. No.: 3141471

Abstract:

The invention relates to anti-PSMA antibodies comprising a heavy chain constant region comprising one or more amino acid substitutions compared to a wild-type IgG, wherein the one or more amino acid substitutions reduce the affinity of the antibody for the neonatal Fc receptor (FcRn), thereby reducing the serum half-life of the modified antibody compared to a wild-type antibody of class IgG. The one or more amino acid modification having the effect of reducing FcRn binding is selected from positions His310, His433, His435, His436, Ile253. Antibodies of the present invention are particularly suited for use in radioimmunotherapy.

ANTIBODIES FOR BINDING PSMA WITH REDUCED AFFINITY FOR THE NEONATAL FC RECEPTOR**Field of the invention**

The invention relates to antibodies, compositions and methods for producing antibodies, in particular antibodies conjugated to a radioisotope, having reduced serum
5 half-life for use in radioimmunotherapy.

Related application

This application claims priority from Australian provisional application AU 2019902344, the entire contents of which are hereby incorporated by reference.

Background of the invention

10 Radiotherapy is an important form of tumour therapy. Various methods of radiotherapy have been developed to treat tumours. Among them, radioimmunotherapy (RAIT) is one emerging approach to the provision of radiotherapy. It employs antibodies or antibody fragments to direct radioisotopes to specific tissues and cells, thus enhancing specificity of tumour treatment and reducing toxicity. RAIT further reduces its
15 side effects by using low dose rate radiation.

Radiation damage to healthy tissues and organs is a major problem associated with radiotherapy. Such damage has been primarily attributed to radiation-generated reactive oxygen species which oxidize functionally important biological molecules, such as nucleic acids, carbohydrates, lipids and lipoproteins, and damage tissues and cells.
20 They have been implicated in a variety of biological processes, e.g., antimicrobial defense, inflammation, carcinogenesis and aging. As reflected by body weight loss, myelosuppression and blood cell loss, such as decreased white blood cell (WBC) and platelet counts and hematopoietic toxicity are the most notable consequences of the radiation damage. The toxicity severely limits the radiation dosage of RAIT and reduces
25 the effectiveness of tumour treatment.

A number of methods have been developed to attempt to mitigate the hematopoietic toxicity of radiation. Stem cell transplantation (SCT) and bone marrow transplantation (BMT) are the most frequently used methods. However, these

approaches are invasive, expensive and may contribute to longer hospitalization of the individual receiving treatment.

Other methods include using cytokines to stimulate the immune system and hemoregulatory proteins such as HP5b to turn off hematopoiesis during the radiation exposure period. These methods have achieved various degrees of success in combating hematopoietic toxicity in small studies but again, require exposing the patient to further medication and treatment, therefore remain largely unused.

There remains a need for new methods for mitigating the toxicity associated with radioimmunotherapy.

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Summary of the invention

The present invention provides a modified antibody of class IgG for use in radioimmunotherapy, comprising a heavy chain constant region having one or more amino acid substitutions compared to a wild-type antibody of the class IgG, wherein the one or more amino acid substitutions reduce the affinity of the antibody for the neonatal Fc receptor (FcRn), thereby reducing the serum half-life of the modified antibody compared to a wild-type antibody of class IgG.

In one embodiment, the one or more amino acid substitutions are selected from substitutions in the heavy chain constant region 2 (CH2) of the IgG molecule, reducing the affinity of the IgG molecule for FcRn. Alternatively, the one or more amino acid substitutions may be in the heavy chain constant region 3 (CH3) of the IgG molecule, thereby reducing the affinity of the IgG molecule for FcRn. Still further, the amino acid substitutions may include at least one substitution in the CH2 region, and at least one substitution in the CH3 region of the IgG molecule, whereby the substitutions reduce the affinity of the IgG for FcRn.

In certain preferred embodiments, the one or more amino acid substitutions may be at one or more of residues His310, His433, His435, His436, or Ile253 of IgG. Preferably, the amino acid substitutions comprise a substitution in the heavy chain constant region at positions His310 or at His435. More preferably, the amino acid substitutions that reduce the affinity of the antibody for FcRn are at both His310 and His435.

In certain embodiments, the modified antibody retains the ability to bind to one or more Fc-gamma receptors and accordingly, in certain embodiments the modified antibody retains the ability to stimulate effector responses (including ADCC).

In alternative embodiments, the one or more amino acid modifications which reduce the affinity for the FcRn receptor also reduce the affinity for the Fc gamma receptors. The modified antibody may further comprise one or more amino acid substitutions compared a wild-type antibody of the class IgG, wherein the amino acid substitutions further reduce the affinity of the antibody for one or more Fc gamma receptors.

In a further embodiment, the modified antibody further comprises one or more amino acid substitutions compared a wild-type antibody of the class IgG, wherein the amino acid substitutions increase the stability of the CH1-CH2 hinge region in the modified antibody compared to a wild-type antibody of the class IgG.

In one embodiment, the modified antibody is conjugated to a diagnostic or therapeutic agent. The diagnostic or therapeutic agent may be conjugated to the antibody directly or indirectly, e.g. by halogenation of amino acid residues. Preferably, the diagnostic or therapeutic agent is indirectly conjugated to the antibody by way of a linker or chelator moiety. In one example, the modified antibody is conjugated to a chelating moiety, selected from the group consisting of: TMT (6,6"-bis[N,N",N""-tetra(carboxymethyl)aminomethyl]-4'-(3-amino-4-methoxyphenyl)-2,2':6',2"-terpyridine), DOTA (1, 4,7,10-tetraazacyclododecane-NN',N"(N""-tetraacetic acid), TCMC, DO3A, CB-DO2A, NOTA, Diamsar, DTPA, CHX-A"-DTPA, TETE, Te2A, HBED, DFO, DFOsq and HOPO or other chelating agent as described herein.

In another example, the modified antibody is conjugated to a bifunctional linker, for example, bromoacetyl, thiols, succinimide ester, TFP ester, a maleimide, or using any amine or thiol- modifying chemistry known in the art.

Preferably, the diagnostic or therapeutic agent is a radioisotope. Examples of suitable isotopes include: actinium-225 (^{225}Ac), astatine-211 (^{211}At), bismuth-212 and bismuth-213 (^{212}Bi , ^{213}Bi), copper-64 and copper-67 (^{64}Cu , ^{67}Cu), gallium-67 and gallium-68 (^{67}Ga and ^{68}Ga), indium-111 (^{111}In), iodine -123, -124, -125 or -131 (^{123}I , ^{124}I , ^{125}I , ^{131}I) (^{123}I), lead-212 (^{212}Pb), lutetium-177 (^{177}Lu), radium-223 (^{223}Ra), samarium-153 (^{153}Sm), scandium-44 and scandium-47 (^{44}Sc , ^{47}Sc), strontium-90 (^{90}Sr), technetium-99 ($^{99\text{m}}\text{Tc}$), yttrium-86 and yttrium-90 (^{86}Y , ^{90}Y), zirconium-89 (^{89}Zr).

The modified antibody of class IgG with reduced FcRn binding affinity compared to an unmodified antibody of class IgG may be any antibody that is useful for targeting a diagnostic or therapeutic agent to a biological site. The antibody may be of any IgG class, including IgG1 (human or murine), IgG2, IgG4, murine IgG2a. In preferred examples, the antibody is any antibody that is useful for targeting or for delivering a diagnostic or therapeutic agent to a cancer cell. Examples of suitable antibodies include the IgG1 antibodies trastuzumab (Herceptin®), rituximab (Rituxan®), bevacizumab (Avastin®), dinutuximab (Unituxin®), the IgG2 antibody panitumumab (Vectibix®), the IgG4 antibodies, pembrolizumab (Keytruda®), nivolumab (Opdivo®), the murine IgG2a antibody tositumomab (Bexxar®) and the murine IgG1 antibody ibritumomab (Zevalin®). Other examples include gemtuzumab (Mylotarg®), brentuximab (Adcetris®), Inotuzumab (Besponsa®), glembatumumab (CDX-011), anetumab (BAY 94-9343), mirvetuximab (IMGN853) depatuxizumab (ABT-414), rovalpituzumab (Rova-T) and vadastuximab talirine (SGN-CD33A).

The present invention also provides a modified antibody of class IgG with reduced FcRn binding affinity compared to an unmodified antibody of class IgG, or compared to a wild-type antibody of the class IgG comprising:

- a heavy chain constant region having one or more amino acid substitutions compared to a wild-type antibody of the class IgG, wherein the one or more amino acid substitutions reduce the affinity of the antibody for the neonatal Fc receptor (FcRn),

thereby reducing the serum half-life of the modified antibody compared to a wild-type antibody of class IgG.

wherein said antibody binds specifically to prostate specific membrane antigen (PSMA) and wherein the antibody comprises:

5 FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 - linker - FR1a - CDR1a - FR2a - CDR2a - FR3a - CDR3a - FR4a

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

10 FR1a, FR2a, FR3a and FR4a are each framework regions;

CDR1a, CDR2a and CDR3a are each complementarity determining regions;

wherein the sequence of any of the complementarity determining regions have an amino acid sequence as described in Table 1 below. Preferably, the framework regions have an amino acid sequence also as described in Table 1 below, including
 15 amino acid variation at particular residues which can be determined by aligning the various framework regions derived from each antibody. The invention also includes where CDR1, CDR2 and CDR3 are sequences from the VH, CDR1a, CDR2a and CDR3a are sequences from VL, or where CDR1, CDR2 and CDR3 are sequences from the VL, CDR1a, CDR2a and CDR3a are sequences from VH.

20 More specifically, the present invention provides a modified antibody of class IgG with reduced FcRn binding affinity compared to an unmodified antibody of class IgG, or compared to a wild-type antibody of the class IgG comprising:

- a heavy chain constant region wherein one or more amino acid residues at positions His310, His433, His435, His436, Ile253 are different from the residues present
 25 in the unmodified antibody, or a wild-type antibody of the class IgG,

wherein said antibody binds specifically to prostate specific membrane antigen (PSMA) and wherein the antibody comprises:

FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4 – linker - FR1a - CDR1a –
FR2a – CDR2a – FR3a – CDR3a – FR4a

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

5 CDR1, CDR2 and CDR3 are each complementarity determining regions;

FR1a, FR2a, FR3a and FR4a are each framework regions;

CDR1a, CDR2a and CDR3a are each complementarity determining regions;

wherein the sequence of any of the complementarity determining regions have
an amino acid sequence as described in Table 1 below. Preferably, the framework
10 regions have an amino acid sequence also as described in Table 1 below, including
amino acid variation at particular residues which can be determined by aligning the
various framework regions derived from each antibody. The invention also includes
where CDR1, CDR2 and CDR3 are sequences from the VH, CDR1a, CDR2a and
CDR3a are sequences from VL, or where CDR1, CDR2 and CDR3 are sequences from
15 the VL, CDR1a, CDR2a and CDR3a are sequences from VH.

In one embodiment, the antibody that specifically binds to PSMA comprises an
antigen binding site that consists essentially of or consists of an amino acids sequence
of (in order of N to C terminus or C to N terminus) SEQ ID NO: 4 or 20.

In a further embodiment, the antibody that specifically binds to PSMA comprises
20 at least one of:

(i) a VH comprising a complementarity determining region (CDR) 1
comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at
least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO 1
or 17, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%,
25 at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in
SEQ ID NO: 2 or 18, and a CDR3 comprising a sequence at least about 80%, at least
85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a
sequence set forth in SEQ ID NO: 3 or 19;

(ii) a VH comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 4 or 20;

(iii) a VL comprising a CDR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 35;

(iv) a VL comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO:36;

(v) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19;

(vi) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20;

(vii) a VL comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 45;

(viii) a VL comprising a sequence set forth in SEQ ID NO: 36;

(ix) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19; and a VL comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 35;

or

(x) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20 and a VL comprising a sequence set forth in SEQ ID NO: 36.

Preferably, the heavy chain constant region comprises amino acid substitutions at both His310 and His435. The antibody may also comprise amino acid substitutions at residues equivalent to Ser228 and Leu235 of the constant heavy chain region.

5 In any embodiment, the antibody comprises a heavy chain constant region that comprises the amino acid sequence as set forth in any one of SEQ ID NOs: 49 to 51, preferably wherein the heavy chain constant region comprises the sequence set forth in SEQ ID NO:50.

In still a further embodiment, the heavy chain of the antibody comprises the sequence set forth in any one of SEQ ID NOs: 49 to 56, preferably as set forth in SEQ
10 ID NO: 53.

Still further, in preferred embodiments, the light chain constant region of the antibody comprises the sequence as set forth in SEQ ID NO: 52. More preferably, the antibody comprises a light chain comprising the amino acid sequence as set forth in SEQ ID NO:57.

15 In a particularly preferred embodiment, the antibody comprises the amino acid sequence set forth in SEQ ID NO: 53 and the sequence set forth in SEQ ID NO: 57.

The present invention also provides a molecule comprising an immunoglobulin moiety and a non-protein agent conjugated thereto,

20 wherein, the immunoglobulin moiety specifically binds to a tumour associated antigen,

wherein the immunoglobulin moiety has reduced or abolished affinity for the FcRn receptor compared to a wild-type immunoglobulin; and

wherein the non-protein agent comprises a therapeutic moiety such as a cytotoxin or a radioactive element.

25 The immunoglobulin moiety may comprise any antibody that is useful for binding to a tumour-associated antigen, included but not limited to trastuzumab (Herceptin®), rituximab (Rituxan®), bevacizumab (Avastin®), dinutuximab (Unituxin®), panitumumab (Vectibix®), pembrolizumab (Keytruda®), nivolumab (Opdivo®), tositumomab

(Bexxar®), ibritumomab (Zevalin®), gemtuzumab (Mylotarg®), brentuximab (Adcetris®), Inotuzumab (Besponsa®), glembatumumab (CDX-011), anetumab (BAY 94-9343), mirvetuximab (IMGN853) depatuxizumab (ABT-414), rovalpituzumab (Rova-T) and vadastuximab talirine (SGN-CD33A), or any other antibody as described herein.

5 The present invention provides a molecule comprising an immunoglobulin moiety and a non-protein agent conjugated thereto,

 wherein, the immunoglobulin moiety specifically binds to PSMA and comprises an antigen binding site including:

 FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4 – linker - FR1a - CDR1a –
10 FR2a – CDR2a – FR3a – CDR3a – FR4a

 wherein:

 FR1, FR2, FR3 and FR4 are each framework regions;

 CDR1, CDR2 and CDR3 are each complementarity determining regions;

 FR1a, FR2a, FR3a and FR4a are each framework regions;

15 CDR1a, CDR2a and CDR3a are each complementarity determining regions;

 wherein the sequence of any of the complementarity determining regions have an amino acid sequence as described in Table 1 below;

 wherein the immunoglobulin moiety has reduced or abolished affinity for the FcRn receptor compared to a wild-type immunoglobulin; and

20 wherein the non-protein agent comprises a therapeutic moiety such as a cytotoxin or a radioactive element.

 Preferably, the framework regions have an amino acid sequence also as described in Table 1 below, including amino acid variation at particular residues which can be determined by aligning the various framework regions derived from each
25 antibody. The invention also includes where CDR1, CDR2 and CDR3 are sequences from the VH, CDR1a, CDR2a and CDR3a are sequences from VL, or where CDR1, CDR2 and CDR3 are sequences from the VL, CDR1a, CDR2a and CDR3a are

sequences from VH. Preferably, the immunoglobulin moiety has amino acid substitutions at residues equivalent to His310 and/or His435 in the constant heavy chain region. The immunoglobulin moiety may also comprise amino acid substitutions at residues equivalent to Ser228 and Leu235 of the constant heavy chain region.

5 Preferably, the non-protein agent comprises a radioactive element.

The present invention also provides a molecule comprising an immunoglobulin moiety and a non-protein agent conjugated thereto,

 wherein, the immunoglobulin moiety specifically binds to PSMA and comprises:
an antigen binding site that consists essentially of or consists of an amino acids
10 sequence of (in order of N to C terminus or C to N terminus) SEQ ID NO: 4 or 20;

 wherein the immunoglobulin moiety has reduced or abolished affinity for the FcRn receptor compared to a wild-type immunoglobulin; and

 wherein the non-protein agent comprises a therapeutic moiety such as a cytotoxin or a radioactive element.

15 Preferably, the immunoglobulin moiety has amino acid substitutions at residues equivalent to His310 and/or His435 in the constant heavy chain region. The immunoglobulin moiety may also comprise amino acid substitutions at residues equivalent to Ser228 and Leu235 of the constant heavy chain region.

 The present invention also provides a molecule comprising an immunoglobulin
20 moiety and a non-protein agent conjugated thereto,

 wherein, the immunoglobulin moiety has reduced or abolished affinity for the FcRn receptor compared to a wild-type immunoglobulin and wherein the immunoglobulin moiety specifically binds to PSMA and comprises at least one of:

 (i) a VH comprising a complementarity determining region (CDR) 1
25 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO 1 or 17, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in

SEQ ID NO: 2 or 18, and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 3 or 19;

5 (ii) a VH comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 4 or 20;

(iii) a VL comprising a CDR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 35;

(iv) a VL comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO: 36;

15 (v) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19;

(vi) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20;

20 (vii) a VL comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 45;

(viii) a VL comprising a sequence set forth in SEQ ID NO: 36;

(ix) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19; and a VL comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 35;
or

(x) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20 and a VL comprising a sequence set forth in SEQ ID NO: 36.

Preferably, the immunoglobulin moiety has amino acid substitutions at residues equivalent to His310 and/or His435 in the constant heavy chain region. The immunoglobulin moiety may also comprise amino acid substitutions at residues equivalent to Ser228 and Leu235 of the constant heavy chain region. Preferably, the non-protein agent comprises a radioactive element.

In any embodiment, the immunoglobulin comprises a heavy chain constant region that comprises the amino acid sequence as set forth in any one of SEQ ID NOs: 49 to 51, preferably wherein the heavy chain constant region comprises the sequence set forth in SEQ ID NO:50.

In still a further embodiment, the immunoglobulin comprises the sequence set forth in any one of SEQ ID NOs: 53 to 56, preferably 53.

In any embodiment, the immunoglobulin comprises a light chain constant region comprising the sequence of SEQ ID NO:52. In an embodiment the immunoglobulin comprises a light chain having the amino acid sequence as set forth in SEQ ID NO:57.

In a particularly preferred embodiment, the immunoglobulin comprises the sequences set forth in SEQ ID NOs:53 and 57.

The present invention also provides a method of treating cancer in an individual, the method comprising administering to an individual in need thereof, a molecule comprising an immunoglobulin moiety and a non-protein agent conjugated as hereinbefore described.

The present invention also provides a method for generating an antibody suitable for use in radioimmunotherapy, the method comprising:

- providing an antibody with an antigen binding site that specifically binds to an epitope present on a cell or tissue requiring radioimmunotherapy;
- introducing at least one amino acid substitution into the heavy chain constant region of the antibody to generate a modified antibody, wherein the at least one amino acid substitution is selected from the group consisting of amino

acid substitutions at residues His310, His435, and Ile253, thereby causing an alteration of the binding affinity for FcRn and/or the serum half-life of said antibody;

- conjugating the modified antibody with a radioactive element

5 thereby generating an antibody suitable for use in radioimmunotherapy.

In certain embodiments, the antibody is an antibody as described herein, including an antibody having any of the complementarity determining regions, framework regions, variable light or variable heavy regions as described in Table 1 below. Preferably, the modified antibody also comprises amino acid substitutions at
10 residues equivalent to Ser228 and Leu235 of the constant heavy chain region. Preferably, the radioactive element is conjugated to the modified antibody using a chelating agent, for example DOTA.

The present invention also provides a method of generating an antibody-radioisotope immunoconjugate for use in the treatment of a disease, the method
15 comprising:

- providing an antibody with an antigen binding site that specifically binds to an epitope present on a cell or tissue requiring radioimmunotherapy;
- introducing at least one amino acid substitution into the heavy chain constant region of the antibody to generate a modified antibody, wherein the at least
20 one amino acid substitution is selected from the group consisting of substitutions at amino acid residues His310, His435, and Ile253, thereby causing an alteration of the binding affinity for FcRn and/or the serum half-life of said antibody;
- conjugating the modified antibody with a radioactive element

25 thereby generating an antibody-radioisotope immunoconjugate for use in the treatment of a disease.

Preferably, the antibody is an antibody as described herein, including an antibody having any of the complementarity determining regions, framework regions, variable light or variable heavy regions as described in Table 1 below. Preferably, the modified
30 antibody also comprises amino acid substitutions at residues equivalent to Ser228 and

Leu235 of the constant heavy chain region. Preferably, the radioactive element is conjugated to the modified antibody using a chelating agent, for example DOTA. More preferably, the modified antibody comprises amino acid substitutions His310Ala and His435Gln.

- 5 Preferably the disease is a cancer, including prostate cancer or renal cell carcinoma.

The present invention further provides for a method of producing a modified antibody with an altered binding affinity for FcRn and/or an altered serum half-life compared with an unmodified form of the antibody, wherein said method comprises:

- 10 (a) providing an expression vector (preferably a replicable expression vector) comprising a suitable promoter operably linked to a nucleic acid molecule encoding at least a constant region of an immunoglobulin heavy chain wherein at least one amino acid from the heavy chain constant region selected from the group consisting of amino acid residues His310, His435, and Ile253 is substituted with an amino acid which is
15 different from that present in an unmodified antibody, thereby causing an alteration in FcRn binding affinity and/or serum half-life;
- (b) transforming host cells with said vector; and
- (c) culturing said transformed host cells to produce said modified antibody.

- Optionally, such a method further comprises: preparing a second expression
20 vector (preferably a replicable expression vector) comprising a promoter operably linked to DNA encoding a complementary immunoglobulin light chain and further transforming said cell line with said second vector.

A method for altering the serum half-life of an antibody for use in radioimmunotherapy, the method comprising:

- 25 - providing an antibody with an antigen binding site that specifically binds to an epitope present on a cell or tissue requiring radioimmunotherapy;
- introducing at least one amino acid substitution into the heavy chain constant region of the antibody to generate a modified antibody, wherein the at least one amino acid substitution is selected from the group consisting of

substitutions at amino acid residues His310, His435, and Ile253, thereby causing an alteration of the binding affinity for FcRn and the serum half-life of said antibody.

Preferably, the method further includes conjugating the modified antibody with a radioactive element. Preferably, the antibody is an antibody as described herein, for binding to PSMA including an antibody having any of the complementarity determining regions, framework regions, variable light or variable heavy regions as described in Table 1 below. Preferably, the modified antibody also comprises amino acid substitutions at residues equivalent to Ser228 and Leu235 of the constant heavy chain region, including Ser228Pro and/or Leu235Glu. More preferably, the modified antibody comprises amino acid substitutions His310Ala and His435Gln.

A method for reducing the toxicity of an antibody for use in radioimmunotherapy, the method comprising

- providing an antibody with an antigen binding site that specifically binds to an epitope present on a cell or tissue requiring radioimmunotherapy;
- introducing at least one amino acid substitution into the heavy chain constant region of the antibody to generate a modified antibody, wherein the at least one amino acid substitution is selected from the group consisting of substitutions at amino acid residues His310, His435, and Ile253, wherein the amino acid substitutions reduce the serum half-life and/or increase the clearance of the modified antibody from the circulation,

thereby reducing the toxicity of the antibody when it is conjugated to a radioactive element for use in radioimmunotherapy.

In any embodiment, reducing the toxicity of an antibody includes reducing a number of toxic effects which would otherwise result from longer-term residence of radioisotope in the circulation (including haematological toxicity, absorption into bone and bone marrow irradiation).

In any embodiment, the toxicity of a radio-labelled antibody or radioimmunoconjugate herein described is assessed by determining the tumour:blood ratio of the antibody or immunoconjugate following administration to an individual.

In any embodiment of the invention, the tumour:blood ratio of the modified antibodies of the present invention is at least 2 times greater, at least 3 times greater, at least 4 times greater, at least 6 times greater, at least 8 times greater or at least 10 times greater, or more, than for unmodified antibodies that do not have the modifications to the heavy chain constant region as herein described, when the ratio is determined at least 8 hours following administration of the antibody. Alternatively, the ratio is determined at least 24, 48, 72 or 120 hours following administration of the antibody to an individual. In certain embodiments, the tumour:blood ratio of the modified antibodies of the present invention is at least 50 times greater, at least 100 times greater, at least 200 times greater or at least 300 times greater than for unmodified antibodies that do not have the modifications to the heavy chain constant region as herein described, when the ratio is determined at least 120 hours following administration of the antibody.

In any embodiment of the invention, the modified antibodies herein described having reduced or altered serum half-life compared to unmodified antibodies, have a serum clearance rate that is at least two times faster, at least three times faster or more, than the unmodified antibodies.

In particularly preferred embodiments of the invention, the antibodies described herein are suitable for use in a theranostic pair, wherein the theranostic pair comprises 1) the antibody coupled to an imaging agent and 2) the antibody coupled to an agent for therapy. For example, the antibody may firstly be used as a diagnostic when coupled to a radioisotope suitable for use in radioimaging, Secondly, the antibody may be used as a therapeutic when coupled to a radioisotope or cytotoxic agent suitable for use in therapy.

The present invention also provides an *in vivo* method of diagnosing, monitoring or prognosing a disease, disorder or infection in a subject comprising:

(a) administering to a subject an effective amount of a modified antibody as herein described, said modified antibody specifically binding to an antigen associated with a disease, disorder or infection;

(b) allowing the modified antibody to concentrate at sites in said subject where said antigen is found; and

(c) detecting said modified antibody;

whereby detection of said modified antibody above a background or standard level indicates that the subject has said disease disorder or infection.

The present invention provides an antigen binding site that binds to or specifically
 5 binds to prostate specific membrane antigen (PSMA). Preferably, the antigen binding site of the invention binds to or specifically binds to human PSMA.

The invention provides an antigen binding site for binding to PSMA, the antigen binding site comprising:

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 - linker - FR1a - CDR1a -
 10 FR2a - CDR2a - FR3a - CDR3a - FR4a

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

FR1a, FR2a, FR3a and FR4a are each framework regions;

15 CDR1a, CDR2a and CDR3a are each complementarity determining regions;

wherein the sequence of any of the framework regions or complementarity determining regions are as described herein.

The invention provides an antigen binding site for binding to PSMA, the antigen binding site including:

20 FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 - linker - FR1a - CDR1a -
 FR2a - CDR2a - FR3a - CDR3a - FR4a

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

FR1a, FR2a, FR3a and FR4a are each framework regions;

CDR1a, CDR2a and CDR3a are each complementarity determining regions;

wherein the sequence of any of the complementarity determining regions have an amino acid sequence as described in Table 1 below. Preferably, the framework regions have an amino acid sequence also as described in Table 1 below, including amino acid variation at particular residues which can be determined by aligning the various framework regions derived from each antibody. The invention also includes where CDR1, CDR2 and CDR3 are sequences from the VH, CDR1a, CDR2a and CDR3a are sequences from VL, or where CDR1, CDR2 and CDR3 are sequences from the VL, CDR1a, CDR2a and CDR3a are sequences from VH.

The invention provides an antigen binding site comprising, consisting essentially of or consisting of an amino acids sequence of (in order of N to C terminus or C to N terminus) SEQ ID NO: 4 or 20.

The present invention also provides an antigen binding site comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to PSMA, wherein the antigen binding domain comprises at least one of:

(i) a VH comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO 1 or 17, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in SEQ ID NO: 2 or 18, and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 3 or 19;

(ii) a VH comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 4 or 20;

(iii) a VL comprising a CDR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical

to a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 35;

(iv) a VL comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO:36;

(v) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19;

(vi) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20;

(vii) a VL comprising a CDR1 comprising a sequence set SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 45;

(viii) a VL comprising a sequence set forth in SEQ ID NO: 36;

(ix) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19; and a VL comprising a CDR1 comprising a sequence set SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 35; or

(x) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20 and a VL comprising a sequence set forth in SEQ ID NO: 36.

In any aspect of the invention, the antigen binding domain further comprises at least one of:

(i) a VH comprising a framework region (FR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 9 or 25, a FR2 comprising a

sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in SEQ ID NO:10 or 26, a FR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO:11 or 27, and a FR4 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 12 or 28;

(ii) a VL comprising a FR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 41, a FR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 42, a FR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO:43, and a FR4 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 44;

(iii) a VH comprising a FR1 comprising a sequence set forth in SEQ ID NO: 9 or 25, a FR2 comprising a sequence set forth between in SEQ ID NO: 10 or 26, a FR3 comprising a sequence set forth in SEQ ID NO: 11 or 27, and a FR4 comprising a sequence set forth in SEQ ID NO: 12 or 28;

(iv) a VL comprising a FR1 comprising a sequence set forth in SEQ ID NO: 41, a FR2 comprising a sequence set forth between in SEQ ID NO: 42, a FR3 comprising a sequence set forth in SEQ ID NO: 43, and a FR4 comprising a sequence set forth in SEQ ID NO: 44; or

(v) a VH comprising a FR1 comprising a sequence set forth in SEQ ID NO: 9 or 25, a FR2 comprising a sequence set forth between in SEQ ID NO: 10 or 26, a FR3 comprising a sequence set forth in SEQ ID NO: 11 or 27, and a FR4 comprising a sequence set forth in SEQ ID NO: 12 or 28; and a VL comprising a FR1 comprising a sequence set forth in SEQ ID NO: 41, a FR2 comprising a sequence set forth between in SEQ ID NO: 42, a FR3 comprising a sequence set forth in SEQ ID NO: 43, and a FR4 comprising a sequence set forth in SEQ ID NO: 44.

In any embodiment, the antigen binding site comprises a heavy chain constant region that comprises the amino acid sequence as set forth in any one of SEQ ID NOs: 49 to 51, preferably wherein the heavy chain constant region comprises the sequence set forth in SEQ ID NO:50.

- 5 In still a further embodiment, the antigen binding site comprises the sequence set forth in any one of SEQ ID NOs:53 to 56, preferably 53.

In any embodiment, the antigen binding site comprises a light chain constant region comprising the sequence of SEQ ID NO:52. In an embodiment the light chain of the antigen binding site comprises the sequence of SEQ ID NO:57.

- 10 In a particularly preferred embodiment, the antigen binding site comprises the sequences set forth in SEQ ID NOs:53 and 57.

The foregoing antigen binding sites can also be referred to as antigen binding domains of antibodies.

- 15 Preferably, an antigen binding site as described herein is an antibody or antigen binding fragment thereof. Typically, the antigen binding site is an antibody, for example, a monoclonal antibody.

As described herein, the antigen binding site may be in the form of:

- (i) a single chain Fv fragment (scFv);
- (ii) a dimeric scFv (di-scFv);
- 20 (iii) one of (i) or (ii) linked to a constant region of an antibody, Fc or a heavy chain constant domain (CH) 2 and/or CH3; or
- (iv) one of (i) or (ii) linked to a protein that binds to an immune effector cell.

Further, as described herein, the antigen binding site may be in the form of:

- (i) a diabody;
- 25 (ii) a triabody;

(iii) a tetrabody;

(iv) a Fab;

(v) a F(ab')₂;

(vi) a Fv;

5 (vii) one of (i) to (vi) linked to a constant region of an antibody, Fc or a heavy chain constant domain (CH) 2 and/or CH3; or

(viii) one of (i) to (vi) linked to a protein that binds to an immune effector cell.

In any aspect or embodiment, the antibody is a naked antibody. Specifically, the antibody is in a non-conjugated form and is not adapted to form a conjugate.

10 The invention also provides a fusion protein comprising an antigen binding site, immunoglobulin variable domain, antibody, dab (single domain antibody), di-scFv, scFv, Fab, Fab', F(ab')₂, Fv fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody molecule, or multispecific antibody as described herein.

The invention also provides a conjugate in the form of an antigen binding site,
15 immunoglobulin variable domain, antibody, dab, di-scFv, scFv, Fab, Fab', F(ab')₂, Fv fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody molecule, or multispecific antibody or fusion protein as described herein conjugated to a label or a cytotoxic agent.

The invention also provides an antibody for binding to an antigen binding site,
20 immunoglobulin variable domain, antibody, dab, di-scFv, scFv, Fab, Fab', F(ab')₂, Fv fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody molecule, or multispecific antibody, fusion protein, or conjugate as described herein.

In a preferred embodiment, the antigen binding site is an IgG immunoglobulin comprising one or more amino acid substitutions in the antibody constant domain, CH2-
25 CH3 region, which modify the binding of the antibody to the neonatal Fc receptor (FcRn) relative to a wild-type antibody Fc region. The one or more amino acid modifications change the affinity of the antibody constant domain, Fc region, or FcRn binding

fragment thereof, for the FcRn and thereby alter the serum half-life of the antigen binding site.

Preferably the substitution alters the binding affinity for FcRn and/or the serum half-life of said modified antibody relative to the unmodified wild-type antibody. The present invention further provides for a modified antibody having a reduced binding affinity for FcRn and/or a reduced serum half-life as compared with the unmodified antibody, wherein any one or more amino acid residues at positions Ile253 or His310 from the CH2 domain and/or residue His435 from the CH3 domain, is substituted with another amino acid which is different from that present in an unmodified antibody or to an unmodified IgG.

In one example, the one or more amino acid modifications is selected from an amino acid substitution at residue equivalent to H310 and H435. In a further example, the antibody comprises amino acid substitutions at both His310 and His435 residues.

The amino acid substitutions may include substitution from a histidine residue to: alanine, glutamine, glutamic acid or aspartic acid. Preferably, the amino acid substitution at His310 is to alanine. Preferably the amino acid substitution at His435 is to glutamine. Preferably, the amino acid substitution at Ile253 is alanine.

In a further embodiment, the antigen binding site is an antibody that comprises one or more amino acid substitutions which modify the binding of the antibody to activating Fc gamma receptors. The one or more amino acid modifications change the affinity of the antibody constant domain, Fc region, or Fc gamma receptor binding fragment, for any one or more Fc gamma receptors. Preferably, the amino acid modification is at a residue equivalent to Leu235. More preferably, the amino acid modification is from Leu235 to glutamic acid.

In one embodiment, the amino acid modification is a hinge stabilising mutation at Ser228. Preferably the amino acid modification at Ser228 is to proline.

In one embodiment of the invention, the antibody comprises mutations at Ser228, Leu235, His310 and His435. Preferably, the amino acid modifications are Ser228Pro, Leu235Glu, His310Ala and His435Gln.

The amino acid modifications are preferably made in an antibody having an IgG1 isotype, or on an IgG4 isotype.

In a preferred embodiment, the antibody comprises a heavy chain constant region as set forth in any one of SEQ ID NOs: 235 to 238.

- 5 The invention also provides a nucleic acid encoding an antigen binding site, immunoglobulin variable domain, antibody, dab, di-scFv, scFv, Fab, Fab', F(ab')₂, Fv fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody molecule, or multispecific antibody, fusion protein or conjugate as described herein.

10 In one example, such a nucleic acid is included in an expression construct in which the nucleic acid is operably linked to a promoter. Such an expression construct can be in a vector, e.g., a plasmid.

In examples of the invention directed to single polypeptide chain antigen binding sites, the expression construct may comprise a promoter linked to a nucleic acid encoding that polypeptide chain.

- 15 In examples directed to multiple polypeptide chains that form an antigen binding site, an expression construct comprises a nucleic acid encoding a polypeptide comprising, e.g., a VH operably linked to a promoter and a nucleic acid encoding a polypeptide comprising, e.g., a VL operably linked to a promoter.

20 In another example, the expression construct is a bicistronic expression construct, e.g., comprising the following operably linked components in 5' to 3' order:

- (i) a promoter
- (ii) a nucleic acid encoding a first polypeptide;
- (iii) an internal ribosome entry site; and
- (iv) a nucleic acid encoding a second polypeptide,

- 25 wherein the first polypeptide comprises a VH and the second polypeptide comprises a VL, or vice versa.

The present invention also contemplates separate expression constructs one of which encodes a first polypeptide comprising a VH and another of which encodes a second polypeptide comprising a VL. For example, the present invention also provides a composition comprising:

- 5 (i) a first expression construct comprising a nucleic acid encoding a polypeptide comprising a VH operably linked to a promoter; and
- (ii) a second expression construct comprising a nucleic acid encoding a polypeptide comprising a VL operably linked to a promoter.

10 The invention provides a cell comprising a vector or nucleic acid described herein. Preferably, the cell is isolated, substantially purified or recombinant. In one example, the cell comprises the expression construct of the invention or:

- (i) a first expression construct comprising a nucleic acid encoding a polypeptide comprising a VH operably linked to a promoter; and
- 15 (ii) a second expression construct comprising a nucleic acid encoding a polypeptide comprising a VL operably linked to a promoter,

wherein the first and second polypeptides associate to form an antigen binding site of the present invention.

Examples of cells of the present invention include bacterial cells, yeast cells, insect cells or mammalian cells.

20 The invention also provides a pharmaceutical composition comprising an antigen binding site, or comprising a CDR and/or FR sequence as described herein, or an immunoglobulin variable domain, antibody, dab (single domain antibody), di-scFv, scFv, Fab, Fab', F(ab')₂, Fv fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody molecule, or multispecific antibody, fusion protein, or conjugate as

25 described herein and a pharmaceutically acceptable carrier, diluent or excipient.

The invention also provides a diagnostic composition comprising an antigen binding site, or comprising a CDR and/or FR sequence as described herein, or antigen binding site, immunoglobulin variable domain, antibody, dab, di-scFv, scFv, Fab, Fab',

F(ab')₂, Fv fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody molecule, or multispecific antibody, fusion protein or conjugate as described herein, a diluent and optionally a label. Preferably, the antigen binding site is a monoclonal antibody conjugated to a radioisotope.

5 The invention also provides a kit or article of manufacture comprising an antigen binding site, or comprising a CDR and/or FR sequence as described herein or an immunoglobulin variable domain, antibody, dab, di-scFv, scFv, Fab, Fab', F(ab')₂, Fv fragment, diabody, triabody, tetrabody, linear antibody, single-chain antibody molecule, or multispecific antibody, fusion protein or conjugate as described herein.

10 Preferably, the antigen binding site is a monoclonal antibody conjugated to a radioisotope.

 An antigen binding site, a protein or antibody as described herein may comprise a human constant region, e.g., an IgG constant region, such as an IgG1, IgG2, IgG3 or IgG4 constant region or mixtures thereof. In the case of an antibody or protein
15 comprising a VH and a VL, the VH can be linked to a heavy chain constant region and the VL can be linked to a light chain constant region.

 In one example a protein or antibody as described herein or a composition of a protein or antibody as described herein, comprises a heavy chain constant region, comprising a stabilized heavy chain constant region, comprising a mixture of sequences
20 fully or partially with or without the C-terminal lysine residue.

 In one example, an antibody of the invention comprises a VH disclosed herein linked or fused to an IgG4 constant region or stabilized IgG4 constant region (e.g., as discussed above) and the VL is linked to or fused to a kappa light chain constant region.

 The functional characteristics of an antigen binding site of the invention will be
25 taken to apply mutatis mutandis to an antibody of the invention.

 An antigen binding site as described herein may be purified, substantially purified, isolated and/or recombinant.

 The present invention also provides a method for treating or preventing cancer in a subject, the method comprising administering an antigen binding site of the invention

to the subject. In this regard, an antigen binding site can be used to prevent a relapse of a condition, and this is considered preventing the condition.

Exemplary cancers include prostate cancer. It will be understood that the antibodies having affinity for PSMA will be useful for this purpose.

5 The present invention also provides an *in vivo* method of diagnosing, monitoring or prognosing a disease, disorder or infection in a subject comprising:

(a) administering to a subject an effective amount of the antibody herein described, said antibody specifically binding to an antigen associated with a disease, disorder or infection;

10 (b) allowing the antibody to concentrate at sites in said subject where said antigen is found; and

(c) detecting said antibody;

whereby detection of said antibody above a background or standard level indicates that the subject has said disease disorder or infection.

15 As used herein, except where the context requires otherwise, the term "comprise" and variations of the term, such as "comprising", "comprises" and "comprised", are not intended to exclude further additives, components, integers or steps.

Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

20

Brief description of the drawings

Figure 1: Average half-life, and Tukey's multiple comparison of half-life, of the antibodies of the invention. Error bars represent standard error of the mean. J591 IgG = control HuJ591 antibody for binding PSMA. ANT4044-K-DOTA = antibody ANT4044 lysine conjugated to DOTA. ANT4044-A2-K-DOTA – antibody ANT4044-A2 lysine conjugated to DOTA. ANT4044-FcRn-K-DOTA = antibody ANT4044 with amino acid substitutions in the FcRn-binding region, lysine conjugated to DOTA. ANT4044-

25

FcRg-K-DOTA = antibody ANT4044 with amino acid substitutions in the FcRn and Fc gamma receptor binding regions, lysine conjugated to DOTA.

Figure 2: Average area under the curve (AUC) and clearance (CL) for selected antibodies of the invention. J591 IgG = control HuJ591 antibody for binding PSMA. ANT4044-K-DOTA = antibody ANT4044 lysine conjugated to DOTA. ANT4044-A2-K-DOTA – antibody ANT4044-A2 lysine conjugated to DOTA. ANT4044-FcRn-K-DOTA = antibody ANT4044 with amino acid substitutions in the FcRn-binding region, lysine conjugated to DOTA. ANT4044-FcRg-K-DOTA = antibody ANT4044 with amino acid substitutions in the FcRn and Fc gamma receptor binding regions, lysine conjugated to DOTA.

Figure 3: Biodistribution of antibodies at 8 hrs as determined by ROI analysis of PET images.

Figure 4: Biodistribution of antibodies at 24 hrs as determined by ROI analysis of PET images.

Figure 5: Biodistribution of all antibodies at 48 hrs as determined by ex vivo gamma count.

Figure 6: Biodistribution of antibodies at 48 hrs as determined by ROI analysis of PET images.

Figure 7: Blood concentration of antibodies out to 5 days post-injection.

Figure 8: Tumour accumulation of antibodies as determined by imaging (8h, 24h, 48hr)

Figure 9: The ratio of antibody in tumour to blood. The tumour:blood ratio (in vivo tumour: tail blood) for each of the antibodies was determined for the 8 hr, 24 hr and 48 hr time points. The ratio is significantly higher for JN006 and JN007 at all time points compared with antibodies JN005 and hJ591.

Figure 10: The ratio of antibody in tumour to blood. The tumour:blood ratio (ex vivo:ex vivo) for each of the antibodies was determined for at 48 hr and 120 hr time points. The ratio is significantly higher for JN006 and JN007 at all time points compared with antibodies JN005 and hJ591.

Figure 11: *In vivo* imaging and *in vivo* distribution of an exemplary antibody of the invention. SPECT imaging in LNCap xenograft mice that received an anti-PSMA, FcRn-K-DOTA-Lu-modified antibody of the invention.

Figure 12: Blood pharmacokinetics of an exemplary antibody of the invention. Levels of radioactivity measured in blood of mice following administration of an anti-PSMA, FcRn-K-DOTA-Lu-modified antibody of the invention.

Figure 13: Efficacy study in LNCap bearing xenograft mice that were treated with an exemplary antibody of the invention. Treatment with an anti-PSMA, K-DOTA-Lu FcRn-modified antibody of the invention significantly suppressed tumour growth as evidenced by no change in tumour volume on day 14 as compared to day 0. In the control (PBS) group, there was an overall increase in tumour volume with tumours becoming significant larger at 9, 12 and 14 days when compared to the corresponding time in FcRn-K-DOTA-Lu treated group.

Figure 14: Tumour:blood ratios in LNCap- bearing xenograft mice following administration of an exemplary antibody of the invention. Tumour:blood ratios are shown for mice that received treatment with an anti-PSMA, K-DOTA-Lu antibody of the invention that is modified to reduce FcRn-binding (HuX592R-DOTA-Lu177). Control mice were administered an anti-PSMA, K-DOTA-Lu antibody (HuJ591-DOTA-Lu177). The ratio is higher for the mice that received the FcRn modified antibody compared to mice that received the unmodified antibody, particularly at 24 hour and 48 hour time points.

Figure 15: ¹⁷⁷Lu-labelled HuX592R and HuJ591 biodistribution in healthy male Balb/c nude mice measured by *ex vivo* gamma counting. A shows HuX592R biodistribution assessed at 24, 48 and 72 hours post administration while B compares biodistribution of HuX592R with HuJ591 at 72 hours post administration.

Figure 16: Regression of LNCaP xenograft tumours in each therapeutic cohort following administration of HuX592R (FcRn-K-DOTA-Lu) or no treatment control.

Figure 17: Plot of cohort survival over the day period of the study following administration of TLX592 (FcRn-K-DOTA-Lu), TLX591 (K-DOTA-Lu antibody, also referred to herein as HuJ591-DOTA-Lu177) or no treatment control.

Detailed description of the embodiments

5 It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

10 Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

15 Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

20 The present invention is directed in part to the identification of a new approach for reducing the toxicity of radioimmunoconjugates for use in radioimmunotherapy. In particular, the method of the present invention reduces toxicity without significantly impacting on the therapeutic potential of the radioimmunoconjugates.

 As previously outlined above, radiation damage to healthy tissues and cells is a major problem associated with radioimmunotherapy. The toxicity severely limits the radiation dosage of RAIT and reduces the effectiveness of tumour treatment.

25 However, the present inventors have developed antibodies for use in radioimmunotherapy that have reduced serum half-life compared to wild-type antibodies by virtue of modifications to the constant heavy chain of the antibody, reducing the affinity of the antibodies for the neonatal Fc receptor (FcRn). The antibodies developed by the present inventors therefore have significant benefits for use in various
30 immunotherapies.

While modification of the FcRn-binding domain of therapeutic antibodies has previously been reported, such modifications have been developed with the aim of *increasing* FcRn affinity, for example to increase serum half-life of therapeutic antibodies and prolong residence of the therapeutic antibodies in the circulation. In contrast to the approaches of the prior art, the present invention is aimed at *reducing* serum half-life of a therapeutic agent.

Surprisingly, the inventors have found that despite reducing serum half-life (and increasing the rate of clearance from the systemic circulation following administration), the antibodies of the present invention have a similar capacity as unmodified antibodies to be delivered to and to accumulate at the tumour site. These results are surprising in that they show that tumour loading for modified and unmodified antibodies is not statistically different, indicating that the approach adopted by the present inventors, while significantly reducing serum half-life and thereby toxicity, does not negatively impact on the ability of the antibodies to bind to their target epitopes, nor on the capacity of the antibodies to be delivered to the target sites.

More importantly, the inventors have shown that the modified antibodies of the present invention remain resident at the tumour site, despite having increased clearance. The work of the present inventors therefore indicates that modification of the FcRn or the FcRn and Fc gamma receptor binding domains of radiolabelled antibodies has significant utility in reducing the amount of radioisotope in the circulation, without impacting on the therapeutic potential of the antibody with respect to its capacity to accumulate in the tumour. This has numerous benefits, including reducing a number of toxic effects which would otherwise result from longer-term residence of radioisotope in the circulation (including haematological toxicity, as a result of bone marrow irradiation and absorption into bone). Moreover, considering that the dose-limiting toxicity for many RAIT therapies thus far has been haematologic toxicities as a direct result of this extended blood circulation and irradiation of the bone marrow, this inventions affords the likelihood of acceptable dosing at higher levels; thus leading to a more effective therapy. absorption into bone and bone marrow irradiation).

Unexpectedly, the inventors have also found that the amino acid modifications to the constant heavy chain, while abrogating and FcRn binding does not impact on the ability of the antibodies to bind to Protein G and some Protein A purification resins.

Thus the antibodies of the present invention, having reduced serum half-life compared to other immunotherapeutics, can be produced using the same existing/ standardised production platform developed for regular antibodies. This is a key advantage over several of the many other engineered antibody formats e.g. minibodies, diabodies etc, which are cumbersome to produce and less stable than IgG molecules. Also, as a molecule format that is 'native' to the body, full-length antibodies also tend to have reduced likelihood of an immunogenic response than engineered antibodies or antibody fragments.

A further advantage of the antibodies of the present invention is their particular suitability for application in the field of theranostics. More specifically, as described above, the reduced serum half-life of the antibodies, when coupled to a radioactive isotope, makes the antibodies particularly useful for therapy, since the antibodies are able to deliver a suitable amount of radioactive agent to the tumour, while being rapidly cleared from the circulation. In addition, the reduced serum half-life of the antibodies makes them particularly suitable for use in diagnostic methods, where rapid clearing of the radioisotope coupled to the antibody and selected for imaging is desirable. The use of the antibody in the first instance as a diagnostic thereby informs the use and dosing of the therapeutic form of the antibody (i.e., when the antibody is coupled to a radioisotope that is suitable for therapy). Thus the antibodies of the invention find utility when coupled to different radioisotopes and subsequently employed as a "theranostic pair".

General

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter. Thus, as used herein, the singular forms "a", "an" and "the" include plural aspects, and vice versa, unless the context clearly dictates otherwise. For example, reference to "a" includes a single as well as two or more; reference to "an" includes a single as well as two or more; reference to "the" includes a single as well as two or more and so forth.

Those skilled in the art will appreciate that the present invention is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present invention. The present invention is in no way limited to the methods and materials described.

All of the patents and publications referred to herein are incorporated by reference in their entirety.

The present invention is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present invention.

Any example or embodiment of the present invention herein shall be taken to apply *mutatis mutandis* to any other example or embodiment of the invention unless specifically stated otherwise.

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991),

D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory
5 Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

The description and definitions of variable regions and parts thereof, immunoglobulins, antibodies and fragments thereof herein may be further clarified by
10 the discussion in Kabat Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md., 1987 and 1991, Bork et al., J Mol. Biol. 242, 309-320, 1994, Chothia and Lesk J. Mol Biol. 196:901 -917, 1987, Chothia et al. Nature 342, 877-883, 1989 and/or or Al-Lazikani et al., J Mol Biol 273, 927-948, 1997.

The term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and
15 Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

As used herein the term "derived from" shall be taken to indicate that a specified integer may be obtained from a particular source albeit not necessarily directly from that source.

20 *Selected Definitions*

As used herein, tumour:blood ratio refers to the ratio of the amount of an antibody (or radiolabelled antibody) to the amount of the same antibody in the blood of an individual. The skilled person will be familiar with standard techniques for calculating tumour:blood ratios. For example, *ex vivo* activity concentrations of radioisotope (or
25 labelled antibody) are measured and expressed as percent of the decay-corrected injected activity per gram of tissue (or blood) and approximated as percentage injected dose/g (%ID/g). The tumour to blood ratio is then calculated as the activity detected in tumour relative to the activity detected in blood.

As used herein, the term 'theranostic' refers to the ability of compounds/materials
30 to be used for diagnosis as well as for therapy. The term "theranostics reagent" relates

to any reagent which is both suitable for detection, diagnostic and/or the treatment of a disease or condition of a patient. The aim of theranostic compounds/materials is to overcome undesirable differences in biodistribution and selectivity, which can exist between distinct diagnostic and therapeutic agents. With a theranostic pair, the theranostic compound containing the imaging radionuclide is first administered to the patient in order to identify the disease or to locate the affected area in the body. Once identified/located, the disease can be treated by administering the theranostic compound containing the therapeutic radionuclide in a target specific way as the biodistribution of the imaging and therapy radionuclides are the same.

In the context of the present invention, the antibodies of the invention are particularly useful for inclusion in theranostic pairs, for example, where the antibody is conjugated to a radioisotope for imaging or diagnostic purposes, and the same antibody is conjugated with a different radioisotope or a cytotoxic agent which is suitable for therapy. The antigen binding site of the antibody directs or targets the diagnostic radioisotope to the site of the tumour to facilitate diagnosis (including tumour distribution, tumour size, tumour density), while the same antigen binding site of the antibody directs the radioisotope to the tumour for therapy.

The term "Fc region", sometimes referred to as "Fc" or "Fc domain", as used herein refers the portion of an IgG molecule that correlates to a crystallizable fragment obtained by papain digestion of an IgG molecule. The Fc region consists of the C-terminal half of the two heavy chains of an IgG molecule that are linked by disulfide bonds. It has no antigen binding activity but contains the carbohydrate moiety and the binding sites for complement and Fc receptors, including the FcRn receptor. The Fc region contains the entire second constant domain CH2 (residues 231-340 of human IgG1, according to the EU Index numbering system, also defined as residues 244 to 360 in the Kabat system) and the third constant domain CH3 (residues 341-447 EU Index/361-478 Kabat) (e.g., see SEQ ID NO 1 of WO2015175874 or Fig. 1C for the sequence of CH2 and SEQ ID NO:2; Fig. 1D for the sequence of CH3, incorporated herein by reference; see also http://www.imgt.org/IMGTScientificChart/Numbering/Hu_IGHGnber.html#refs for a comparison of the numbering conventions used for various residues in the Fc region of immunoglobulins).

As used herein, the "EU index" or "EU numbering scheme" refers to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85, hereby entirely incorporated by reference.) As used herein, the "Kabat system" refers to the Kabat Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md., 1987 and 1991. The skilled person will be able to readily determine whether a given amino acid sequence is numbered according to either EU or Kabat systems.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally-associated components that accompany it in its native state; is substantially free of other proteins from the same source. A protein may be rendered substantially free of naturally associated components or substantially purified by isolation, using protein purification techniques known in the art. By "substantially purified" is meant the protein is substantially free of contaminating agents, e.g., at least about 70% or 75% or 80% or 85% or 90% or 95% or 96% or 97% or 98% or 99% free of contaminating agents.

The term "recombinant" shall be understood to mean the product of artificial genetic recombination. Accordingly, in the context of a recombinant protein comprising an antibody antigen binding domain, this term does not encompass an antibody naturally-occurring within a subject's body that is the product of natural recombination that occurs during B cell maturation. However, if such an antibody is isolated, it is to be considered an isolated protein comprising an antibody antigen binding domain. Similarly, if nucleic acid encoding the protein is isolated and expressed using recombinant means, the resulting protein is a recombinant protein comprising an antibody antigen binding domain. A recombinant protein also encompasses a protein expressed by artificial recombinant means when it is within a cell, tissue or subject, e.g., in which it is expressed.

The term "protein" shall be taken to include a single polypeptide chain, i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). For example, the series of polypeptide chains can be covalently linked using a suitable chemical or a disulphide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions.

The term "polypeptide" or "polypeptide chain" will be understood from the foregoing paragraph to mean a series of contiguous amino acids linked by peptide bonds.

As used herein, the term "antigen binding site" is used interchangeably with "antigen binding domain" and shall be taken to mean a region of an antibody that is capable of specifically binding to an antigen, i.e., a VH or a VL or an Fv comprising both a VH and a VL. The antigen binding domain need not be in the context of an entire antibody, e.g., it can be in isolation (e.g., a domain antibody) or in another form, e.g., as described herein, such as a scFv.

For the purposes for the present disclosure, the term "antibody" includes a protein capable of specifically binding to one or a few closely related antigens by virtue of an antigen binding domain contained within a Fv. This term includes four chain antibodies (e.g., two light chains and two heavy chains), recombinant or modified antibodies (e.g., chimeric antibodies, humanized antibodies, human antibodies, CDR-grafted antibodies, primatized antibodies, de-immunized antibodies, synhumanized antibodies, half-antibodies, bispecific antibodies). An antibody generally comprises constant domains, which can be arranged into a constant region or constant fragment or fragment crystallizable (Fc). Exemplary forms of antibodies comprise a four-chain structure as their basic unit. Full-length antibodies comprise two heavy chains (~50 to 70 kD) covalently linked and two light chains (~23 kDa each). A light chain generally comprises a variable region (if present) and a constant domain and in mammals is either a κ light chain or a λ light chain. A heavy chain generally comprises a variable region and one or two constant domain(s) linked by a hinge region to additional constant domain(s). Heavy chains of mammals are of one of the following types α , δ , ϵ , γ , or μ . Each light chain is also covalently linked to one of the heavy chains. For example, the two heavy chains and the heavy and light chains are held together by inter-chain disulfide bonds and by non-covalent interactions. The number of inter-chain disulfide bonds can vary among different types of antibodies. Each chain has an N-terminal variable region (VH or VL wherein each are ~110 amino acids in length) and one or more constant domains at the C- terminus. The constant domain of the light chain (CL which is ~110 amino acids in length) is aligned with and disulfide bonded to the first constant domain of the heavy chain (CH1 which is 330 to 440 amino acids in

length). The light chain variable region is aligned with the variable region of the heavy chain. The antibody heavy chain can comprise 2 or more additional CH domains (such as, CH2, CH3 and the like) and can comprise a hinge region between the CH1 and CH2 constant domains. Antibodies can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass. In one example, the antibody is a murine (mouse or rat) antibody or a primate (such as, human) antibody. In one example the antibody heavy chain is missing a C-terminal lysine residue. In one example, the antibody is humanized, synhumanized, chimeric, CDR-grafted or deimmunized.

The terms "full-length antibody", "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antigen binding fragment of an antibody. Specifically, whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be wild-type sequence constant domains (e.g., human wild-type sequence constant domains) or amino acid sequence variants thereof.

As used herein, "variable region" refers to the portions of the light and/or heavy chains of an antibody as defined herein that is capable of specifically binding to an antigen and, includes amino acid sequences of complementarity determining regions (CDRs); i.e., CDR1, CDR2, and CDR3, and framework regions (FRs). For example, the variable region comprises three or four FRs (e.g., FR1, FR2, FR3 and optionally FR4) together with three CDRs. VH refers to the variable region of the heavy chain. VL refers to the variable region of the light chain.

As used herein, the term "complementarity determining regions" (syn. CDRs; i.e., CDR1, CDR2, and CDR3) refers to the amino acid residues of an antibody variable region the presence of which are major contributors to specific antigen binding. Each variable region domain (VH or VL) typically has three CDRs identified as CDR1, CDR2 and CDR3. The CDRs of VH are also referred to herein as CDR H1, CDR H2 and CDR H3, respectively, wherein CDR H1 corresponds to CDR 1 of VH, CDR H2 corresponds to CDR 2 of VH and CDR H3 corresponds to CDR 3 of VH. Likewise, the CDRs of VL are referred to herein as CDR L1, CDR L2 and CDR L3, respectively, wherein CDR L1 corresponds to CDR 1 of VL, CDR L2 corresponds to CDR 2 of VL and CDR L3 corresponds to CDR 3 of VL. In one example, the amino acid positions assigned to

CDRs and FRs are defined according to Kabat Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, Md., 1987 and 1991 (also referred to herein as "the Kabat numbering system"). In another example, the amino acid positions assigned to CDRs and FRs are defined according to the Enhanced Chothia Numbering Scheme (<http://www.bioinfo.org.uk/mdex.html>). The present invention is not limited to FRs and CDRs as defined by the Kabat numbering system, but includes all numbering systems, including the canonical numbering system or of Chothia and Lesk J. Mol. Biol. 196: 901-917, 1987; Chothia et al., Nature 342: 877-883, 1989; and/or Al-Lazikani et al., J. Mol. Biol. 273: 927-948, 1997; the numbering system of Honnegger and Plückthun J. Mol. Biol. 309: 657-670, 2001; or the IMGT system discussed in Giudicelli et al., Nucleic Acids Res. 25: 206-211 1997. In one example, the CDRs are defined according to the Kabat numbering system. Optionally, heavy chain CDR2 according to the Kabat numbering system does not comprise the five C-terminal amino acids listed herein or any one or more of those amino acids are substituted with another naturally-occurring amino acid. In this regard, Padlan et al., FASEB J., 9: 133-139, 1995 established that the five C-terminal amino acids of heavy chain CDR2 are not generally involved in antigen binding.

"Framework regions" (FRs) are those variable region residues other than the CDR residues. The FRs of VH are also referred to herein as FR H1, FR H2, FR H3 and FR H4, respectively, wherein FR H1 corresponds to FR 1 of VH, FR H2 corresponds to FR 2 of VH, FR H3 corresponds to FR 3 of VH and FR H4 corresponds to FR 4 of VH. Likewise, the FRs of VL are referred to herein as FR L1, FR L2, FR L3 and FR L4, respectively, wherein FR L1 corresponds to FR 1 of VL, FR L2 corresponds to FR 2 of VL, FR L3 corresponds to FR 3 of VL and FR L4 corresponds to FR 4 of VL.

As used herein, the term "Fv" shall be taken to mean any protein, whether comprised of multiple polypeptides or a single polypeptide, in which a VL and a VH associate and form a complex having an antigen binding domain, i.e., capable of specifically binding to an antigen. The VH and the VL which form the antigen binding domain can be in a single polypeptide chain or in different polypeptide chains. Furthermore, an Fv of the invention (as well as any protein of the invention) may have multiple antigen binding domains which may or may not bind the same antigen. This term shall be understood to encompass fragments directly derived from an antibody as

well as proteins corresponding to such a fragment produced using recombinant means. In some examples, the VH is not linked to a heavy chain constant domain (CH) 1 and/or the VL is not linked to a light chain constant domain (CL). Exemplary Fv containing polypeptides or proteins include a Fab fragment, a Fab' fragment, a F(ab') fragment, a scFv, a diabody, a triabody, a tetrabody or higher order complex, or any of the foregoing linked to a constant region or domain thereof, e.g., CH2 or CH3 domain, e.g., a minibody. A "Fab fragment" consists of a monovalent antigen-binding fragment of an immunoglobulin, and can be produced by digestion of a whole antibody with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain or can be produced using recombinant means. A "Fab' fragment" of an antibody can be obtained by treating a whole antibody with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain comprising a VH and a single constant domain. Two Fab' fragments are obtained per antibody treated in this manner. A Fab' fragment can also be produced by recombinant means. A "F(ab')₂ fragment" of an antibody consists of a dimer of two Fab' fragments held together by two disulfide bonds, and is obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A "Fab₂" fragment is a recombinant fragment comprising two Fab fragments linked using, for example a leucine zipper or a CH3 domain. A "single chain Fv" or "scFv" is a recombinant molecule containing the variable region fragment (Fv) of an antibody in which the variable region of the light chain and the variable region of the heavy chain are covalently linked by a suitable, flexible polypeptide linker.

As used herein, the term "binds" in reference to the interaction of an antigen binding site or an antigen binding domain thereof with an antigen means that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the antigen. For example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody binds to epitope "A", the presence of a molecule containing epitope "A" (or free, unlabelled "A"), in a reaction containing labeled "A" and the protein, will reduce the amount of labelled "A" bound to the antibody.

As used herein, the term "specifically binds" or "binds specifically" shall be taken to mean that an antigen binding site of the invention reacts or associates more

frequently, more rapidly, with greater duration and/or with greater affinity with a particular antigen or cell expressing same than it does with alternative antigens or cells. For example, an antigen binding site binds to PSMA with materially greater affinity (e.g., 1.5 fold or 2 fold or 5 fold or 10 fold or 20 fold or 40 fold or 60 fold or 80 fold to 100 fold or 150 fold or 200 fold) than it does to other antigens.

As used herein, the term "epitope" (syn. "antigenic determinant") shall be understood to mean a region of a cell surface protein (such as PSMA) to which an antigen binding site comprising an antigen binding domain of an antibody binds.

As used herein, the term "condition" refers to a disruption of or interference with normal function, and is not to be limited to any specific condition, and will include diseases or disorders.

As used herein, the terms "preventing", "prevent" or "prevention" include administering an antigen binding site of the invention to thereby stop or hinder the development of at least one symptom of a condition. This term also encompasses treatment of a subject in remission to prevent or hinder relapse.

As used herein, the terms "treating", "treat" or "treatment" include administering an antigen binding site described herein to thereby reduce or eliminate at least one symptom of a specified disease or condition.

As used herein, the term "subject" shall be taken to mean any animal including humans, for example a mammal. Exemplary subjects include but are not limited to humans and non-human primates. For example, the subject is a human.

Modified antibodies

The present invention relates in part to modifications to IgG antibodies, which include one or more amino acid substitutions to a region of the antibody which reduces or abolishes the affinity of the antibody for FcRn, thereby reducing the serum half-life of the antibodies.

It will be understood that in accordance with the present invention, any antibody for which reduced serum half-life is desired can be modified according to the methods described herein. Moreover, it will be understood that in preferred embodiments, the

antibody that is modified to reduce serum half-life is an antibody that is useful in diagnostic or therapeutic applications, and more specifically in theranostic applications.

Examples of suitable antibodies for use in accordance with the methods of the present invention include, trastuzumab (Herceptin®), rituximab (Rituxan®),
5 bevacizumab (Avastin®), dinutuximab (Unituxin®), panitumumab (Vectibix®), pembrolizumab (Keytruda®), nivolumab (Opdivo®), tositumomab (Bexxar®) ibratumomab (Zevalin®). However, it will also be understood that the present invention is not intended to be limited to the specific antibody, provided that the antibody would otherwise be susceptible to binding by FcRn.

10 The present invention also contemplates the use of antibody drug conjugates for targeting tumour antigens, wherein the conjugates include cytotoxic payload. Examples of such antibodies include gemtuzumab (Mylotarg®), brentuximab (Adcetris®), Inotuzumab (Besponsa®), glembatumumab (CDX-011), anetumab (BAY 94-9343), mirvetuximab (IMGN853) depatuxizumab (ABT-414), rovalpituzumab (Rova-T) and
15 vadastuximab talirine (SGN-CD33A). Further examples are described in Lambert et al., 2017, Adv Ther (2017) 34:1015–1035, incorporated herein by reference.

In certain embodiments, the antibodies suitable for modification according the present invention, to reduce affinity for FcRn, are antibodies having one or more of the sequences as shown in Table 1.

20 The present invention also provides an antigen binding site or a nucleic acid encoding same having at least 80% identity to a sequence disclosed herein. In one example, an antigen binding site or nucleic acid of the invention comprises sequence at least about 85% or 90% or 95% or 97% or 98% or 99% identical to a sequence disclosed herein.

25 Alternatively, or additionally, the antigen binding site comprises a CDR (e.g., three CDRs) at least about 80% or 85% or 90% or 95% or 97% or 98% or 99% identical to CDR(s) of a V_H or V_L as described herein according to any example.

In another example, a nucleic acid of the invention comprises a sequence at least about 80% or 85% or 90% or 95% or 97% or 98% or 99% identical to a sequence
30 encoding an antigen binding site having a function as described herein according to any

example. The present invention also encompasses nucleic acids encoding an antigen binding site of the invention, which differs from a sequence exemplified herein as a result of degeneracy of the genetic code.

5 The % identity of a nucleic acid or polypeptide is determined by GAP (Needleman and Wunsch. *Mol. Biol.* 48, 443-453, 1970) analysis (GCG program) with a gap creation penalty=5, and a gap extension penalty=0.3. The query sequence is at least 50 residues in length, and the GAP analysis aligns the two sequences over a region of at least 50 residues. For example, the query sequence is at least 100 residues in length and the GAP analysis aligns the two sequences over a region of at
10 least 100 residues. For example, the two sequences are aligned over their entire length.

The present invention also contemplates a nucleic acid that hybridizes under stringent hybridization conditions to a nucleic acid encoding an antigen binding site described herein. A "moderate stringency" is defined herein as being a hybridization
15 and/or washing carried out in 2 x SSC buffer, 0.1% (w/v) SDS at a temperature in the range 45°C to 65°C, or equivalent conditions. A "high stringency" is defined herein as being a hybridization and/or wash carried out in 0.1 x SSC buffer, 0.1% (w/v) SDS, or lower salt concentration, and at a temperature of at least 65°C, or equivalent conditions. Reference herein to a particular level of stringency encompasses equivalent conditions
20 using wash/hybridization solutions other than SSC known to those skilled in the art. For example, methods for calculating the temperature at which the strands of a double stranded nucleic acid will dissociate (also known as melting temperature, or T_m) are known in the art. A temperature that is similar to (e.g., within 5°C or within 10°C) or equal to the T_m of a nucleic acid is considered to be high stringency. Medium
25 stringency is to be considered to be within 10°C to 20°C or 10°C to 15°C of the calculated T_m of the nucleic acid.

The present invention also contemplates mutant forms of an antigen binding site of the invention comprising one or more conservative amino acid substitutions compared to a sequence set forth herein. In some examples, the antigen binding site
30 comprises 10 or fewer, e.g., 9 or 8 or 7 or 6 or 5 or 4 or 3 or 2 or 1 conservative amino acid substitutions. A "conservative amino acid substitution" is one in which the amino

acid residue is replaced with an amino acid residue having a similar side chain and/or hydrophobicity and/or hydrophilicity.

Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), β -branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Hydrophobic indices are described, for example in Kyte and Doolittle *J. Mol. Biol.*, 157: 105-132, 1982 and hydrophilic indices are described in, e.g., US4554101.

The present invention also contemplates non-conservative amino acid changes. For example, of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or positively charged amino acids. In some examples, the antigen binding site comprises 10 or fewer, e.g., 9 or 8 or 7 or 6 or 5 or 4 or 3 or 2 or 1 non-conservative amino acid substitutions.

In one example, the mutation(s) occur within a FR of an antigen binding domain of an antigen binding site of the invention. In another example, the mutation(s) occur within a CDR of an antigen binding site of the invention.

Exemplary methods for producing mutant forms of an antigen binding site include:

- mutagenesis of DNA (Thie *et al.*, *Methods Mol. Biol.* 525: 309-322, 2009) or RNA (Kopsidas *et al.*, *Immunol. Lett.* 107:163-168, 2006; Kopsidas *et al.* *BMC Biotechnology*, 7: 18, 2007; and WO1999/058661);
- introducing a nucleic acid encoding the polypeptide into a mutator cell, e.g., XL-1Red, XL-mutS and XL-mutS-Kanr bacterial cells (Stratagene);
- DNA shuffling, e.g., as disclosed in Stemmer, *Nature* 370: 389-91, 1994; and

- site directed mutagenesis, e.g., as described in Dieffenbach (ed) and Dveksler (ed) (*In: PCR Primer: A Laboratory Manual*, Cold Spring Harbor Laboratories, NY, 1995).

Exemplary methods for determining biological activity of the mutant antigen binding sites of the invention will be apparent to the skilled artisan and/or described herein, e.g., antigen binding. For example, methods for determining antigen binding, competitive inhibition of binding, affinity, association, dissociation and therapeutic efficacy are described herein.

Constant Regions

10 The present invention encompasses antigen binding sites and/or antibodies described herein comprising a constant region of an antibody. This includes antigen binding fragments of an antibody fused to an Fc.

Sequences of constant regions useful for producing the proteins of the present invention may be obtained from a number of different sources. In some examples, the constant region or portion thereof of the protein is derived from a human antibody. The constant region or portion thereof may be derived from any antibody class, including IgM, IgG, IgD, IgA and IgE, and any antibody isotype, including IgG1, IgG2, IgG3 and IgG4. In one example, the constant region is human isotype IgG4 or a stabilized IgG4 constant region.

20 Preferred Modifications

The present invention specifically contemplates modifications to an antibody or antigen binding site comprising an Fc region or constant region.

The neonatal Fc-receptor (FcRn) is important for the metabolic fate of antibodies of the IgG class *in vivo*. The FcRn functions to salvage IgG from the lysosomal degradation pathway, resulting in reduced clearance and increased half-life. It is a heterodimeric protein consisting of two polypeptides: a 50 kDa class I major histocompatibility complex-like protein (α -FcRn) and a 15 kDa p2-microglobulin (β 2m). FcRn binds with high affinity to the CH2-CH3 portion of the Fc-region of an antibody of the class IgG. The interaction between an antibody of the class IgG and the FcRn is pH

dependent and occurs in a 1 :2 stoichiometry, i.e. one IgG antibody molecule can interact with two FcRn molecules via its two heavy chain Fc-region polypeptides (see e.g. Huber, A.H., et al, J. Mol. Biol. 230 (1993) 1077-1083).

Thus, an IgG's *in vitro* FcRn binding properties/characteristics are indicative of its
5 in vivo pharmacokinetic properties in the blood circulation. In the interaction between the FcRn and the Fc-region of an antibody of the IgG class different amino acid residues of the heavy chain CH2- and CH3 -domain are participating.

Different mutations that influence the FcRn binding and therewith the half-live in the blood circulation are known. Fc-region residues critical to the mouse Fc-region-
10 mouse FcRn interaction have been identified by site-directed mutagenesis (see e.g. Dall'Acqua, W.F., et al. J. Immunol 169 (2002) 5171-5180). Residues Ile253, His310, His433, Asn434 and His435 (numbering according to EU index numbering system) are involved in the interaction (Medesan, C, et al., Eur. J. Immunol. 26 (1996) 2533-2536; Firan, M., et al, Int. Immunol. 13 (2001) 993-1002; Kim, J.K., et al, Eur. J. Immunol. 24
15 (1994) 542-548). (Using the Kabat system, the relevant residues are Ile266, His329, His464, Asn465 and His466). Residues Ile253, His310, and His435 were found to be critical for the interaction of human Fc-region with murine FcRn (Kim, J.K., et al, Eur. J. Immunol. 29 (1999) 2819-2885).

More specifically, the antibody may comprise one or more amino acid
20 substitutions that decrease the half-life of the protein. For example, the antibody comprises a Fc region comprising one or more amino acid substitutions that decrease the affinity of the Fc region for the neonatal Fc region (FcRn).

The present invention also provides for an antibody having a constant region substantially identical to a naturally occurring class IgG antibody constant region
25 wherein at least one amino acid residue selected from the group consisting of residues His310, His435, and Ile253 is different from that present in the naturally occurring class IgG antibody, thereby altering FcRn binding affinity and/or serum half-life of said antibody relative to the naturally occurring antibody. In preferred embodiments, the naturally occurring class IgG antibody comprises a heavy chain constant region of a
30 human IgG1, IgG2, IgG2M3, IgG3 or IgG4 molecule.

Also in preferred embodiments, amino acid residue 310 or residue 435 from the heavy chain constant region of the antibody having a constant region substantially identical to the naturally occurring class IgG antibody is any amino acid that is not histidine and which reduces the affinity of the constant region for FcRn. For example, the amino acid at residue 310 or 435 may be alanine, glutamic acid, aspartic acid, leucine, isoleucine, arginine, proline, glutamine, methionine, serine, threonine, lysine, asparagine, phenylalanine, tyrosine, tryptophan, cysteine, valine or glycine.

Preferably, the residue at position 310 is selected from alanine, or glutamic acid or glutamine; or amino acid residue 435 from the heavy chain constant region is selected from arginine, glutamine or alanine. In other preferred embodiments, the antibody having a constant region substantially identical to a naturally occurring class IgG antibody has an alanine residue at position 310 and glutamine residue at position 435.

In a preferred embodiment of the present invention, the binding affinity for FcRn and/or the serum half-life of the modified antibody is decreased by at least about 30%, 50%, 80%, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold, 25-fold, 30-fold, 40-fold, 50-fold, 60-fold, 70-fold, 80-fold, 90-fold, or 100-fold. In a preferred embodiment of the present invention, the binding affinity for FcRn and/or the serum half-life of said modified antibody is reduced by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 98%, or 99%.

In addition, the antibodies of the present invention may comprise one or more mutations which modify the affinity of the antibodies for any one or more Fc gamma receptors.

In preferred embodiments of the invention, the Fc region of the constant region retains the ability to induce effector functions. In one example, the Fc region of the constant region contains one or more amino acid substitutions that modulate effector function, including increasing effector function compared to a wild-type IgG.

In one example, the Fc region of the constant region has a reduced ability to induce effector function, e.g., compared to a native or wild-type human IgG1 or IgG3 Fc region. In one example, the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC) and/or antibody-dependent cell-mediated phagocytosis (ADCP)

and/or complement-dependent cytotoxicity (CDC). Methods for assessing the level of effector function of an Fc region containing protein are known in the art and/or described herein.

5 In one example, the amino acid substitution that modifies that ability of the antibody to induce effector function is an amino acid substitution at residue Ile253 from the heavy chain constant region. In one example, the substitution is to any amino acid selected from be alanine, glutamic acid, aspartic acid, leucine, isoleucine, arginine, proline, glutamine, methionine, serine, threonine, lysine, asparagine, phenylalanine, tyrosine, tryptophan, cysteine, valine or glycine, wherein the substitution reduces the
10 ability of the antibody to induce effector function. In preferred embodiments, the substitution from Ile at residue 253 is to arginine, proline, or aspartate, more preferably alanine.

In one example, the Fc region is an IgG4 Fc region (i.e., from an IgG4 constant region), e.g., a human IgG4 Fc region. Sequences of suitable IgG4 Fc regions will be
15 apparent to the skilled person and/or available in publicly available databases (e.g., available from National Center for Biotechnology Information).

In one example, the constant region is a stabilized IgG4 constant region. The term "stabilized IgG4 constant region" will be understood to mean an IgG4 constant region that has been modified to reduce Fab arm exchange or the propensity to
20 undergo Fab arm exchange or formation of a half-antibody or a propensity to form a half antibody. "Fab arm exchange" refers to a type of protein modification for human IgG4, in which an IgG4 heavy chain and attached light chain (half-molecule) is swapped for a heavy-light chain pair from another IgG4 molecule. Thus, IgG4 molecules may acquire two distinct Fab arms recognizing two distinct antigens (resulting in bispecific
25 molecules). Fab arm exchange occurs naturally *in vivo* and can be induced *in vitro* by purified blood cells or reducing agents such as reduced glutathione. A "half antibody" forms when an IgG4 antibody dissociates to form two molecules each containing a single heavy chain and a single light chain.

In one example, a stabilized IgG4 constant region comprises a proline at position
30 241 of the hinge region according to the system of Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest Washington DC United States Department of Health

and Human Services, 1987 and/or 1991). This position corresponds to position 228 of the hinge region according to the EU numbering system. In human IgG4, this residue is generally a serine. Following substitution of the serine for proline, the IgG4 hinge region comprises a sequence CPPC. In this regard, the skilled person will be aware that the

5 “hinge region” is a proline-rich portion of an antibody heavy chain constant region that links the Fc and Fab regions that confers mobility on the two Fab arms of an antibody. The hinge region includes cysteine residues which are involved in inter-heavy chain disulfide bonds. It is generally defined as stretching from Glu226 to Pro243 of human IgG1 according to the numbering system of Kabat (or Glu216 to Pro230 using the EU

10 index). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain disulphide (S-S) bonds in the same positions (see for example WO2010/080538).

Additional examples of stabilized IgG4 antibodies are antibodies in which arginine at position 409 in a heavy chain constant region of human IgG4 (according to

15 the EU numbering system) is substituted with lysine, threonine, methionine, or leucine (e.g., as described in WO2006/033386). The Fc region of the constant region may additionally or alternatively comprise a residue selected from the group consisting of: alanine, valine, glycine, isoleucine and leucine at the position corresponding to 405 (according to the EU numbering system). Optionally, the hinge region comprises a

20 proline at position 241 (i.e., a CPPC sequence) (as described above).

In another example, the Fc region is a region modified to have reduced effector function, i.e., a “non-immunostimulatory Fc region”. For example, the Fc region is an IgG1 Fc region comprising a substitution at one or more positions selected from the group consisting of 268, 309, 330 and 331. In another example, the Fc region is an

25 IgG1 Fc region comprising one or more of the following changes E233P, L234V, L235A and deletion of G236 and/or one or more of the following changes A327G, A330S and P331S (Armour *et al.*, *Eur J Immunol.* 29:2613-2624, 1999; Shields *et al.*, *J Biol Chem.* 276(9):6591-604, 2001). Additional examples of non-immunostimulatory Fc regions are described, for example, in Dall'Acqua *et al.*, *J Immunol.* 177 : 1129-1138 2006; and/or

30 Hezareh *J Virol* ;75: 12161-12168, 2001).

In another example, the Fc region is a chimeric Fc region, e.g., comprising at least one CH2 domain from an IgG4 antibody and at least one CH3 domain from an IgG1

antibody, wherein the Fc region comprises a substitution at one or more amino acid positions selected from the group consisting of 240, 262, 264, 266, 297, 299, 307, 309, 323, 399, 409 and 427 (EU numbering) (e.g., as described in WO2010/085682). Exemplary substitutions include 240F, 262L, 264T, 266F, 297Q, 299A, 299K, 307P, 5 309K, 309M, 309P, 323F, 399S, and 427F.

Antibody Production

Preferably, an antigen binding site described herein according to any example is recombinant.

In the case of a recombinant protein, nucleic acid encoding same can be cloned 10 into expression constructs or vectors, which are then transfected into host cells, such as *E. coli* cells, yeast cells, insect cells, or mammalian cells, such as simian COS cells, Chinese Hamster Ovary (CHO) cells, human embryonic kidney (HEK) cells, or myeloma cells that do not otherwise produce the protein. Exemplary cells used for expressing a protein are CHO cells, myeloma cells or HEK cells. Molecular cloning techniques to 15 achieve these ends are known in the art and described, for example in Ausubel *et al.*, (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) or Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989). A wide variety of cloning and *in vitro* amplification methods are suitable for the construction of 20 recombinant nucleic acids. Methods of producing recombinant antibodies are also known in the art, see, e.g., US4816567 or US5530101.

Following isolation, the nucleic acid is inserted operably linked to a promoter in an expression construct or expression vector for further cloning (amplification of the DNA) or for expression in a cell-free system or in cells.

25 As used herein, the term "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a genomic gene, including the TATA box or initiator element, which is required for accurate transcription initiation, with or without additional regulatory elements (e.g., upstream activating sequences, transcription factor binding sites, enhancers and silencers) that alter expression of a 30 nucleic acid, e.g., in response to a developmental and/or external stimulus, or in a tissue specific manner. In the present context, the term "promoter" is also used to

describe a recombinant, synthetic or fusion nucleic acid, or derivative which confers, activates or enhances the expression of a nucleic acid to which it is operably linked. Exemplary promoters can contain additional copies of one or more specific regulatory elements to further enhance expression and/or alter the spatial expression and/or
5 temporal expression of said nucleic acid.

As used herein, the term "operably linked to" means positioning a promoter relative to a nucleic acid such that expression of the nucleic acid is controlled by the promoter.

Many vectors for expression in cells are available. The vector components
10 generally include, but are not limited to, one or more of the following: a signal sequence, a sequence encoding a protein (e.g., derived from the information provided herein), an enhancer element, a promoter, and a transcription termination sequence. The skilled artisan will be aware of suitable sequences for expression of a protein. Exemplary signal sequences include prokaryotic secretion signals (e.g., pelB, alkaline
15 phosphatase, penicillinase, lpp, or heat-stable enterotoxin II), yeast secretion signals (e.g., invertase leader, α factor leader, or acid phosphatase leader) or mammalian secretion signals (e.g., herpes simplex gD signal).

Exemplary promoters active in mammalian cells include cytomegalovirus immediate early promoter (CMV-IE), human elongation factor 1- α promoter (EF1), small
20 nuclear RNA promoters (U1a and U1b), α -myosin heavy chain promoter, Simian virus 40 promoter (SV40), Rous sarcoma virus promoter (RSV), Adenovirus major late promoter, β -actin promoter; hybrid regulatory element comprising a CMV enhancer/ β -actin promoter or an immunoglobulin promoter or active fragment thereof. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40
25 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture; baby hamster kidney cells (BHK, ATCC CCL 10); or Chinese hamster ovary cells (CHO).

Typical promoters suitable for expression in yeast cells such as for example a yeast cell selected from the group comprising *Pichia pastoris*, *Saccharomyces*
30 *cerevisiae* and *S. pombe*, include, but are not limited to, the *ADH1* promoter, the *GAL1*

promoter, the *GAL4* promoter, the *CUP1* promoter, the *PHO5* promoter, the *nmt* promoter, the *RPR1* promoter, or the *TEF1* promoter.

Means for introducing the isolated nucleic acid or expression construct comprising same into a cell for expression are known to those skilled in the art. The technique used for a given cell depends on the known successful techniques. Means for introducing recombinant DNA into cells include microinjection, transfection mediated by DEAE-dextran, transfection mediated by liposomes such as by using lipofectamine (Gibco, MD, USA) and/or cellfectin (Gibco, MD, USA), PEG-mediated DNA uptake, electroporation and microparticle bombardment such as by using DNA-coated tungsten or gold particles (Agracetus Inc., WI, USA) amongst others.

The host cells used to produce the protein may be cultured in a variety of media, depending on the cell type used. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing mammalian cells. Media for culturing other cell types discussed herein are known in the art.

Isolation of Proteins

Methods for isolating a protein are known in the art and/or described herein.

Where an antigen binding site is secreted into culture medium, supernatants from such expression systems can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants. Alternatively, or additionally, supernatants can be filtered and/or separated from cells expressing the protein, e.g., using continuous centrifugation.

The antigen binding site prepared from the cells can be purified using, for example, ion exchange, hydroxyapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, affinity chromatography (e.g., protein A affinity chromatography or protein G chromatography), or any combination of the

foregoing. These methods are known in the art and described, for example in WO99/57134 or Ed Harlow and David Lane (editors) *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, (1988).

The skilled artisan will also be aware that a protein can be modified to include a tag to facilitate purification or detection, e.g., a poly-histidine tag, e.g., a hexa-histidine tag, or an influenza virus hemagglutinin (HA) tag, or a Simian Virus 5 (V5) tag, or a FLAG tag, or a glutathione S-transferase (GST) tag. The resulting protein is then purified using methods known in the art, such as, affinity purification. For example, a protein comprising a hexa-his tag is purified by contacting a sample comprising the protein with nickel-nitrilotriacetic acid (Ni-NTA) that specifically binds a hexa-his tag immobilized on a solid or semi-solid support, washing the sample to remove unbound protein, and subsequently eluting the bound protein. Alternatively, or in addition a ligand or antibody that binds to a tag is used in an affinity purification method.

Linking of radioisotopes to antibodies

In any embodiment of the invention, the antibodies herein described may be directly or indirectly linked to a diagnostic or therapeutic agent. Preferably, the diagnostic or therapeutic agent is a radioisotope.

Examples of suitable isotopes include: actinium-225 (^{225}Ac), astatine-211 (^{211}At), bismuth-212 and bismuth-213 (^{212}Bi , ^{213}Bi), copper-64 and copper-67 (^{64}Cu , ^{67}Cu), gallium-67 and gallium-68 (^{67}Ga and ^{68}Ga), indium-111 (^{111}In), iodine -123, -124, -125 or -131 (^{123}I , ^{124}I , ^{125}I , ^{131}I) (^{123}I), lead-212 (^{212}Pb), lutetium-177 (^{177}Lu), radium-223 (^{223}Ra), samarium-153 (^{153}Sm), scandium-44 and scandium-47 (^{44}Sc , ^{47}Sc), strontium-90 (^{90}Sr), technetium-99 ($^{99\text{m}}\text{Tc}$), yttrium-86 and yttrium-90 (^{86}Y , ^{90}Y), zirconium-89 (^{89}Zr). The skilled person will be familiar with which radioisotopes are preferable for use as diagnostic agents and which are preferably for use as therapeutics.

It will be understood that the radioisotopes may be conjugated to the antibodies of the invention directly (via a chelating agent or prosthetic group or linker) or indirectly via binding to single or multiple amino acid residues in the antibody (e.g. halogenation of tyrosine residues).

In alternative embodiments, chelating agents or linkers may be used in order to conjugate the radioisotope to the antibody. In one example, the antibodies can be conjugated to a chelating moiety, selected from the group consisting of: TMT (6,6"-bis[N,N",N""-tetra(carboxymethyl)aminomethyl]-4'-(3-amino-4-methoxyphenyl)-2,2':6',2"-terpyridine), DOTA (1, 4,7,10-tetraazacyclododecane-NN',N"(N""-tetraacetic acid, also known as tetraxetan), TCMC (the tetra-primary amide of DOTA), DO3A (1,4,7,10-Tetraazacyclododecane-1,4,7-tris(acetic acid)-10-(2-thioethyl)acetamide), CB-DO2A (4,10-bis(carboxymethyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecan), NOTA (1,4,7-triazacyclononane-triacetic acid) Diamsar (3,6,10,13,16,19-hexaazabicyclo[6.6.6]eicosane-1,8-diamine), DTPA (Pentetic acid or diethylenetriaminepentaacetic acid), CHX-A"-DTPA ([[(R)-2-Amino-3-(4-isothiocyanatophenyl)propyl]-trans-(S,S)-cyclohexane-1,2-diamine-pentaacetic acid), TETA (1,4,8,11-tetraazacyclotetradecane-1,4,8), 11-tetraacetic acid, Te2A (4,11 - bis(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane), HBED, DFO (Desferrioxamine), DFOsq (DFO-squaramide) and HOPO (3,4,3-(LI-1,2-HOPO) or other chelating agent as described herein.

Chelators with radiometals and other halogenated radioisotopes may be bound to the antibodies of the invention via one or more amino acid residues or reactive moieties in the antibody, including but not limited to one or more lysine residues, tyrosine residues or thiol moieties.

In another example, the modified antibody is conjugated to a bifunctional linker, for example, bromoacetyl, thiols, succinimide ester, TFP ester, a maleimide, or using any amine or thiol- modifying chemistry known in the art.

The skilled person will be familiar with standard methods for conjugating chelating agents to antibodies and derivatives or fragments thereof. In addition, the skilled person will be familiar with approaches for selecting a relevant chelating agent for pairing with a radiometal, for example as described in Chem. Soc. Rev., 2014,43, 260, incorporated herein by reference.

Assaying Activity of an Antigen Binding Site

Binding to PSMA

It will be apparent to the skilled artisan from the disclosure herein that the preferred antigen binding sites of the present invention bind to PSMA. Methods for assessing binding to a protein are known in the art, e.g., as described in Scopes (*In: Protein purification: principles and practice*, Third Edition, Springer Verlag, 1994). Such a method generally involves immobilizing the antigen binding site and contacting it with labeled antigen. Following washing to remove non-specific bound protein, the amount of label and, as a consequence, bound antigen is detected. Of course, the antigen binding site can be labeled and the antigen immobilized. Panning-type assays can also be used. Alternatively, or additionally, surface plasmon resonance assays can be used.

Therapeutic, diagnostic and theranostic methods

The antibodies of the present invention are useful for treating a number of conditions requiring treatment by radioimmunotherapy. Typically, such conditions include cancer.

Exemplary cancers include cystic and solid tumours, bone and soft tissue tumours, including tumours in anal tissue, bile duct, bladder, blood cells, bowel, brain, breast, carcinoid, cervix, eye, esophagus, head and neck, kidney, larynx, leukemia, liver, lung, lymph nodes, lymphoma, melanoma, mesothelioma, myeloma, ovary, pancreas, penis, prostate, skin (e.g. squamous cell carcinoma), sarcomas, stomach, testes, thyroid, vagina, vulva. Soft tissue tumours include Benign schwannoma Monosomy, Desmoid tumour, lipo-blastoma, lipoma, uterine leiomyoma, clear cell sarcoma, dermatofibrosarcoma, Ewing sarcoma, extraskelatal myxoid chondrosarcoma, liposarcoma myxoid, Alveolar rhabdomyosarcoma and synovial sarcoma. Specific bone tumours include nonossifying fibroma, unicameral bone cyst, enchondroma, aneurismal bone cyst, osteoblastoma, chondroblastoma, chondromyxofibroma, ossifying fibroma and adamantinoma, Giant cell tumour, fibrous dysplasia, Ewing's sarcoma eosinophilic granuloma, osteosarcoma, chondroma, chondrosarcoma, malignant fibrous histiocytoma and metastatic carcinoma. Leukemias include acute lymphoblastic, acute myeloblastic, chronic lymphocytic and chronic myeloid.

Other examples include breast tumours, colorectal tumours, adenocarcinomas, mesothelioma, bladder tumours, prostate tumours, germ cell tumour, hepatoma/cholangio, carcinoma, neuroendocrine tumours, pituitary neoplasm, small round cell tumour, squamous cell cancer, melanoma, atypical fibroxanthoma, seminomas, nonseminomas, stromal leydig cell tumours, Sertoli cell tumours, skin tumours, kidney tumours, testicular tumours, brain tumours, ovarian tumours, stomach tumours, oral tumours, bladder tumours, bone tumours, cervical tumours, esophageal tumours, laryngeal tumours, liver tumours, lung tumours, vaginal tumours and Wilm's tumour.

Preferably, the antigen binding sites of the present invention are useful for treating cancer that are characterised by the presence of PSMA. For example, the antibodies that bind to PSMA are useful for treating cancers characterised by increased expression of PSMA, including prostate cancer.

The skilled person will be familiar with methods for selecting suitable diagnostic agents for use with the antibodies of the invention, including radioisotopes for use in radioimaging for diagnosing conditions described herein. Further, the skilled person will be familiar with imaging techniques for use in conjunction with the diagnostic reagents described herein.

As used herein, a theranostic method is a method for the *in vitro* and/or *in vivo* visualization, identification and/or detection of tumour cells and/or metastases as well as a method of treatment of cancer.

In one embodiment, the present invention includes a theranostic method that comprises:

(1) administering a diagnostically-effective amount an antibody of the present invention to a patient or subject,

wherein the antibody comprises at least one diagnostically useful label, and,

(2) administering a therapeutically-effective amount of an antibody of the present invention to a patient or subject in need thereof,

wherein the antibody comprises a tumour therapeutic(s) (e.g, radioisotope, toxin(s), drug(s)).

Preferably, step 1 and step 2 are conducted sequentially and the antibody in step 1 and step 2 are the same.

5 **Antibody Binding Domain Containing Proteins**

Single-Domain Antibodies

In some examples, an antigen binding site or protein of the invention is or comprises a single-domain antibody (which is used interchangeably with the term “domain antibody” or “dAb”). A single-domain antibody is a single polypeptide chain
10 comprising all or a portion of the heavy chain variable region of an antibody. In certain examples, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., US6248516).

Diabodies, Triabodies, Tetrabodies

In some examples, a protein of the invention is or comprises a diabody, triabody,
15 tetrabody or higher order protein complex such as those described in WO98/044001 and/or WO94/007921.

For example, a diabody is a protein comprising two associated polypeptide chains, each polypeptide chain comprising the structure V_L -X- V_H or V_H -X- V_L , wherein V_L is an antibody light chain variable region, V_H is an antibody heavy chain variable region,
20 X is a linker comprising insufficient residues to permit the V_H and V_L in a single polypeptide chain to associate (or form an Fv) or is absent, and wherein the V_H of one polypeptide chain binds to a V_L of the other polypeptide chain to form an antigen binding domain, i.e., to form a Fv molecule capable of specifically binding to one or more antigens. The V_L and V_H can be the same in each polypeptide chain or the V_L and V_H
25 can be different in each polypeptide chain so as to form a bispecific diabody (i.e., comprising two Fvs having different specificity).

Single Chain Fv (scFv)

The skilled artisan will be aware that scFvs comprise V_H and V_L regions in a single polypeptide chain and a polypeptide linker between the V_H and V_L which enables the scFv to form the desired structure for antigen binding (i.e., for the V_H and V_L of the single polypeptide chain to associate with one another to form a Fv). For example, the linker comprises in excess of 12 amino acid residues with (Gly₄Ser)₃ being one of the more favored linkers for a scFv.

The present invention also contemplates a disulfide stabilized Fv (or diFv or dsFv), in which a single cysteine residue is introduced into a FR of V_H and a FR of V_L and the cysteine residues linked by a disulfide bond to yield a stable Fv.

Alternatively, or in addition, the present invention encompasses a dimeric scFv, i.e., a protein comprising two scFv molecules linked by a non-covalent or covalent linkage, e.g., by a leucine zipper domain (e.g., derived from Fos or Jun). Alternatively, two scFvs are linked by a peptide linker of sufficient length to permit both scFvs to form and to bind to an antigen, e.g., as described in US20060263367.

Heavy Chain Antibodies

Heavy chain antibodies differ structurally from many other forms of antibodies, in so far as they comprise a heavy chain, but do not comprise a light chain. Accordingly, these antibodies are also referred to as "heavy chain only antibodies". Heavy chain antibodies are found in, for example, camelids and cartilaginous fish (also called IgNAR).

The variable regions present in naturally occurring heavy chain antibodies are generally referred to as "V_{HH} domains" in camelid antibodies and V-NAR in IgNAR, in order to distinguish them from the heavy chain variable regions that are present in conventional 4-chain antibodies (which are referred to as "V_H domains") and from the light chain variable regions that are present in conventional 4-chain antibodies (which are referred to as "V_L domains").

A general description of heavy chain antibodies from camelids and the variable regions thereof and methods for their production and/or isolation and/or use is found *inter alia* in the following references WO94/04678, WO97/49805 and WO 97/49805.

5 A general description of heavy chain antibodies from cartilaginous fish and the variable regions thereof and methods for their production and/or isolation and/or use is found *inter alia* in WO2005/118629.

Other Antibodies and Proteins Comprising Antigen Binding Domains Thereof

The present invention also contemplates other antibodies and proteins comprising antigen-binding domains thereof, such as:

- 10 (i) "key and hole" bispecific proteins as described in US5731168;
- (ii) heteroconjugate proteins, e.g., as described in US4676980;
- (iii) heteroconjugate proteins produced using a chemical cross-linker, e.g., as described in US4676980; and
- (iv) Fab₃ (e.g., as described in EP19930302894).

15 **Compositions**

In some examples, an antigen binding site as described herein can be administered orally, parenterally, by inhalation spray, adsorption, absorption, topically, rectally, nasally, buccally, vaginally, intraventricularly, via an implanted reservoir in dosage formulations containing conventional non-toxic pharmaceutically-acceptable

20 carriers, or by any other convenient dosage form. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intraperitoneal, intrathecal, intraventricular, intrasternal, and intracranial injection or infusion techniques.

Methods for preparing an antigen binding site into a suitable form for administration to a subject (e.g. a pharmaceutical composition) are known in the art and

25 include, for example, methods as described in Remington's Pharmaceutical Sciences (18th ed., Mack Publishing Co., Easton, Pa., 1990) and U.S. Pharmacopeia: National Formulary (Mack Publishing Company, Easton, Pa., 1984).

The pharmaceutical compositions of this invention are particularly useful for parenteral administration, such as intravenous administration or administration into a body cavity or lumen of an organ or joint. The compositions for administration will commonly comprise a solution of an antigen binding site dissolved in a pharmaceutically acceptable carrier, for example an aqueous carrier. A variety of aqueous carriers can be used, e.g., buffered saline and the like. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of an antigen binding site of the present invention in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the patient's needs. Exemplary carriers include water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as mixed oils and ethyl oleate may also be used. Liposomes may also be used as carriers. The vehicles may contain minor amounts of additives that enhance isotonicity and chemical stability, e.g., buffers and preservatives.

Dosages and Timing of Administration

Suitable dosages of an antigen binding site of the present invention will vary depending on the specific an antigen binding site, the condition to be treated and/or the subject being treated. It is within the ability of a skilled physician to determine a suitable dosage, e.g., by commencing with a sub-optimal dosage and incrementally modifying the dosage to determine an optimal or useful dosage. Alternatively, to determine an appropriate dosage for treatment/prophylaxis, data from the cell culture assays or animal studies are used, wherein a suitable dose is within a range of circulating concentrations that include the ED₅₀ of the active compound with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. A therapeutically/prophylactically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration or amount of the compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more

accurately determine useful doses in humans. Levels in plasma maybe measured, for example, by high performance liquid chromatography.

In some examples, a method of the present invention comprises administering a prophylactically or therapeutically effective amount of a protein described herein.

5 The term “therapeutically effective amount” is the quantity which, when administered to a subject in need of treatment, improves the prognosis and/or state of the subject and/or that reduces or inhibits one or more symptoms of a clinical condition described herein to a level that is below that observed and accepted as clinically diagnostic or clinically characteristic of that condition. The amount to be administered to
10 a subject will depend on the particular characteristics of the condition to be treated, the type and stage of condition being treated, the mode of administration, and the characteristics of the subject, such as general health, other diseases, age, sex, genotype, and body weight. A person skilled in the art will be able to determine appropriate dosages depending on these and other factors. Accordingly, this term is
15 not to be construed to limit the present invention to a specific quantity, e.g., weight or amount of protein(s), rather the present invention encompasses any amount of the antigen binding site(s) sufficient to achieve the stated result in a subject.

As used herein, the term “prophylactically effective amount” shall be taken to mean a sufficient quantity of a protein to prevent or inhibit or delay the onset of one or
20 more detectable symptoms of a clinical condition. The skilled artisan will be aware that such an amount will vary depending on, for example, the specific antigen binding site(s) administered and/or the particular subject and/or the type or severity or level of condition and/or predisposition (genetic or otherwise) to the condition. Accordingly, this term is not to be construed to limit the present invention to a specific quantity, e.g.,
25 weight or amount of antigen binding site(s), rather the present invention encompasses any amount of the antigen binding site(s) sufficient to achieve the stated result in a subject.

Kits

The present invention additionally comprises a kit comprising one or more of the
30 following:

- (i) an antibody of the invention or expression construct(s) encoding same;
- (ii) a molecule of the invention;
- (iii) a complex of the invention; or
- (iii) a pharmaceutical composition of the invention.

5 In the case of a kit for detecting cancer, the kit can additionally comprise a detection means, e.g., linked to an antigen binding site of the invention.

In the case of a kit for therapeutic/prophylactic use, the kit can additionally comprise a pharmaceutically acceptable carrier.

Optionally a kit of the invention is packaged with instructions for use in a method
10 described herein according to any example.

Table 1: Summary of amino acid and nucleotide sequences for PSMA-binding antibodies of the invention

Antibody ID	Region	SEQ ID NO:	Amino acid or nucleotide sequence
ANT4044 Variable Heavy chain	HCDR1 (protein)	1	EYTIH
	HCDR2 (protein)	2	NINPNNGGTTYNQKFED
	HCDR3 (protein)	3	GWNFDY
	VH (protein)	4	EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYTIHWVRQA PGKGLEWIGNINPNNGGTTYNQKFEDRVITITVDKSTSTAYM ELSSLRSEDVAVYYCAAGWNFDYWGGQTTVTVSS
	HCDR1 (DNA)	5	GAATACACCATCCAC
	HCDR2 (DNA)	6	AACATTAATCCTAACAATGGTGGTACTACCTACAACCAGA AGTTCGAGGAC
	HCDR3 (DNA)	7	GGTTGGAACCTTGACTAC
	VH (DNA)	8	GAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAA GCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTG GATACACATTCAGTGAATACACCATCCACTGGGTGAGGC AGGCCCTGGAAAGGGCCTTGAGTGGATTGGAAACATTA ATCCTAACAATGGTGGTACTACCTACAACCAGAAGTTCCG AGGACAGAGTCACAATCACTGTAGACAAGTCCACCAGCA CAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGATA CTGCAGTCTATTACTGTGCAGCTGGTTGGAACCTTGACT ACTGGGGCCAAGGCACCGGTACCGTCTCCTCA
	HFR1 (protein)	9	EVQLVQSGAEVKKPGASVKVSCKASGYTFT
	HFR2 (protein)	10	WVRQAPGKGLEWIG
	HFR3 (protein)	11	RVITITVDKSTSTAYMELSSLRSEDVAVYYCAA
	HFR4 (protein)	12	WGQGTITVTVSS
	HFR1 (DNA)	12	GAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAA GCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTG GATACACATTCAGT
	HFR2 (DNA)	14	TGGGTGAGGCAGGCCCTGGAAAGGGCCTTGAGTGGAT TGGA
	HFR3 (DNA)	15	AGAGTCACAATCACTGTAGACAAGTCCACCAGCACAGCC TACATGGAGCTCAGCAGCCTGAGATCTGAGGATACTGCA GTCTATTACTGTGCAGCT

Antibody ID	Region	SEQ ID NO:	Amino acid or nucleotide sequence
	HFR4 (DNA)	16	TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA
ANT4044-A2 Variable Heavy chain	HCDR1 (protein)	17	EYTIH
	HCDR2 (protein)	18	NINPNNGGTTYNQKFED
	HCDR3 (protein)	19	YWLFDY
	VH (protein)	20	EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYTIHWVRQA PGKGLEWIGNINPNNGGTTYNQKFEDRVITITVDKSTSTAYM ELSSLRSEDTAVYYCAAYWLFDYWGGGTTVTVSS
	HCDR1 (DNA)	21	GAATACACCATCCAC
	HCDR2 (DNA)	22	AACATTAATCCTAACAATGGTGGTACTACCTACAACCAGA AGTTCCGAGGAC
	HCDR3 (DNA)	23	TACTGGCTGTTCCGACTAC
	VH (DNA)	24	GAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAA GCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTG GATACACATTCACCTGAATACACCATCCACTGGGTGAGGC AGGCCCTGGAAAGGGCCTTGAGTGGATTGGAACATTA ATCCTAACAATGGTGGTACTACCTACAACCAGAAGTTCC AGGACAGAGTCACAATCACTGTAGACAAGTCCACCAGCA CAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGATA CTGCAGTCTATTACTGTGCAGCTTACTGGCTGTTCCGACT ACTGGGGCCAAGGCACCACGGTCACCGTCTCCTCA
	HFR1 (protein)	25	EVQLVQSGAEVKKPGASVKVSCKASGYTFT
	HFR2 (protein)	26	WVRQAPGKGLEWIG
	HFR3 (protein)	27	RVITITVDKSTSTAYMELSSLRSEDTAVYYCAA
	HFR4 (protein)	28	WGQGTTVTVSS
	HFR1 (DNA)	29	GAGGTCCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAA GCCTGGGGCCTCAGTGAAGGTCTCCTGCAAGGCTTCTG GATACACATTCACCT
	HFR2 (DNA)	30	TGGGTGAGGCAGGCCCTGGAAAGGGCCTTGAGTGGAT TGGA
	HFR3 (DNA)	31	AGAGTCACAATCACTGTAGACAAGTCCACCAGCACAGCC TACATGGAGCTCAGCAGCCTGAGATCTGAGGATACTGCA GTCTATTACTGTGCAGCT
	HFR4 (DNA)	32	TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA

Antibody ID	Region	SEQ ID NO:	Amino acid or nucleotide sequence
ANT4044/ ANT4044-A2 Variable Light chain	LCDR1 (protein)	33	KASQDVGTAVD
	LCDR2 (protein)	34	WASTRHT
	LCDR3 (protein)	35	QQYNSYPLT
	VL (protein)	36	DIQMTQSPSTLSASVGDRTITCKASQDVGTAVDWYQQKP GQAPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISRLQPE DFAVYYCQQYNSYPLTFGQGTKVDIK
	LCDR1 (DNA)	37	AAGGCCAGTCAGGATGTGGGTACTGCTGTAGAC
	LCDR2 (DNA)	38	TGGGCATCCACCCGGCACACT
	LCDR3 (DNA)	39	CAGCAATATAACAGCTATCCTCTCAGG
	VL (DNA)	40	GACATTCAGATGACCCAGTCTCCCAGCACCCCTGTCCGCA TCAGTAGGAGACAGGGTCACCATCACTTGCAAGGCCAGT CAGGATGTGGGTACTGCTGTAGACTGGTATCAACAGAAA CCAGGGCAAGCTCCTAACTACTGATTTACTGGGCATCC ACCCGGCACACTGGAGTCCCTGATCGCTTCAGCGGCAG TGGATCTGGGACAGATTTCACTCTCACCATCAGCAGACT GCAGCCTGAAGACTTTGCAGTTTATTACTGTCAGCAATAT AACAGCTATCCTCTCACGTTTCGGCCAGGGGACCAAGGT GGATATCAAA
	LFR1 (protein)	41	DIQMTQSPSTLSASVGDRTITC
	LFR2 (protein)	42	WYQQKPGQAPKLLIY
	LFR3 (protein)	43	GVPDRFSGSGSGTDFTLTISRLQPEDFAVYYC
	LFR4 (protein)	44	FGQGTKVDIK
	LFR1 (DNA)	45	GACATTCAGATGACCCAGTCTCCCAGCACCCCTGTCCGCA TCAGTAGGAGACAGGGTCACCATCACTTGC
	LFR2(DNA)	46	TGGTATCAACAGAAACCAGGGCAAGCTCCTAACTACTG ATTTAC
	LFR3 (DNA)	47	GGAGTCCCTGATCGCTTCAGCGGCAGTGGATCTGGGAC AGATTTCACTCTCACCATCAGCAGACTGCAGCCTGAAGA CTTTGCAGTTTATTACTGT
	LFR4	48	TTCGGCCAGGGGACCAAGGTGGATATCAAA

Antibody ID	Region	SEQ ID NO:	Amino acid or nucleotide sequence
	(DNA)		
ANT4044/ANT4044-A2__unmodified human IgG1 heavy chain constant region	IgG1 HC (protein)	49	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
ANT4044/ANT4044-A2 modified human IgG1 heavy chain constant region	IgG1 H310A H435Q HC (protein)	50	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLAQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNQYTQ KSLSLSPGK
ANT4044/ANT4044-A2__modified human IgG4 constant chain region	IgG4 S228P L235E H310A H435Q HC (protein)	51	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYT CNVDHKPSNTKVDKRVEPKYGPCCPPCPAPEFEGGSPVFL FPPKPKDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGV EVHNAKTKPREEQFNSTYRVVSVLTVLAQDWLNGKEYKCK VSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS FFLYSRLTVDKSRWQEGNVFSCSVMHEALHNQYTQKSLSL SLGK
ANT4044/ANT4044-A2 kappa light chain constant region	Human κ LC constant region	52	RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
ANT4044 RADmAb heavy chain (HuX592r)	FcRn-null, IgG1 allotype G1m(3) H310A H435Q	53	EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYTIHWVRQA PGKGLEWIGNINPNNGGTTYNQKFEDRVITITVDKSTSTAYM ELSSLRSEDTAVYYCAAGWNFDYWGGQTTVTVSSASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF

Antibody ID	Region	SEQ ID NO:	Amino acid or nucleotide sequence
			PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLAQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFF LYSKLTVDKSRWQQGNVFSVMSVMHEALHNQYTKQSLSLSP GK
ANT4044 RADmAb IgG4 heavy chain	FcRn+FcRy- null, IgG4 S228P L235E H310A H435Q	54	EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYTIHWVRQA PGKGLEWIGNINPNNGGTTYNQKFEDRVITITVDKSTSTAYM ELSSLRSEDTAVYYCAAGWNFDYWGGQTTVTVSSASTKG PSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDH KPSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKP KDTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLAQDWLNGKEYKCKVSNKG LPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYS RLTVDKSRWQEGNVFSVMSVMHEALHNQYTKQSLSLSLGK
ANT4044-A2 RADmAb IgG1 heavy chain	FcRn-null, IgG1 H310A H435Q	55	EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYTIHWVRQA PGKGLEWIGNINPNNGGTTYNQKFEDRVITITVDKSTSTAYM ELSSLRSEDTAVYYCAAYWLFYDWGGQTTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTQYICNVNH KPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLAQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFL YSKLTVDKSRWQQGNVFSVMSVMHEALHNQYTKQSLSLSP GK
ANT4044-A2 RADmAb IgG4 heavy chain	FcRn+FcRy- null IgG4 S228P L235E H310A H435Q	56	EVQLVQSGAEVKKPGASVKVSCKASGYTFTEYTIHWVRQA PGKGLEWIGNINPNNGGTTYNQKFEDRVITITVDKSTSTAYM ELSSLRSEDTAVYYCAAYWLFYDWGGQTTVTVSSASTKGP SVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALT SGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTKYTCNVDHK PSNTKVDKRVESKYGPPCPPCPAPEFEGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSDQEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLAQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV

Antibody ID	Region	SEQ ID NO:	Amino acid or nucleotide sequence
			KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSR LTVDKSRWQEGNVFSCSVMHEALHNQYTKSLSLSLGK
<u>ANT4044-</u> <u>ANT4044-A2</u> Vk <u>Light chain</u>	Vk Light chain	57	DIQMTQSPSTLSASVVGDRVTITCKASQDVGTAVDWYQQKP GQAPKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISRLQPE DFAVYYCQQYNISYPLTFGQGTKVDIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNFPYAPREKVKQWVDNALQSGNSQE SVTEQDSKSTYSLSSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC

Examples

Example 1: Antibodies for binding to PSMA

Summary

5 The antibody VH gene sequences for the two antibodies, ANT4044 and ANT4044-A2, were cloned into three different human IgG dual expression vectors encoding unmodified IgG1, IgG1 harbouring the mutations H310A and H435Q (that abolish FcRn binding and Protein A binding (Andersen, et al., 2012)) (referred to as IgG1 (H310A, H435Q)) and a modified IgG4 with the same FcRn abolishing mutations
10 described above, together with the hinge stabilising S228P mutation (Angal, et al., 1993) and the Fc silencing L235E mutation (Reddy, et al., 2000) (referred to as IgG4 (S228P, L235E, H310A, H435Q)). Each dual expression vector also contained the antibody Vk gene sequence common to both ANT4044 and ANT4044-A2.

15 A total of five antibodies were transiently transfected and expressed in CHO cells and purified using either Protein A (ANT4044-A2 IgG1) or Protein G (both ANT4044 and ANT4044-A2 as both IgG1 (H310A, H435Q) and IgG4 (S228P, L235E, H310A, H435Q)). Affinity chromatography was followed by preparative size exclusion chromatography (SEC).

20 Antibody integrity was assessed by SDS-PAGE, analytical SEC, thermal stability and antigen binding to PSMA by Biacore. Additional testing was conducted against a panel of human Fc gamma receptors (FcγRIIIA176F, FcγRIIIA176V, FcγRIIIB,

FcγRIIA167R, FcγRIIA167H, FcγRIIB, FcγRI) as well as the neonatal receptor, FcRn, using Biacore single cycle analysis.

Methods and results

Construction of antibody-expression plasmids

5 The VH and Vk sequences of the humanised antibody ANT4044 and the affinity matured, humanised antibody ANT4044-A2 were used to generate DNA fragments with flanking restriction enzyme sites for cloning into the pANT dual expression vector for IgG1 (pANT18), IgG1 (H310A, H435Q) (pANT71) and IgG4 (S228P, L235E, H310A, H435Q) (pANT73). The VH regions were cloned between the Mlu I and Hind III
10 restriction sites, and the Vk regions were cloned between the Pte I and BamH I restriction sites within each isotype vector. All five constructs were confirmed by DNA sequencing.

Transient expression of antibodies

 Endotoxin-free DNA corresponding to the five antibody constructs was prepared
15 and transiently transfected into CHO-S cells (ThermoFisher, Loughborough, UK) using a MaxCyte STX® electroporation system (MaxCyte Inc., Gaithersburg, USA). Following recovery, cells were diluted to 3×10^6 cells/ml in CD OptiCHO medium (ThermoFisher, Loughborough, UK) containing 8 mM L-Glutamine (ThermoFisher, Loughborough, UK) and 1x Hypoxanthine- Thymidine (ThermoFisher, Loughborough, UK). 24 hours post-
20 transfection, the culture temperature was reduced to 32°C and 1 mM sodium butyrate (Sigma, Dorset, UK) was added.

 Cultures were fed daily by the addition of 3.6% (of the starting volume) feed (2.5% CHO CD Efficient Feed A (ThermoFisher, Loughborough, UK), 0.5% Yeastolate (BD Biosciences, Oxford, UK), 0.25 mM Glutamax (ThermoFisher, Loughborough, UK)
25 and 2 g/L Glucose (Sigma, Dorset, UK)). IgG supernatant titres were monitored by IgG ELISA and transfected cells were cultured for up to 14 days prior to harvesting supernatants.

Antibody purification

Cell culture supernatants were passed over either Protein A (ANT4044-A2 IgG1) or Protein G (both ANT4044 and ANT4044-A2 as both IgG1 (H310A, H435Q) and IgG4 (S228P, L235E, H310A, H435Q)) Sepharose columns (GE Healthcare, Little Chalfont, UK). All antibodies were buffer exchanged into 1x PBS, pH 7.2. Protein A or Protein G purified material was run on a HiLoad™ 26/600 Superdex™ 200 pg preparative SEC column (GE Healthcare, Little Chalfont, UK) using 1x PBS as mobile phase during which monomeric fractions were collected, pooled and filter sterilised. SEC profiles revealed that antibodies, particularly ANT4044-A2, when expressed as IgG4 (S228P, L235E, H310A, H435Q) showed greater levels of aggregation (up to 22%) as compared with antibodies expressed as other IgG isotypes.

Antibodies were quantified by measuring the OD280nm and using extinction coefficients (Ec(0.1%)) based on their predicted amino acid sequences.

Analytical SEC and SDS-PAGE

Stock ANT4044 IgG1 and Protein A or Protein G followed by preparative SEC purified material was analysed by analytical SEC using a Superdex™ 200 Increase 10/300 GL analytical column (GE Healthcare, Little Chalfont, UK) and 1x PBS as mobile phase. The elution profiles were typical for correctly folded monomeric species of IgG. Antibodies were also analysed by non-reducing and reducing SDS-PAGE. Bands corresponding to the predicted sizes of VH and Vk chains were observed

Thermostability analysis

To assess the thermostability of the five purified antibodies together with ANT4044 IgG1 from stock, melting temperatures (the temperature at which 50% of a protein domain is unfolded) were determined using a fluorescence-based thermal shift assay. All antibodies were diluted to a working concentration of 100 µg/ml in 1x PBS containing SYPRO® Orange (ThermoFisher, Loughborough, UK) and subjected to a temperature gradient from 25°C to 99°C on a StepOnePlus real-time PCR system (ThermoFisher, Loughborough, UK) over a period of 56 minutes. The melting curves were analysed using protein thermostability software (version 1.2) and Tms were calculated based on first derivative data.

For both ANT4044 and ANT4044-A2, the IgG1 backbone appeared the most thermally stable with the lowest melting temperatures for both being 69.3°C. When comparing the different IgG backbones, ANT4044 exhibited greater thermal stability as compared with ANT4044-A2.

5 *Assessment of binding to PSMA*

Multi-cycle kinetic analysis was performed on each of the purified antibodies in order to assess binding to prostate-specific membrane antigen (PSMA). The analysis was performed using a Biacore T200 (serial no. 1909913) instrument running Biacore T200 Evaluation Software V3.0.1 (Uppsala, Sweden. For direct comparison, all
10 antibodies were captured on a Protein G chip (GE Healthcare, Uppsala, Sweden).

Purified antibodies were diluted to a concentration of 1 µg/ml in HBS-EP+. At the start of each cycle, each antibody was captured on the Protein G surface to give an RL of ~ 50 RU. Following capture, the surface was allowed to stabilise. Kinetic data was obtained using a flow rate of 35 µl/min to minimise any potential mass transfer effects.
15 For the kinetic analysis, PSMA (R&D Systems, Minneapolis, U.S.A) was used. Multiple repeats of the blank (PSMA) and a repeat of a single concentration of the analyte were programmed into the kinetic run to check the stability of both the surface and analyte over the kinetic cycles. For kinetic analysis, a twofold dilution range was selected from 25 nM to 1.5625 nM PSMA. The association phase of PSMA was monitored for 600
20 seconds and the dissociation phase was monitored for 2400 seconds. Regeneration of the Protein G surface was conducted using two injections of 10 mM glycine-HCL pH 1.5 containing 0.5% P20 at the end of each cycle.

The signal from the reference channel Fc1 was subtracted from that of Fc2, Fc3 and Fc4 to correct for differences in non-specific binding to a reference surface, and a
25 global Rmax parameter was used in the 1-to-1 binding model. The relative KD was calculated by dividing the KD of each antibody by that of ANT4044 IgG1 on the same chip.

Assessment of binding to human FcRn

The binding of the purified antibodies to FcRn was assessed by steady state
30 affinity analysis using a Biacore T200 (serial no. 1909913) instrument running Biacore

T200 Evaluation Software V3.0.1 (Uppsala, Sweden). FcRn (Sino Biological, Beijing, China) was coated onto a CM5 chip at 10 µg/mL in sodium acetate pH 5.5 using standard amine coupling to 300 RU.

Purified antibodies were titrated in a five-point dilution from 37 nM to 3000 nM in
5 PBS containing 0.05% P20 at either pH 6.0 or pH7.4. Antibodies were passed over the chip in increasing concentrations at a flow rate of 30 µl/min and at 25°C. The injection time was 30s and the dissociation time was 100s. Following a single dissociation, the chip was regenerated with 0.1M Tris pH 8.0.

As expected, the H310A H435Q mutations significantly reduced antibody binding
10 to FcRn at pH 6.0. Both ANT4044 and ANT4044-A2 antibodies tested as unmodified IgG1 showed similar affinities toward FcRn at pH 6.0. Very little binding was observed for any of the antibodies at pH 7.4.

Assessment of binding to human Fc gamma receptors

Binding of purified antibodies to high and low affinity Fc gamma receptors was
15 assessed by single cycle analysis using a Biacore T200 (serial no. 1909913) instrument running Biacore T200 Evaluation Software V3.0.1 (Uppsala, Sweden) running at a flow rate of 30 µl/min. The human Fc receptors, FcγRI, FcγRIIa (both 167R and 167H polymorphisms), FcγRIIb, FcγRIIIa (both 176F and 176V polymorphisms) and FcγRIIIb were obtained from Sino Biological (Beijing, China). FcγR were captured on a CM5
20 sensor chip pre-coupled using a Hiscapture kit (GE Healthcare, Little Chalfont, UK) using standard amine chemistry.

At the start of each cycle His-tagged Fc gamma receptors diluted in HBS-P+ were loaded to a specific RU level. A five point, three-fold dilution range of antibody without regeneration between each concentration was used for each receptor. In all
25 cases, following dissociation the chip was regenerated with two injections of Glycine pH 1.5. The signal from the reference channel Fc1 (blank) was subtracted from that of the Fc loaded with receptor to correct for differences in non-specific binding to the reference surface.

Sensorgrams were analysed for 1:1 kinetics for the high affinity Fc gamma receptor FcγRI and by steady state binding for the low affinity Fc gamma receptors. Representative sensorgrams

ANT4044 and ANT4044-A2 expressed as unmodified IgG1 bound to all high affinity and low affinity human activating Fcγ receptors. Introduction of the H310A and H435Q mutations in IgG1 did not affect this binding although a small trend towards slightly lower binding to low affinity human Fcγ receptors was observed. As anticipated, the IgG4 (S228P, L235E, H310A, H435Q) antibodies showed markedly reduced binding to all activating Fcγ receptors. All antibodies tested showed low affinity binding to the inhibitory receptor FcγRIIB. Thus, both IgG1 and IgG1 (H310A, H435Q) are potentially able to stimulate effector function whereas IgG4 (S228P, L235E, H310A, H435Q) is unlikely to stimulate effector function.

Conclusion

The variable heavy and light chain sequences corresponding to the humanised anti-PSMA antibody ANT4044 and its affinity matured variant ANT4044-A2 were cloned into dual expression vectors encoding either unmodified human IgG1, human IgG1 with the mutations H310A and H435Q (which abolish FcRn binding), or human IgG4 (S228P, L235E, H310A, H435Q). CHO cells were transiently transfected and five antibodies purified by Protein A or Protein G affinity chromatography and preparative SEC. Analytical SEC revealed profiles that were consistent with monomeric IgG with little evidence of aggregation. All antibodies including ANT4044 IgG1 (from stock) were characterised in terms of their thermostability and binding to PSMA, human FcRn and human Fcγ receptors.

For both ANT4044 and ANT4044-A2, the IgG1 backbone appeared the most thermally stable while ANT4044 antibodies exhibited greater thermal stability as compared with ANT4044-A2 antibodies. All antibodies in both a IgG1 (H310A, H435Q) and a IgG4 (S228P, L235E, H310A, H435Q) format showed similar binding to PSMA as their counterparts expressed as unmodified IgG1. Affinity matured ANT4044-A2 antibodies showed a 2-3-fold greater affinity for PSMA as compared with ANT4044.

Binding to FcRn at pH 6.0 was significantly reduced by the introduction of the H310A, H435Q mutations while very little binding to FcRn was observed for any of the antibodies at pH 7.4.

Analysis of antibody binding to human Fcγ receptors confirmed the abolition of binding by the L235E mutation in antibodies expressed as IgG4 (S228P, L235E, H310A, H435Q). The mutations H310A and H435Q did not significantly impact the binding of antibodies to human Fcγ receptors and thus, both IgG1 and IgG1 (H310A, H435Q) are anticipated to show similar effector function properties.

Example 2: Conjugation of antibodies

Antibodies ANT4044-IgG1, ANT4044-A2-IgG1, ANT4044-IgG1 H310A H435Q (a.k.a. ANT4044-IgG1-2M) and ANT4044-IgG4 S228P L235E H310A H435Q (a.k.a. ANT4044-IgG4-4M) were conjugated to either a ThioBridge™-PEG(6u)-DOTA reagent or an NHS-DOTA reagent.

ThioBridge™ is PolyTherics' proprietary disulfide conjugation linker and is described in

DOTA is a chelator payload, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono amide.

ThioBridge™ DOTA conjugation evaluation: ANT4044-IgG1 was prepared as a 6-10 mg/mL solution in reaction buffer (20 mM Sodium Phosphate, pH 7.5, 150 mM NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA). To ANT4044-IgG1 in reaction buffer (6-10 mg/mL, 40 °C) was added 6-10 equivalents of tris(2-carboxyethyl)phosphine (TCEP) per antibody or DTT 10 mM. The antibody concentration was adjusted to 5 mg/mL by dilution with reaction buffer. The reduction mixture was incubated for 1 hour at 37-40 °C. The reduction mixture was cooled down to 22 °C prior to addition of reagent. 5.6-8 equivalents of ThioBridge™-PEG(6u)-DOTA in acetonitrile were added to the mixture, which was further diluted to 4 mg/mL with reaction buffer. Percentage of acetonitrile in the mixture was 5%. The reaction mixture was incubated for up to 22 h at 22 °C. Buffer exchange and excess reagent removal was carried out by ultracentrifugation at 14,000 rcf with Vivaspinn 20 filters (30 kDa MWCO, PES membrane, Generon). The sample was buffer exchanged 7-9x into

Dulbecco's PBS pH 7.2-7.5 with Vivaspin 20 filters (30 kDa MWCO, PES membrane, Generon). Antibody-ThioBridge™ DOTA conjugate concentration was measured by UV-vis, corrected to 4.0 mg/mL with Dulbecco's-PBS pH 7.2-7.5, sterile filtered (0.22 µm cellulose acetate filters) and stored at -80 °C.

5 **Lysine-DOTA conjugation evaluation:** ANT4044-IgG1 was prepared as a 6 mg/mL solution in 0.1 M NaHCO₃ and 20 mM ethylenediaminetetraacetic acid (EDTA), pH 8-9 (reaction buffer). Next, prepared 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono-N-hydroxysuccinimide ester hexafluorophosphate trifluoroacetate salt (NHS-DOTA reagent) in Dulbecco's PBS pH 7.2-7.5 at 5.0 mg/mL. Added 10-25
10 equivalents of NHS-DOTA reagent solution and corrected antibody concentration to 4.0 mg/mL by addition of reaction buffer. Incubated at 22 °C for 2-3 h. Next, quenched by addition of 0.2 M sodium acetate, pH 5.5 (4:1 v/v) and ultracentrifuged at 14,000 rcf with Vivaspin 20 filters (30 kDa MWCO, PES membrane, Generon). Repeated dilution and ultracentrifugation 2x more. Then, buffer exchanged 4x into Dulbecco's PBS pH 7.2-7.5
15 with Vivaspin 20 filters (30 kDa MWCO, PES membrane, Generon). Antibody-DOTA conjugate concentration was measured by UV-vis, corrected to 4.0 mg/mL with Dulbecco's-PBS pH 7.2-7.5, sterile filtered (0.22 µm cellulose acetate filters) and stored at -80 °C. Small-scale reactions and purifications were first carried out to identify appropriate conjugation conditions. Analytical SEC and analytical LC-MS methods were
20 developed to confirm extent of conjugation, purity and residual reagent present. It was found that conjugation was efficient for both reagents. Neither reagent resulted in aggregation during or after conjugation. Lysine conjugates displayed a wider range of differently DOTA loaded species and required higher amounts for LC-MS analysis than ThioBridge™ conjugates. All samples tested were shown to have an average DAR
25 between 4.0 and 4.2 (ThioBridge™ conjugates) and average DAR between 3.8 and 4.9 (Lysine conjugates) by LC-MS.

Example 3: Pharmacokinetic analysis of antibodies for binding to PSMA

The serum half lives of the following antibodies was assessed using a similar methodology to that described in Example 1: J591 IgG lysine DOTA conjugate (control
30 antibody for binding PSMA), ANT4044 lysine DOTA conjugate (ANT4044-K-DOTA), ANT4044-A2 lysine DOTA conjugate (ANT4044-A2-K-DOTA), ANT4044 with amino acid substitutions in the FcRn-binding region, lysine DOTA conjugate (ANT4044-FcRn-

K-DOTA) and ANT4044 with amino acid substitutions in the FcRn and Fc gamma receptor binding regions, lysine DOTA conjugate (ANT4044-FcRg-K-DOTA). The results are shown in Figure 1.

Figure 2 shows the average area under the curve (AUC, top) and clearance (CL, bottom) for each test antibody. Error bars represent standard error of the mean.

Example 4: Biodistribution and tumour accumulation studies

Quantitative analysis of the comparative targeting of different antibodies of the invention to LNCaP cells in mouse models was assessed.

Radiolabelling and TLC analysis of antibodies.

The test antibodies were:

1. ANT4044-IgG1+DOTA ("JN005")
2. ANT4044-A2-IgG1+DOTA ("JN008")
3. ANT4044-IgG1(FcRn)+DOTA ("JN007")
4. ANT4044-IgG4(FcRn/FcRgamma)+DOTA ("JN006")
5. HuJ591 IgG1+DOTA ("J591" – control for binding to PSMA)

All antibodies were incubated with ^{64}Cu at a 200-fold excess of biomolecule in 0.1 M pH 5.5 Ammonium Acetate buffer for 45 minutes at room temperature. Samples of each solution were taken and mixed 1:1 with 50 mM EDTA. 5 μL of each solution was spotted on TLC paper (Agilent iTLC-SG Glass microfiber chromatography paper impregnated with silica gel) and run with 50:50 H_2O :Ethanol. Plates were then imaged on a Carestream MSFX imaging system using a radioisotopic phosphor screen. Where necessary, unbound copper was removed by purification using 7 K MWCO Zeba Spin Columns (Thermo Scientific) as per manufacturers protocols. All samples showed >95% labelling.

Control experiments were conducted to monitor the elution behaviour of free Cu-^{64} and Cu-^{64} bound to EDTA for quality control.

Tumour Initiation and Growth

8 week old male Balb/c nude mice were injected (27G needle) subcutaneously with 5×10^6 LNCaP cells in 100 μ L phosphate buffered saline into the right flank of each mouse.

- 5 Antibody solutions were injected via the tail vein (29G) and then mice were imaged using the Siemens Inveon PET-CT instrument, or blood collected via tail snip and activity measured via gamma counter for blood concentration analysis at indicated timepoints.

Imaging protocol

- 10 Mice were anaesthetised with isoflurane (IsoFlo, Abbott Laboratories) at a dose of 2% in a closed anaesthetic induction chamber. Mice were monitored using ocular and pedal reflexes to ensure deep anaesthesia. Once the mouse was deeply anaesthetised, it was placed on an appropriate animal bed, where the anaesthetic air mixture (1%) was delivered to its nose and mouth through a nose cone. Physiological monitoring
15 (respiratory using a sensor probe) was achieved throughout all experiments using an animal monitoring system (the BioVet™ system, m2m Imaging, Australia). Images were acquired using a Siemens Inveon PET-CT scanner following tail vein intravenous injection of the antibodies.

- 20 The injection syringe was filled with the radioisotope solution (approximately 150 μ L) and the activity in the syringe was measured using a dose calibrator (Capintec CRC-25) with a calibration factor of 35. The activity left in the syringe after the tail vein injection was measured using the same dose calibrator and the total volume injected in each mouse was calculated.

- 25 Calibration of the PET/CT scanner was performed with an in-house manufactured phantom containing a dose of Cu-64 solution as a radiation source.

The mice were positioned on the scanner bed (n=4 per scan using a bed developed in-house) and micro-CT scans were acquired for anatomical co-registration. The CT images of the mice were acquired through an X-ray source with the voltage set to 80 kV and the current set to 500 μ A. The scans were performed using 360° rotation

with 120 rotation steps with a low magnification and a binning factor of four. The exposure time was 230 ms with an effective pixel size of 106 μm . The CT images were reconstructed using Feldkamp reconstruction software (Siemens). Following CT imaging, PET scans were acquired at, 8 hours, 24 hours and 48 hours after injection of the radiotracer, using 30 - 60 minute static acquisitions. The PET Images were reconstructed using an ordered-subset expectation maximisation (OSEM2D) algorithm and analysed using the Inveon Research Workplace software (IRW 4.1) (Siemens) which allows fusion of CT and PET images and definition of regions of interest (ROIs). CT and PET datasets of each individual animal were aligned using IRW software (Siemens) to ensure good overlap of the organs of interest. Three dimensional ROIs were placed within the whole body, as well as all the organs of interest, such as heart, kidney, lungs, bladder, liver, spleen, intestines and tumour, using morphologic CT information to delineate organs. Activity per voxel was converted to nci/cc using a conversion factor obtained by scanning a cylindrical phantom filled with a known activity of Cu-64 to account for PET scanner efficiency. Activity concentrations were then expressed as percent of the decay-corrected injected activity per cm^3 of tissue that can be approximate as percentage injected dose/g (%ID/g).

The tumour to blood ratio was then calculated as the activity detected in tumour relative to the activity detected in blood.

Results

Mice were imaged at 8, 24 and 48 hrs post-injection. Following the 48 hr timepoint, organs were harvested for gamma counting and quantification of organ distribution.

Regions of interest were drawn around the tumour margins (as delineated from the CT scan) and the concentration of antibody calculated for each mouse in the imaging study (based on % injected dose). Figures 3 to 6, and 8 show organ accumulation as measured *in vivo* and *ex vivo* (by gamma counter). Variability in quantitation between *in vivo* and *ex vivo* arises due to ROI and background signal for the *in vivo* plots. ns $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$

Figure 7 shows blood concentration of antibodies out to 5 days post-injection.

In vivo imaging showed tumour accumulation and long term localisation (over 2 days) for all antibodies. There was no statistical difference between the different antibodies and the amount in the tumours was approximately 5% ID/g out to 48 hrs.

Ex vivo analyses showed biodistribution at 48 hrs for all antibodies. At this timepoint, the tumour accumulation had the highest concentration of antibody, followed by the liver, spleen and lungs.

JN005 shows lower liver and spleen accumulation than the other variants (except J591). This is also evident in the much longer circulation time – there is still ~ 10% ID/g of JN005 circulating at 120hrs.

Pharmacokinetic evaluation was undertaken by taking blood samples out to 120hrs. The most notable observation was the faster clearance of JN007 compared to the other antibodies.

Blood samples were also used to calculate the tumour:blood ratios of the antibodies. The tumour:blood ratio (in vivo: tail bleed) for each of the antibodies was determined for the 8 hr, 24 hr and 48 hr time points and the results are shown in Figures 9. Figure 10 shows the tumour:blood ratio (ex vivo:ex vivo) at 48 hrs and 120 hrs.

The tumour:blood ratio is significantly higher for antibodies JN006 (ANT4044-IgG4(FcRn/FcRgamma)+DOTA) and JN007 (ANT4044-IgG1(FcRn)+DOTA) at all time points compared with antibodies JN005 (ANT4044-IgG1+DOTA) and J591. The ratio at 120 hr is particularly striking, with the tumour:blood ratio for FcRn-binding modified and for FcRn/Rf gamma receptor-binding modified antibodies exceeding that of non-modified antibodies by approximately 200-fold.

Discussion

Despite some of the tested antibodies having significantly reduced serum half-lives (and increased clearance), the amount of all antibodies accumulating in the tumours was not statistically different. These results are surprising in that they show that tumour loading for each of the antibodies was the same, indicating that all antibodies had similar binding affinities for their target epitopes, and all antibodies had a similar capacity to be delivered to the target sites, even though the FcRn and Fc gamma

receptor modifications significantly increased clearance and reduced the serum half life of the JN007 and JN006 antibodies.

The results indicate that modification of the FcRn or the FcRn and Fc gamma receptor binding domains of radiolabelled antibodies has significant utility in reducing the amount of radioisotope in the circulation, without impacting on the therapeutic potential of the antibody with respect to its capacity to accumulate in the tumour. This has numerous benefits, including reducing a number of toxic effects which would otherwise result from longer-term residence of radioisotope in the circulation (including haematological toxicity, absorption into bone and bone marrow irradiation).

10 **Example 5: ¹⁷⁷Lu imaging and radiotherapy efficacy study of an exemplary antibody of the invention**

Test Articles

1. TXP02-JN007 (ANT4044-FcRn-K-DOTA)
2. TXP02-JN005 (ANT4044-K-DOTA)
- 15 3. PSMA-617 (PSMA-binding peptide)

Phase 1 Chemistry: Imaging Study

Labelling Overview: JN007, JN005.

ANT4044 is an anti-PSMA antibody as described herein. ANT4044-FcRn is a modified form of the antibody, in which the FcRn-binding region of the heavy constant chain has been modified to reduce serum half-life. ANT4044-FcRn-K-DOTA is ANT4044-FcRn, conjugated to the chelator DOTA via lysine residues.

150 µg of ANT4044-FcRn was labelled with 500 MBq of Lu-177 with a 2-hour incubation at 37 °C. Labelling efficiency was 81%. The reaction mixture was purified on a NAP-5 column into PBS with a subsequent labelling efficiency of 98%. Fractions containing the labelled antibody were collated. Doses containing 10 µg, 100 µg and 370 µg each labelled with 20 MBq of Lu-177 were prepared by addition of the appropriate amounts of unlabelled ANT4044-FcRn antibody and PBS.

500 µg of ANT4044 antibody was labelled with 100 MBq of Lu-177 with a 2-hour incubation at 37 °C. Labelling efficiency was 98.2%. The preparation was diluted in PBS and used without further purification.

Phase 2 Chemistry: Efficacy Study

5 *Labelling Overview: PSMA-617, ANT4044-FcRn.*

6 µg (4 nmol) of PSMA-617 (6 µl of 1 mg/ml solution in water) was labelled with 200 MBq Lu-177 in 0.1 M ammonium acetate buffer (pH 5.5) and heated to 95 °C for 10 minutes. Labelling efficiency was 97.6%. The preparation was diluted in PBS and used without further purification.

10 300 µg ANT4044-FcRn was labelled with 120 MBq Lu-177. Labelling efficiency after 2 hours incubation at 37 °C was 96.5%. The preparation was quenched by addition of 5 µl 0.1 M EDTA, diluted in PBS and used without further purification.

Phase 1 – Imaging Study Design

1. For the phase 1 study, animals with the appropriate tumour size (150-300 mm³) were placed on study into 4 groups of n=3 with dosing as follows
 - a. Group 1: 10 µg [¹⁷⁷Lu]TXP02- ANT4044-FcRn in 200 µL, intravenous (IV) injection into the lateral tail vein.
 - b. Group 2: 100 µg [¹⁷⁷Lu]TXP02- ANT4044-FcRn in 200 µL, IV injection into the lateral tail vein.
 - 20 c. Group 3: 370 µg [¹⁷⁷Lu]TXP02- ANT4044-FcRn in 200 µL, IV injection into the lateral tail vein.
 - d. Group 4: 100 µg [¹⁷⁷Lu]TXP02- ANT4044 in 200 µL, IV injection into the lateral tail vein.
2. Antibody biodistribution was assessed at 4, 24, and 48 hours post antibody injection via SPECT/CT imaging:
 - 25 a. A whole-body static SPECT image was acquired followed by a whole-body CT for anatomical reference.

b. SPECT scan duration was 40 minutes, CT scan duration was 12 minutes per scan.

c. Animals were imaged three at a time in a multi-mouse hotel

3. Tumour volume was assessed via calliper measurements 3 times a week.

5 Animals were inspected for any adverse effects and weighed regularly.

4. For select animals tissues were collected for additional *ex vivo* analysis.

5. Remaining animals were culled and carcasses discarded.

Phase 2 – Efficacy Study Design

1. For the main efficacy study, animals with the appropriate tumour size (150-300 mm³) were placed on study in 3 groups of n=6 with dosing as follows

10

a. Group 1: 50 µg [¹⁷⁷Lu]TXP02- ANT4044-FcRn in 200 µL, IV

b. Group 2: 600 ng [¹⁷⁷Lu]PSMA-617 in 200 µL, IV

c. Group 3: 200 µL PBS, IV

2. Tumour size was assessed via calliper measurements 3 times a week for 3 weeks following test agent injections.

15

3. Animals were inspected for any adverse effects and weighed regularly.

4. Blood samples were taken from animals via tail prick method in groups 1 and 2 at 0.5, 4, 8, 24, 48, 72, 96, 120 hours and assessed via gamma counting.

5. Whole blood samples were weighed and then counted in the gamma counter with reference standards.

20

6. Initial study aim was to monitor the tumour growth for 5 weeks post-treatment. This time frame was reduced due to the radiation sickness observed in some of the [¹⁷⁷Lu]TXP02- ANT4044-FcRn mice.

7. For select animals tissues were collected for additional *ex vivo* analysis.

8. Remaining animals were culled and carcasses discarded.

Results and Discussion

Figure 11 is an exemplary image of [^{177}Lu]TXP02- ANT4044-FcRn distribution in the xenograft mice.

5 Figure 12 is a plot of [^{177}Lu]TXP02-ANT4044-FcRn radioactivity levels measured in blood from the mice.

Figure 13 is a plot of tumour growth as determined in the present study. ANT4044-FcRn-DOTA-Lu treatment significantly suppressed tumour growth as evidence by no change in tumour volume on day 14 as compared to day 0. In the control (PBS) group, there was an overall increase in tumour volume, with tumours becoming significantly larger at 9, 12 and 14 days when compared to the corresponding time in ANT4044-FcRn-DOTA-Lu treated group.

Radiolabelling of both test (JN007) and comparator (JN005) antibodies was successful (>96 % labelling efficiency) with a maximum specific activity of 2 MBq/ μg achieved.

The imaging study showed uptake of both test and comparator antibodies in the LNCaP tumour xenografts (see Table 2).

Blood clearance, as inferred by a small ROI drawn in the left ventricle of the heart, was faster for the JN007 antibody for all groups at 48 hours post-injection, compared to the same time point for the JN005 antibody (see Table 3).

Table 2: [^{177}Lu] TXP02-JN007 and [^{177}Lu]TXP02-JN005 Tumour uptake at 4, 24 and 48 hours

Group	Mass dose ($\mu\text{g}/\text{animal}$)	Imaging tracer	Dose Radioactivity (Mbq/animal, Mean $\pm$ SD)	4hrs: Tumour %ID/g (Mean $\pm$ SD)	24hrs: Tumour %ID/g (Mean $\pm$ SD)	48hrs: Tumour %ID/g (Mean $\pm$ SD)
1	100	[^{177}Lu] TXP02-JN007	19.4 $\pm$ 0.6	3.2 $\pm$ 0.4	6.9 $\pm$ 1.2	8.3 $\pm$ 1.7
2	100	[^{177}Lu]	19.7 $\pm$ 0.7	4.5 $\pm$ 0.2	16.8 $\pm$ 1.2	23.7 $\pm$ 0.8

		TXP02-JN005				
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Table 3: [^{177}Lu] TXP02-JN007 and [^{177}Lu]TXP02-JN005 Heart (blood) uptake at 4, 24 and 48 hours

Group	Mass dose ($\mu\text{g}/\text{animal}$)	Imaging tracer	Dose Radioactivity (Mbq/animal, Mean $\pm$ SD)	4hrs: blood %ID/g (Mean $\pm$ SD)	24hrs: blood %ID/g (Mean $\pm$ SD)	48hrs: blood %ID/g (Mean $\pm$ SD)
1	100	[^{177}Lu] TXP02-JN007	19.4 $\pm$ 0.6	11.7 $\pm$ 1.7	3.0 $\pm$ 0.2	1.7 $\pm$ 0.1
2	100	[^{177}Lu] TXP02-JN005	19.7 $\pm$ 0.7	16.5 $\pm$ 1.1	10.0 $\pm$ 0.6	7.8 $\pm$ 0.9

5 Figure 14 is a plot of the tumour:blood ratios in mice following administration the JN007 antibody (anti-PSMA, K-DOTA-Lu antibody of the invention modified to reduce FcRn-binding- also referred to as HuX592R-DOTA-Lu177).

Compared to control mice (which received JN005, i.e., anti-PSMA, K-DOTA-Lu antibody HuJ591-DOTA-Lu177 which does not have modifications in the FcRn-binding
10 region), mice that received the FcRn modified antibody had a higher ratio of antibody in tumour compared to blood.

Example 6

Test Antibodies

1. HuX592R (ANT4044-FcRN)
- 15 2. HuJ591 (ANT4044)

Radiolabelling and TLC analysis of antibodies

All antibodies were incubated with ^{177}Lu at a 50-fold or 100-fold excess of biomolecule for HuX592R and HuJ591 respectively in 0.1 M pH 5.5 ammonium acetate buffer for 45 minutes at room temperature. Samples of each solution were taken and
20 mixed 1:1 with 50 mM DTPA. 5 μL of each DTPA incubated sample or neat solution was

spotted on TLC paper (Agilent iTLC-SG Glass microfiber chromatography paper impregnated with silica gel) and run with 50:50 H₂O:ethanol. Detection of radiolabelled species migration was then achieved using an Eckert and Ziegler Mini-Scan and Flow-Count system. All samples showed ≥90% labelling. Control experiments were conducted to monitor the elution behaviour of free ¹⁷⁷Lu and ¹⁷⁷Lu bound to DTPA for quality control.

Cell binding

Lu-labelled constructs HuX592R and HuJ591 were assessed for cell binding.

In summary, Eppendorf tubes containing 1.25 x 10⁵ PC-3 tumour cells (negative control) or 1.25 x 10⁵ LNCaP tumour cells in 0.100 mL PBS are incubated at 37 °C with 5 µL (0.030 MBq) of labelled antibody. The incubations were stopped at the following time points for analysis: 1 hour, 2 hours, 4 hours and 24 hours.

Unbound fraction determination.

At the end of each incubation period, the Eppendorf tubes are centrifuged for 5 min at 500g. The supernatants containing the free Lu-177 antibody were recovered in separate tubes and counted using gamma analysis. The pellets containing the cells associated with Lu-177 labelled antibody were washed with 0.200 mL of PBS solution and centrifuged for 5 min at 500g for 3 repeats. Supernatants of each wash were collected and counted, values combined with the recovered incubation supernatant to result a total free unbound antibody.

Surface bound fraction determination.

Pellets were resuspended in 0.1 mL PBS at pH 4.0 for 20 min at ice cold temperature. Eppendorf tubes were then centrifuged for 5 min at 500g at 4°C and supernatants collected for counting. Following a further three washes with ice cold PBS pH 4.0, the supernatants were collected for gamma counting.

Internalised fraction determination.

After washing and centrifugation, the cell pellets were counted as internalised fraction.

Protein concentration

Cells were solubilised by 1 M NaOH and protein concentration determined.

Calculation

5 The binding of Lu-177 antibody to the tumour cells (surface bound and internalised fractions) and unbound antibodies were calculated and reported as a percentage of the total radioactivity incubated per mg of protein.

Animals

10 Healthy male Balb/C nude mice (~20g) from 6 weeks old were obtained from the ARC (Western Australia) and used for this study. Mice were monitored for 1 week prior to the study in order to acclimatise to the environment prior to injection of cells. All animals were provided with free access to food and water before and during the experiments.

Dosing

15 The injection syringes were filled with the radiolabelled antibody solution (approximately 100 μ L) and the activity in the syringe was measured using a dose calibrator (Capintec CRC-25) with a calibration factor of 35. The activity left in the syringe after the tail vein injection was measured using the same dose calibrator and the total volume injected in each mouse was calculated.

Tolerability

20 Healthy male Balb/C nude mice (n=3 per dosing cohort) were injected (29 G, tail vein injection in ~100 μ L saline) a total of 3 times 7 days apart with ^{177}Lu -labelled HuX592R to give 6, 9 or 12 MBq injected dose at a mass dose of approximately 100 μ g per mouse. Mouse health was then monitored following injection, and at 28 days post initial injection all mice were culled and organs fixed in PFA, transferred to 30% sucrose
25 and allowed to decay before being stored frozen for potential future analysis if required.

Biodistribution

Healthy male Balb/C nude mice (n=3 per cohort) were injected (29 G, tail vein injection saline) with ^{177}Lu -labelled constructs to give 6 MBq injected dose at a mass dose of 100 (HuX592R) or 140 (HuJ591) μg per mouse. Animals were then sacrificed at 24, 48 and 72 hours post injection (for HuX592R) and 72 hrs (for HuJ591). Blood was sampled and tissues collected and cleaned of excess blood and weighed for ex vivo analysis. A Perkin PerkinElmer 2480 Automatic Gamma Counter was used to measure radioactivity in tissues. The gamma counter was calibrated using known samples of ^{177}Lu and measured activity presented as %ID/g of tissue weight based on injected activities.

Tumour Initiation and Growth

For the autoradiography and therapeutic studies, 8-12 week old male Balb/c nude mice were injected (27G needle) subcutaneously with 4×10^6 LNCaP cells in 50 μL 50:50 phosphate buffered saline:matrigel into the right flank of each mouse. There was no evidence of ulceration at the time of cell injection; the animals were closely monitored and tumour measured by callipers and remained in good condition apart from the growth of solid tumours. The tumour growth was sporadic as is often observed for LNCaP tumours and mice were enrolled in appropriate study arms as tumours reached 100-200 mm^3 .

Autoradiography

Tumour bearing male Balb/C nude mice (n=2 per cohort) were injected (29 G, tail vein injection in $\sim 100 \mu\text{L}$ saline) with ^{177}Lu -labelled constructs to give 6 MBq injected dose at a mass dose of 100 (HuX592R) or 140 (HuJ591) μg per mouse. Animals were then sacrificed at 7 days post injection, tumour samples were snap frozen in an isopentane-dry ice slurry, then embedded in OCT compound for sectioning. 20 μm sections were collected onto slides and air dried, then exposed on a phosphor screen in a closed cassette for approximately 3.5 hours. Images were then obtained using an Amersham Typhoon Phosphorimager using a pixel size of 50 μm (scan time 20-30 min) as optimized for ^{177}Lu with a sensitivity setting of 1000. ImageQuant TL software was used to analyze images.

Therapeutic study

Tumour bearing male Balb/C nude mice (n=6 per cohort) were injected (29 G, tail vein injection in ~100 μ L saline) with ^{177}Lu -labelled constructs to give 6 or 8 MBq for HuX592R or 6 MBq for HuJ591 injected dose at a mass dose of 100 for HuX592R or 140 for HuJ591 μ g per mouse each week for 3 weeks. Tumour growth in response to therapy was monitored over 90 days in comparison to vehicle only control.

RESULTS

Both HuX592R and HuJ591 showed successful ^{177}Lu labelling. These ^{177}Lu labelled constructs were used directly for the cell binding, tolerability, biodistribution and therapeutic efficacy studies.

Cell binding

Binding of ^{177}Lu -labelled HuX592R and HuJ591 was assessed against LNCaP (PSMA+) and PC3 (PSMA-) cell lines. The unbound, surface bound and cell pellet associated fractions were assessed at 1, 2 and 4 hours after addition of the constructs to the cells. The cell assay showed that both HuJ591 and HuX592R showed enhanced accumulation in PSMA expressing cell lines, with the highest concentration observed in the cell pellet indicating successful internalisation during the assay period (at all timepoints). Minimal association/internalisation was generally observed in the PSMA-negative cell line (PC3), indicating that binding was receptor-dependent and the labelling did not interfere significantly with the antibody binding to the receptor.

Tolerability

Tolerability of ^{177}Lu -labelled HuX592R in healthy male Balb/c nude mice (n=3 per cohort) was assessed at 3 dosing levels, 12, 9, and 6 MBq administered 3 times one week apart. Animal health was assessed by animal weight and animal score sheet for 4 weeks including 3 weeks of therapeutic administration. In this study, it was found that the 12 MBq dose too high, so the full study was then undertaken using doses of 6MBq and 8 MBq.

Biodistribution

Biodistribution of ^{177}Lu -labelled constructs in healthy male Balb/c nude mice (n=3 per cohort) was assessed at 24, 48 and 72 hrs post administration for HuX592R (Figure 15A) and at 72 hrs only post administration for HuJ591 (Figure 15B). The results show significantly higher circulation time for HuJ591 compared to HuX592R with commensurate lower levels of accumulation in clearance organs such as the liver and spleen suggesting more rapid clearance by the system.

Autoradiography

^{177}Lu -labelled constructs were administered into tumour-bearing male Balb/c nude mice. Animals were sacrificed 1 week post injection, tumours frozen in OCT, sectioned and autoradiography images acquired. Overall results are in agreement with tumour accumulation and penetration by both constructs, with enhanced accumulation for HuJ591 compared to HuX592R at this timepoint. Moreover, distribution of the radiotherapeutic appears to be well-dispersed throughout the tumour for both antibodies.

Therapeutic study

Male Balb/c nude mice bearing flank LNCaP xenograft tumours were assigned to therapeutic cohorts and administered 3 doses 1 week apart of either;

1. Vehicle control
2. 6 MBq [^{177}Lu]-HuX592R
3. 8 MBq [^{177}Lu]-HuX592R
4. 6 MBq [^{177}Lu]-HuJ591

Tumour regression (Figure 17) and animal health as assessed by animal weight was monitored for 100 days. Results show significant tumour growth inhibition for both HuX592R (Figure 16) with 2 mice in the HuX592R 8 MBq and 4 mice in the HuJ591 cohort (not shown) showing complete tumour regression where tumour burden was not measurable by callipers. At 37 days after commencing therapy (the last datapoint at which all cohorts were n=6 allowing statistical comparison) all 3 treatments produced

significant reductions in tumour growth compared to the control cohort as determined by 2 way ANOVA, with HuX592R at 6 MBq (p 0.0105), HuX592R at 8 MBq (p 0.0086) and HuJ591 at 6 MBq (p 0.0056).

5 No dosing group showing significant ill-health as assessed by change in body weight.

Mouse survival (Figure 17) is statistically longer ($p < 0.05$ by Mantel-Cox test) for all 3 treatment cohorts (median survival > study period) compared to the control cohort (median survival 83.5 days). Upward tick indicates animal euthanised due to non-tumour/health related issues.

10 Both formulations (HuJ591 at 6 MBq) and HuX592R (at both 6 MBq and 8 MBq) showed efficacy against PSMA expressing tumours. Both HuJ591 (at 6 MBq) and HuX592R (at 8 MBq) had no treatment/tumour related deaths during the 100 days of the study, and 2 mice in the HuX592R 8 MBq and 4 mice in the HuJ591 groups showed absolute regression of the tumour (immeasurable by calipers). In terms of the survival
15 plot, all treatment groups with antibody were statistically better than the control group.

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

20

CLAIMS

1. A modified antibody with reduced FcRn binding affinity compared to an unmodified form of the antibody, or compared to a wild-type antibody of the class IgG, comprising:

- 5 - a heavy chain constant region wherein one or more amino acid residues at positions His310, His433, His435, His436, Ile253 are different from the residues present in the unmodified antibody or a wild-type antibody of the class IgG,

wherein said antibody binds specifically to prostate specific membrane antigen (PSMA) and wherein the antibody comprises:

- 10 FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4 – linker - FR1a - CDR1a – FR2a – CDR2a – FR3a – CDR3a – FR4a

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

- 15 FR1a, FR2a, FR3a and FR4a are each framework regions;

CDR1a, CDR2a and CDR3a are each complementarity determining regions;

wherein the sequence of any of the complementarity determining regions have an amino acid sequence as described in Table 1.

2. The modified antibody of claim 1, wherein the antibody that specifically binds to PSMA comprises an antigen binding site that comprises or consists essentially of or consists of an amino acids sequence of (in order of N to C terminus or C to N terminus) SEQ ID NO: 4 or 20.

3. The modified antibody of claim 1 or 2, wherein the antibody comprises at least one of:

- 25 (i) a VH comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at

least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO 1 or 17, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in SEQ ID NO: 2 or 18, and a CDR3 comprising a sequence at least about 80%, at least
5 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 3 or 19;

(ii) a VH comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 4 or 20;

(iii) a VL comprising a CDR1 comprising a sequence at least about 80%, at
10 least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at
15 least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 35;

(iv) a VL comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO:36;

(v) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a
20 CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19;

(vi) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20;

(vii) a VL comprising a CDR1 comprising a sequence set SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 45;

25 (viii) a VL comprising a sequence set forth in SEQ ID NO: 36;

(ix) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19; and a VL comprising a CDR1 comprising a sequence set SEQ ID NO: 33, a CDR2 comprising a sequence set

forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 35;
or

(x) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20 and a VL comprising a sequence set forth in SEQ ID NO: 36.

- 5 4. The modified antibody of any one of claims 1 to 3, wherein the heavy chain constant region of the antibody comprises amino acid substitutions at both His310 and His435.
5. The modified antibody of any one of claims 1 to 4, wherein the heavy chain constant region of the antibody comprises amino acid substitutions at residues
10 equivalent to Ser228 and Leu235 of the constant heavy chain region.
6. The modified antibody of any one of claims 1 to 5, wherein the heavy chain constant region of the antibody comprises the amino sequence set forth in SEQ ID NO:50 or in SEQ ID NO: 51.
7. The modified antibody of any one of claims 1 to 6 wherein the antibody
15 comprises the sequences of SEQ ID NO:53 and 57.
8. A molecule comprising an immunoglobulin moiety and a non-protein agent conjugated thereto,

wherein, the immunoglobulin moiety specifically binds to PSMA and comprises an antigen binding site including:

- 20 FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4 – linker - FR1a - CDR1a – FR2a – CDR2a – FR3a – CDR3a – FR4a

wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

- 25 FR1a, FR2a, FR3a and FR4a are each framework regions;

CDR1a, CDR2a and CDR3a are each complementarity determining regions;

wherein the sequence of any of the complementarity determining regions have an amino acid sequence as described in Table 1;

wherein the immunoglobulin moiety has reduced or abolished affinity for the FcRn receptor compared to a wild-type immunoglobulin; and

5 wherein the non-protein agent comprises a therapeutic moiety, preferably a cytotoxin or a radioactive element.

9. The molecule of claim 8, wherein the immunoglobulin moiety comprises an antigen binding site that consists essentially of or consists of an amino acids sequence of (in order of N to C terminus or C to N terminus) SEQ ID NO: 4 or 20.

10 10. The molecule of claim 8 or 9, wherein the immunoglobulin moiety comprises at least one of:

(i) a VH comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO 1
15 or 17, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in SEQ ID NO: 2 or 18, and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 3 or 19;

20 (ii) a VH comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 4 or 20;

(iii) a VL comprising a CDR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence at least
25 about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 35;

(iv) a VL comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO:36;

(v) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a
5 CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19;

(vi) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20;

(vii) a VL comprising a CDR1 comprising a sequence set SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 45;

10 (viii) a VL comprising a sequence set forth in SEQ ID NO: 36;

(ix) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19; and a VL comprising a CDR1 comprising a sequence set SEQ ID NO: 33, a CDR2 comprising a sequence set
15 forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 35;
or

(x) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20 and a VL comprising a sequence set forth in SEQ ID NO: 36.

11. The molecule of any one of claims 8 to 10, wherein the immunoglobulin moiety
20 comprises amino acid substitutions at residues equivalent to His310 and/or His435 in the constant heavy chain region, thereby reducing or abolishing the affinity of the immunoglobulin moiety for the FcRn receptor.

12. The molecule of any one of claims 8 to 11, wherein the immunoglobulin moiety further comprises one or more amino acid substitutions at residues equivalent to Ser228
25 and Leu235 of the constant heavy chain region, thereby reducing the affinity of the moiety for an activating Fc gamma receptor or stabilising the moiety.

13. The molecule of any one of claims 8 to 12 wherein the non-protein agent comprises a therapeutic agent in the form of a radioisotope.

14. The molecule of any one of claims 8 to 13 wherein the non-protein agent comprises a radioisotope selected from the group consisting of: actinium-225 (^{225}Ac), astatine-211 (^{211}At), bismuth-212 and bismuth-213 (^{212}Bi , ^{213}Bi), copper-64 and copper-67 (^{64}Cu , ^{67}Cu), gallium-67 and gallium-68 (^{67}Ga and ^{68}Ga), indium-111 (^{111}In), iodine -
 5 123, -124, -125 or -131 (^{123}I , ^{124}I , ^{125}I , ^{131}I) (^{123}I), lead-212 (^{212}Pb), lutetium-177 (^{177}Lu),
 radium-223 (^{223}Ra), samarium-153 (^{153}Sm), scandium-44 and scandium-47 (^{44}Sc , ^{47}Sc),
 strontium-90 (^{90}Sr), technetium-99 ($^{99\text{m}}\text{Tc}$), yttrium-86 and yttrium-90 (^{86}Y , ^{90}Y), and
 zirconium-89 (^{89}Zr).
15. The molecule of claim 13 or 14 wherein the radioisotope is selected from:
 10 actinium-225 (^{225}Ac), astatine-211 (^{211}At), and iodine-123 (^{123}I).
16. A method of treating prostate cancer in an individual, the method comprising administering to an individual in need thereof, an antibody of any one of claims 1 to 7, or a molecule of any one of claims 8 to 15.
17. Use of an antibody of any one of claims 1 to 7, or of a molecule of any one of
 15 claims 8 to 15, in the manufacture of a medicament for the treatment of prostate cancer.
18. An antibody of any one of claims 1 to 7, or a molecule of any one of claims 8 to 15, for use in the treatment of prostate cancer.
19. A pharmaceutical composition comprising an antibody of any one of claims 1 to 7, or a molecule of any one of claims 8 to 15.
- 20 20. A therapeutic IgG molecule comprising:
- heavy and light chains, wherein each chain comprises a variable region and a constant region;
 - wherein the constant region of the heavy chain comprises one or more amino acid substitutions compared to a wild-type antibody of the class IgG, wherein the one or
 25 more amino acid substitutions reduce the affinity of the antibody for the neonatal Fc receptor (FcRn), thereby reducing the serum half-life of the modified monoclonal antibody compared to a wild-type antibody of class IgG; and

- a radioisotope conjugated to one or more amino acid residues of the heavy or light chains;
- wherein the IgG molecule comprises:

(i) a VH comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO 1 or 17, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set in SEQ ID NO: 2 or 18, and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 3 or 19;

(ii) a VH comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO: 4 or 20;

(iii) a VL comprising a CDR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 35;

(iv) a VL comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO:36;

(v) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19;

(vi) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20;

(vii) a VL comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 45;

(viii) a VL comprising a sequence set forth in SEQ ID NO: 36;

(ix) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 1 or 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 2 or 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 3 or 19; and a VL comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 35; or

(x) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20 and a VL comprising a sequence set forth in SEQ ID NO: 36.

21. The therapeutic IgG molecule of claim 20 wherein the variable region of the heavy chain (VH) comprises an amino acid sequence as set forth in any one of SEQ ID NO: 4.
22. The therapeutic IgG molecule of any one of claims 20 to 21 wherein the variable region of the light chain comprises an amino acid sequence as set forth in any one of: SEQ ID NO: 36.
23. The therapeutic IgG molecule of any one of claims 20 to 22, wherein the one or more amino acid substitutions comprise a substitution in the heavy chain constant region at position His310 or at His435, preferably, wherein the amino acid substitutions are at both His310 and His435.
24. The therapeutic IgG molecule of any one of claims 20 to 23, wherein the modified antibody further comprises: (a) one or more amino acid substitutions that reduce the affinity of the antibody for one or more Fc gamma receptors compared to a wild-type antibody of the class IgG; and/or (b) one or more amino acid substitutions that increase the stability of the CH1-CH2 hinge region in the modified antibody compared to a wild-type antibody of the class IgG.
25. The therapeutic IgG molecule of claim 24 wherein the one or more amino acid substitutions that reduce affinity of the antibody for an Fc gamma receptor comprises a substitution at position Leu235.

26. The therapeutic IgG molecule of claim 24 or 25 wherein the one or more amino acid substitutions that increase the stability of the CH1-CH2 hinge region in the antibody comprises a substitution at IgG molecule.

27. The therapeutic antibody of any one of claims 20 to 26 wherein the heavy chain
5 comprises an amino acid sequence as set forth in SEQ ID NO: 53.

28. The therapeutic IgG molecule of any one of claims 20 to 27 wherein the light chain comprises an amino acid sequence as set forth in SEQ ID NO: 57.

29. The therapeutic IgG molecule of any one of claims 20 to 28 wherein the antibody comprises the amino acid sequence as set forth in 53 and the antibody comprises the
10 amino acid sequence set forth in SEQ ID NO: 57.

30. An antigen binding site that binds to or specifically binds to prostate specific membrane antigen (PSMA) comprising:

FR1 - CDR1 – FR2 – CDR2 – FR3 – CDR3 – FR4 – linker - FR1a - CDR1a –
FR2a – CDR2a – FR3a – CDR3a – FR4a

15 wherein:

FR1, FR2, FR3 and FR4 are each framework regions;

CDR1, CDR2 and CDR3 are each complementarity determining regions;

FR1a, FR2a, FR3a and FR4a are each framework regions;

CDR1a, CDR2a and CDR3a are each complementarity determining regions;

20 wherein the sequence of any of the complementarity determining regions have an amino acid sequence as described in Table 1.

31. The antigen binding site of claim 30, comprising, consisting essentially of or consisting of an amino acids sequence of (in order of N to C terminus or C to N terminus) SEQ ID NO: 20.

32. The antigen binding site of claim 30 or 31 comprising an antigen binding domain of an antibody, wherein the antigen binding domain binds to or specifically binds to PSMA, wherein the antigen binding domain comprises at least one of:

- (i) a VH comprising a complementarity determining region (CDR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 17, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 18, and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 19;
- (ii) a VH comprising a sequence at least about 95% or 96% or 97% or 98% or 99% identical to a sequence set forth in SEQ ID NO 4 or 20;
- (iii) a VL comprising a CDR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 35;
- (iv) a VL comprising a sequence at least about 95% identical to a sequence set forth in SEQ ID NO:36;
- (v) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 19;
- (vi) a VH comprising a sequence set forth in SEQ ID NO: 4 or 20;
- (vii) a VL comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 45;

(viii) a VL comprising a sequence set forth in SEQ ID NO: 36;

(ix) a VH comprising a CDR1 comprising a sequence set forth in SEQ ID NO: 17, a CDR2 comprising a sequence set forth between in SEQ ID NO: 18 and a CDR3 comprising a sequence set forth in SEQ ID NO: 19; and a VL comprising a CDR1
5 comprising a sequence set forth in SEQ ID NO: 33, a CDR2 comprising a sequence set forth in SEQ ID NO: 34 and a CDR3 comprising a sequence set forth in SEQ ID NO: 35; or

(x) a VH comprising a sequence set forth in SEQ ID NO: 20 and a VL comprising a sequence set forth in SEQ ID NO: 36.

33. The antigen binding site of any one of claims 30 to 32 wherein the antigen
10 binding domain further comprises at least one of:

(i) a VH comprising a framework region (FR) 1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 25, a FR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at
15 least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 26, a FR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 27, and a FR4 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in
20 SEQ ID NO: 28;

(ii) a VL comprising a FR1 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 41, a FR2 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%
25 identical to a sequence set forth in SEQ ID NO: 42, a FR3 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 43, and a FR4 comprising a sequence at least about 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99% identical to a sequence set forth in SEQ ID NO: 44;

(iii) a VH comprising a FR1 comprising a sequence set forth in SEQ ID NO: 25, a FR2 comprising a sequence set forth between in SEQ ID NO: 26, a FR3 comprising a sequence set forth in SEQ ID NO: 27, and a FR4 comprising a sequence set forth in SEQ ID NO: 28;

5 (iv) a VL comprising a FR1 comprising a sequence set forth in SEQ ID NO: 41, a FR2 comprising a sequence set forth between in SEQ ID NO: 42, a FR3 comprising a sequence set forth in SEQ ID NO: 43, and a FR4 comprising a sequence set forth in SEQ ID NO: 44; or

(v) a VH comprising a FR1 comprising a sequence set forth in SEQ ID NO: 25, a
10 FR2 comprising a sequence set forth between in SEQ ID NO: 26, a FR3 comprising a sequence set forth in SEQ ID NO: 27, and a FR4 comprising a sequence set forth in SEQ ID NO: 28; and a VL comprising a FR1 comprising a sequence set forth in SEQ ID NO: 41, a FR2 comprising a sequence set forth between in SEQ ID NO: 42, a FR3 comprising a sequence set forth in SEQ ID NO: 43, and a FR4 comprising a sequence
15 set forth in SEQ ID NO: 44.

34. The antigen binding site of any one of claims 30 to 33 wherein the antigen binding site is an antibody, preferably a naked antibody.

35. The antigen binding site of any one of claims 30 to 33, wherein the antigen binding site is conjugated to a label, including a radiolabel or a cytotoxic agent.

20 36. The antigen binding site of any one of claims 30 to 35, wherein the antigen binding site is an IgG immunoglobulin comprising one or more amino acid substitutions in the antibody constant domain, CH2-CH3 region, which reduce the binding affinity of the antibody to the neonatal Fc receptor (FcRn) relative to a wild-type antibody Fc region.

25 37. The antigen binding site of claim 36, wherein the IgG immunoglobulin comprises one or more amino acid substitutions at positions His310, His435, Ile253 of the heavy chain constant region compared to wild-type IgG immunoglobulin.

38. The antigen binding site of claim 37, wherein the one or more amino acid modifications is selected from an amino acid substitution at residue equivalent to H310

and H435, preferably wherein the amino acid substitutions are at both H310 and H435 residues.

39. The antigen binding site of claim 38, wherein the amino acid substitutions are His310Ala and/or His435Gln.

5 40. The antigen binding site of any one of claims 30 to 39, wherein the antigen binding site is an IgG immunoglobulin comprising one or more amino acid substitutions which modify the binding of the antibody to activating Fc gamma receptors, preferably wherein the amino acid modification is from Leu235 to glutamic acid.

41. The antigen binding site of any one of claims 30 to 40 wherein the antigen
10 binding site comprises a hinge stabilising amino acid modification at Ser228, wherein preferably the amino acid modification at Ser228 is to proline.

42. An *in vivo* method of diagnosing, monitoring or prognosing a disease, disorder or infection in a subject comprising:

(a) administering to a subject an effective amount of the antibody according to
15 any one of claims 1 to 7 or the antigen binding site according to any one of claims 30 to 41, said antibody or antigen binding site specifically binding to an antigen associated with a disease, disorder or infection;

(b) allowing the antibody or antigen binding site to concentrate at sites in said subject where said antigen is found; and

20 (c) detecting said antibody or antigen binding site;

whereby detection of said antibody or antigen binding site above a background or standard level indicates that the subject has said disease disorder or infection.

43. The method of claim 42 wherein the antibody or antigen binding site is conjugated to a radiolabel, preferably wherein the radiolabel is selected from the group
25 consisting of: actinium-225 (^{225}Ac), astatine-211 (^{211}At), bismuth-212 and bismuth-213 (^{212}Bi , ^{213}Bi), copper-64 and copper-67 (^{64}Cu , ^{67}Cu), gallium-67 and gallium-68 (^{67}Ga and ^{68}Ga), indium-111 (^{111}In), iodine -123, -124, -125 or -131 (^{123}I , ^{124}I , ^{125}I , ^{131}I) preferably (^{124}I), lead-212 (^{212}Pb), lutetium-177 (^{177}Lu), radium-223 (^{223}Ra), samarium-

153 (^{153}Sm), scandium-44 and scandium-47 (^{44}Sc , ^{47}Sc), strontium-90 (^{90}Sr), technetium-99 ($^{99\text{m}}\text{Tc}$), yttrium-86 and yttrium-90 (^{86}Y , ^{90}Y), and zirconium-89 (^{89}Zr).

44. The method of claim 43 wherein the radiolabel is selected from: gallium-67 and gallium-68 (^{67}Ga and ^{68}Ga), iodine -124 (^{124}I), and zirconium-89 (^{89}Zr).

Figure 1

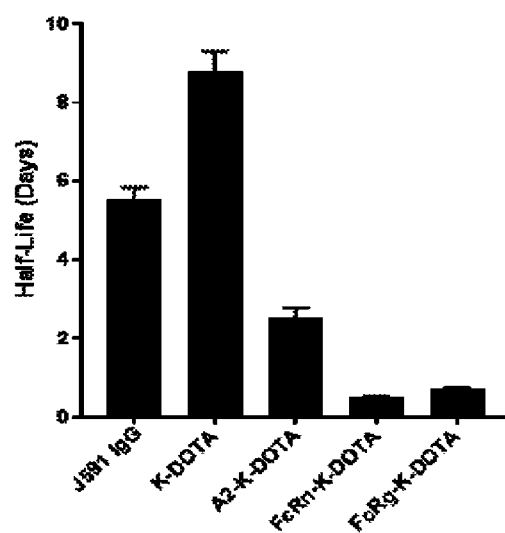


Figure 2

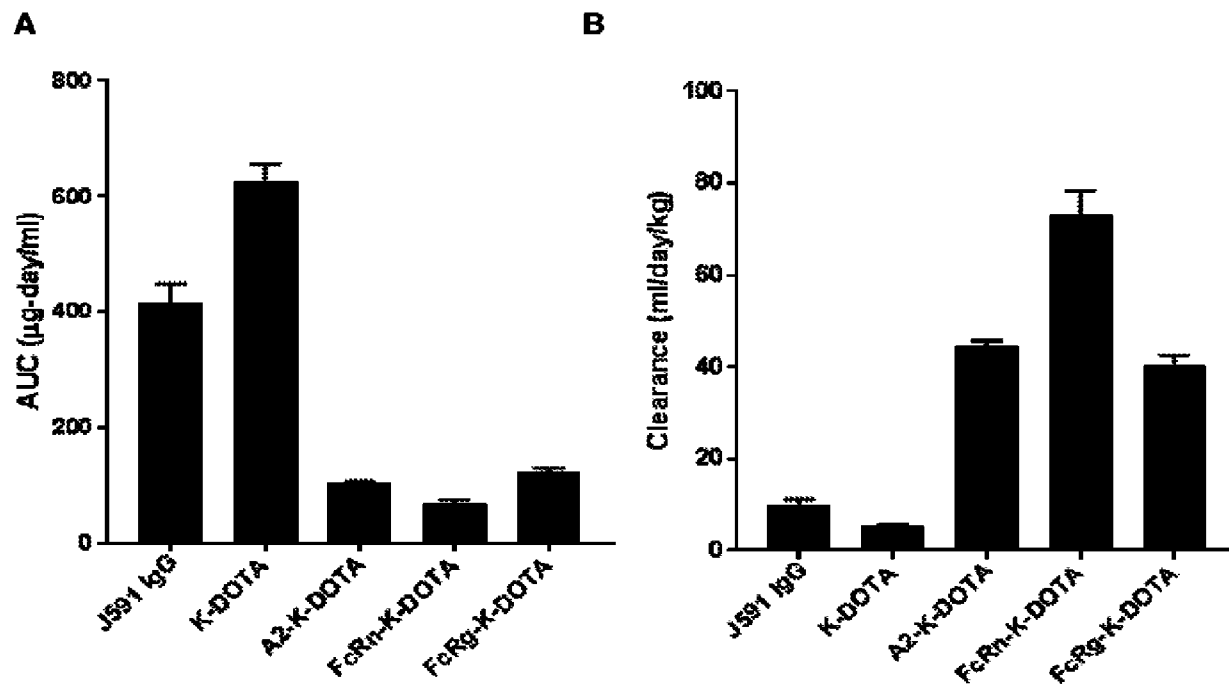


Figure 3

In Vivo Biodistribution at 8 hours

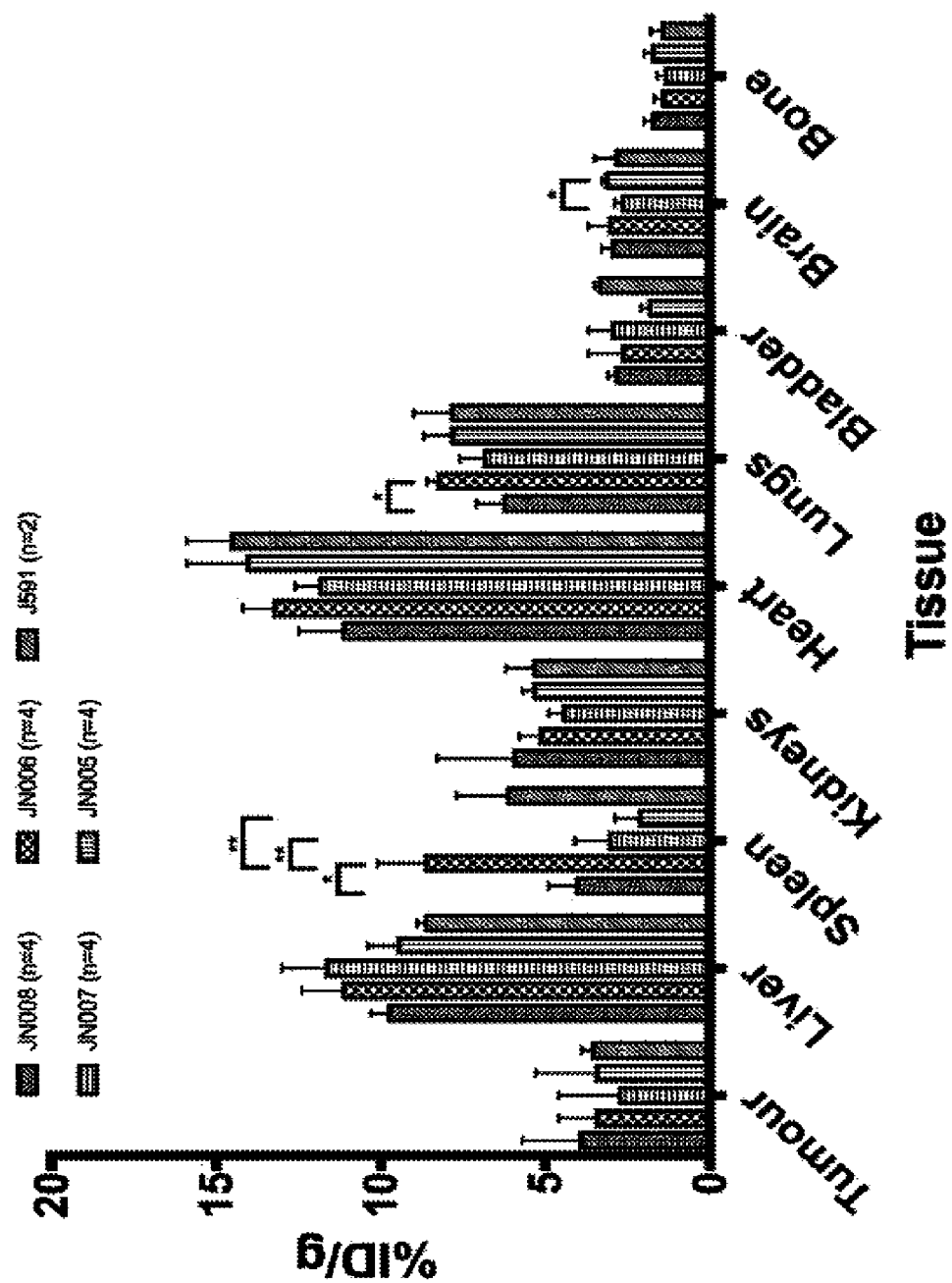
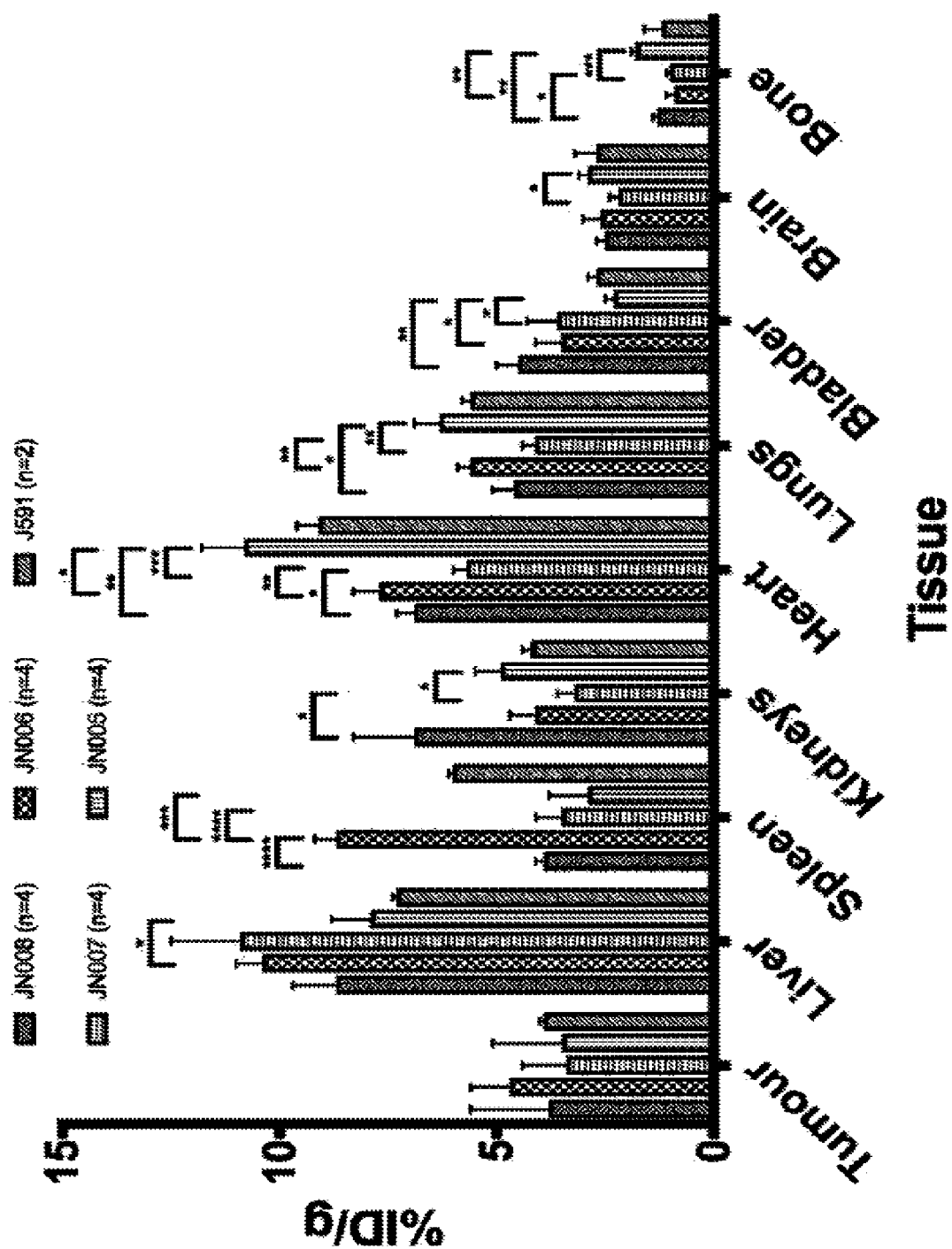


Figure 4

In Vivo Biodistribution at 24 hours



Ex Vivo Biodistribution at 48 hours

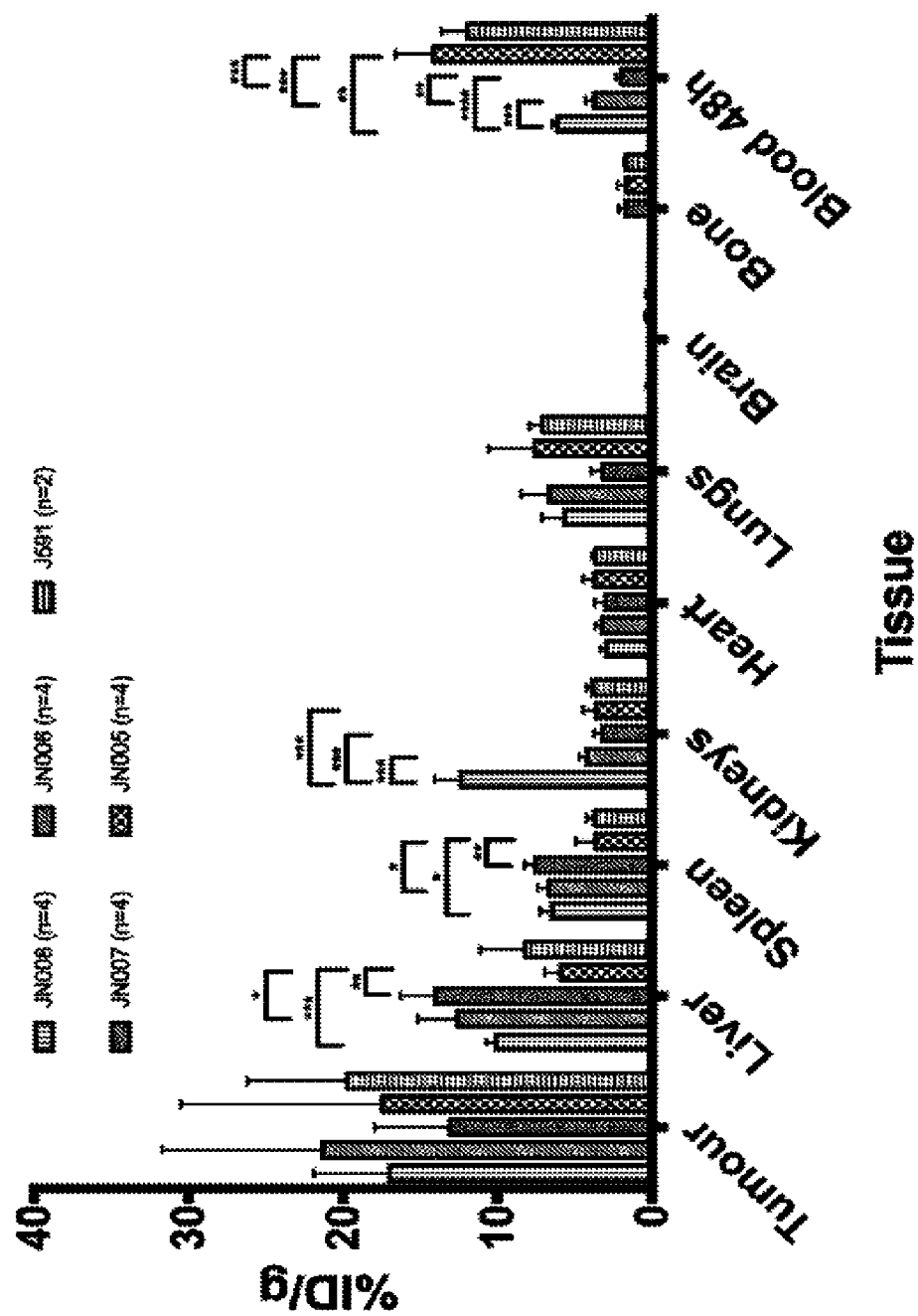


Figure 6

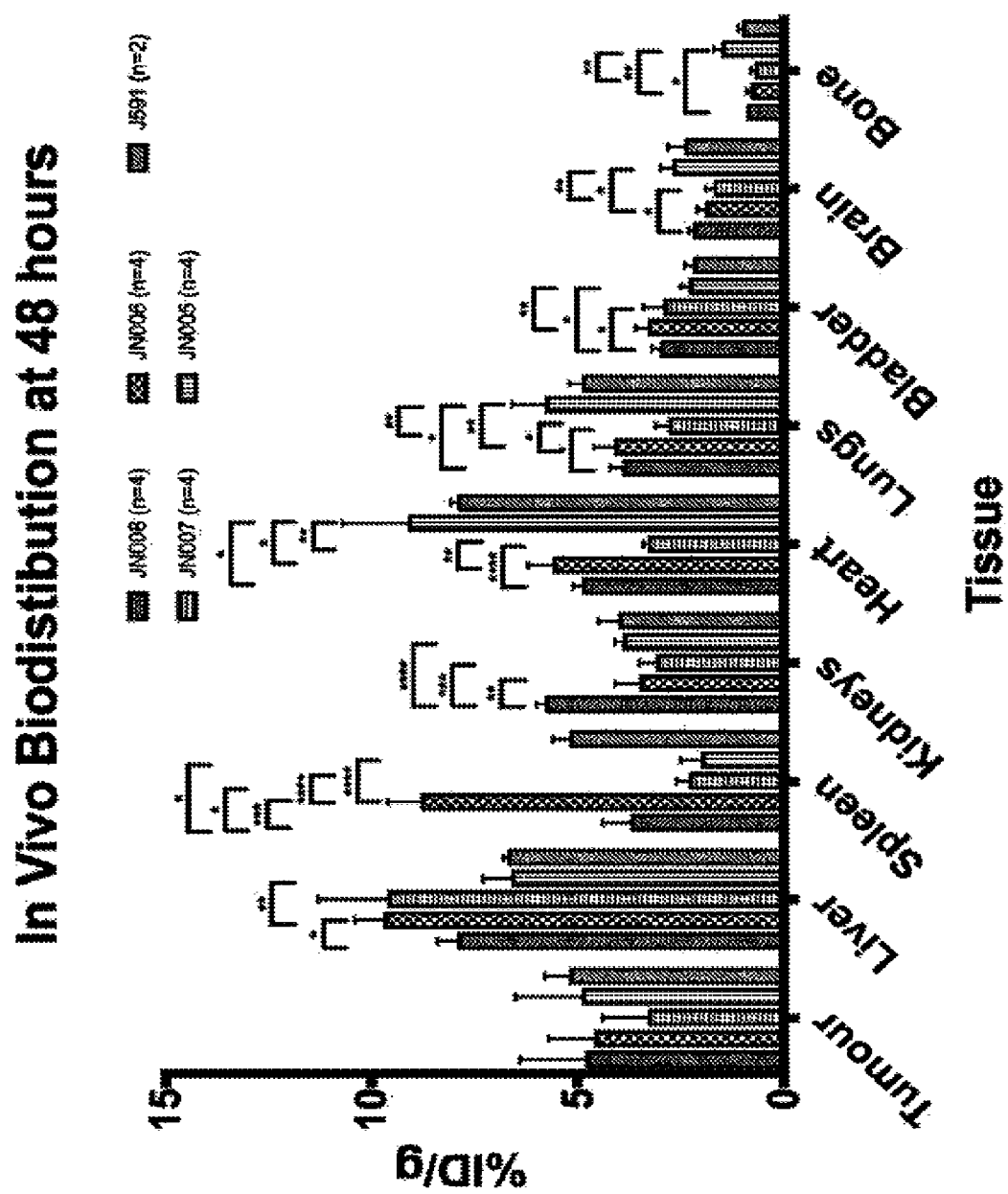


Figure 7

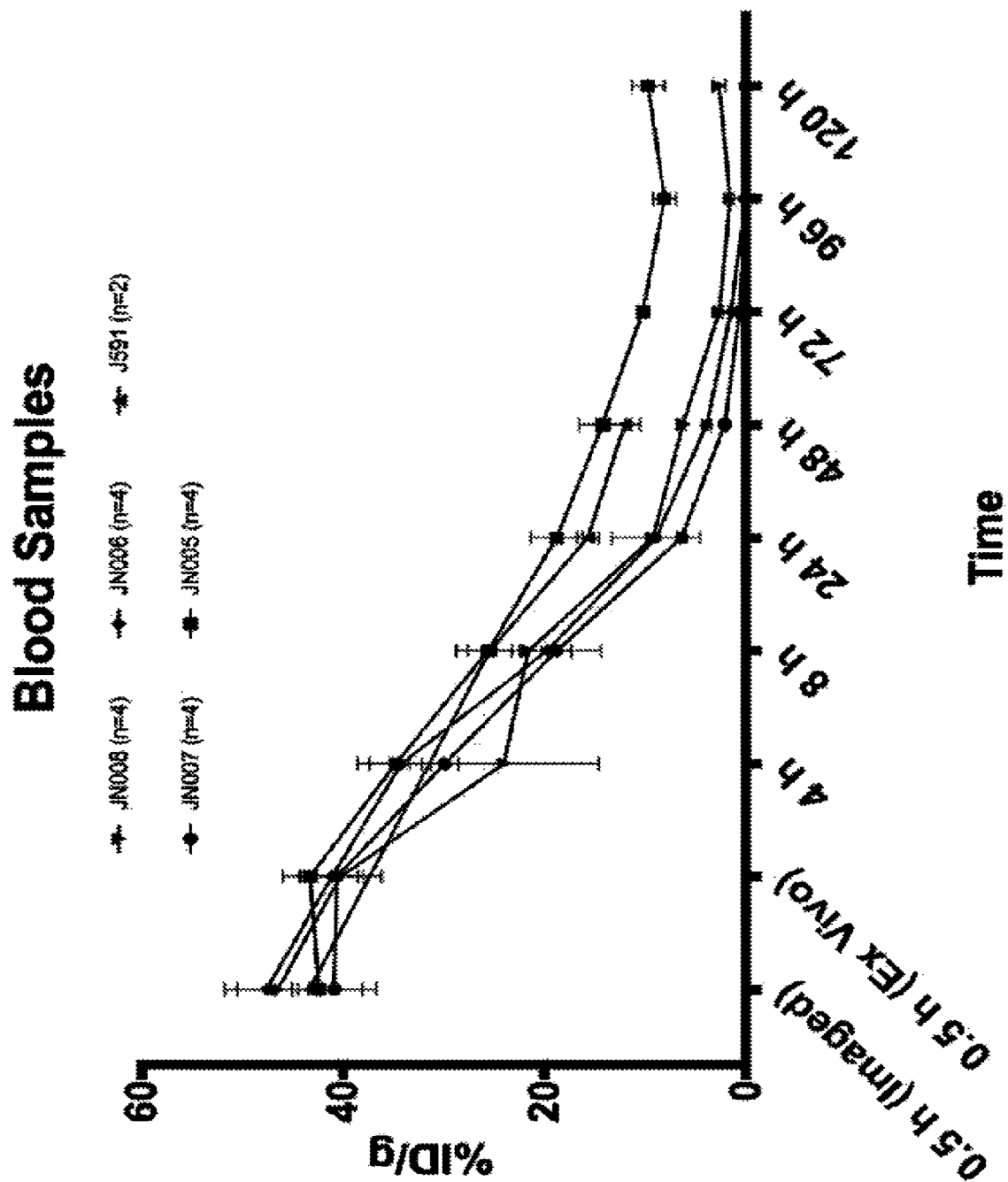


Figure 8

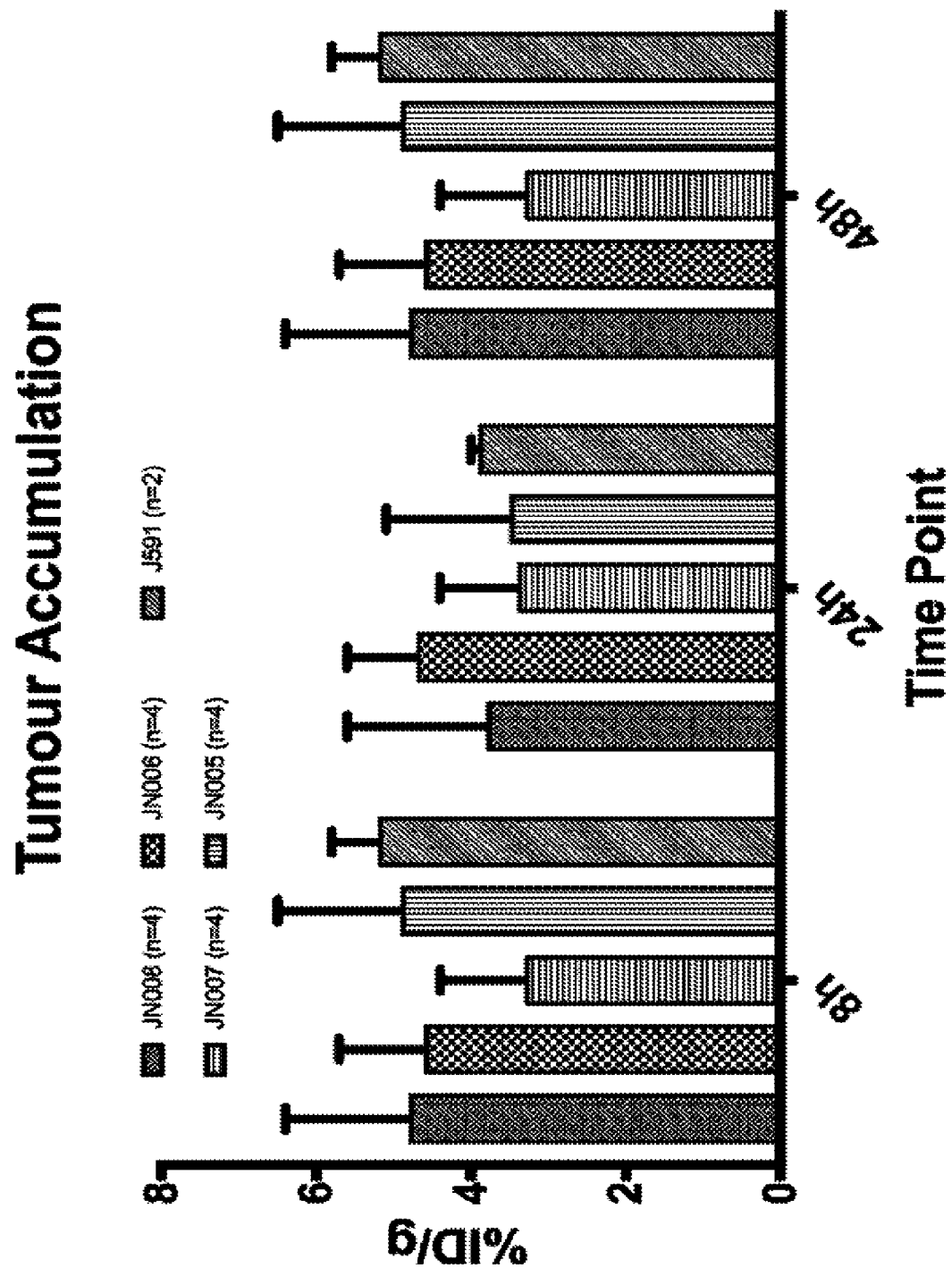


Figure 9

Tumour to Blood from (In Vivo:Tail Bleed)

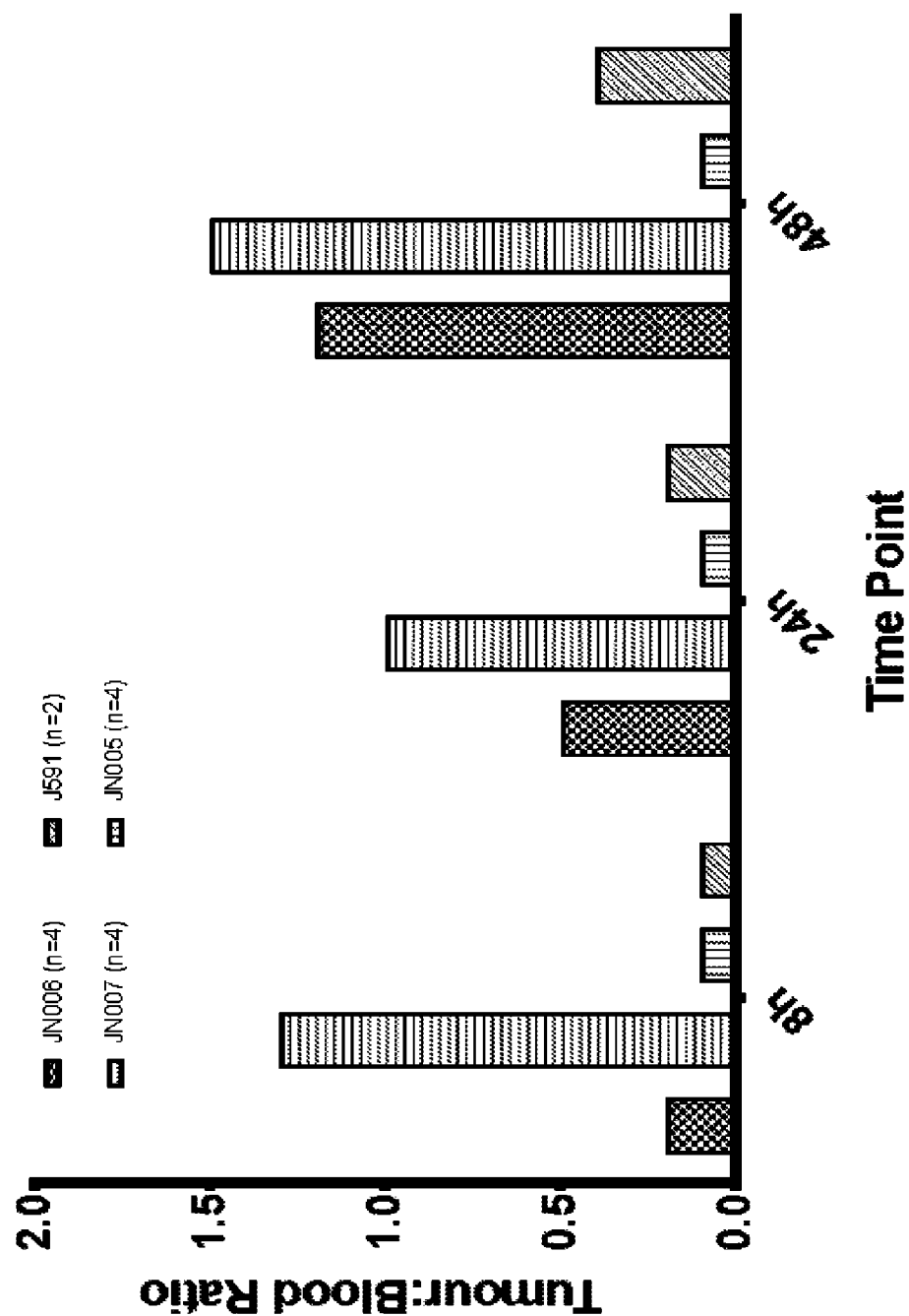


Figure 10

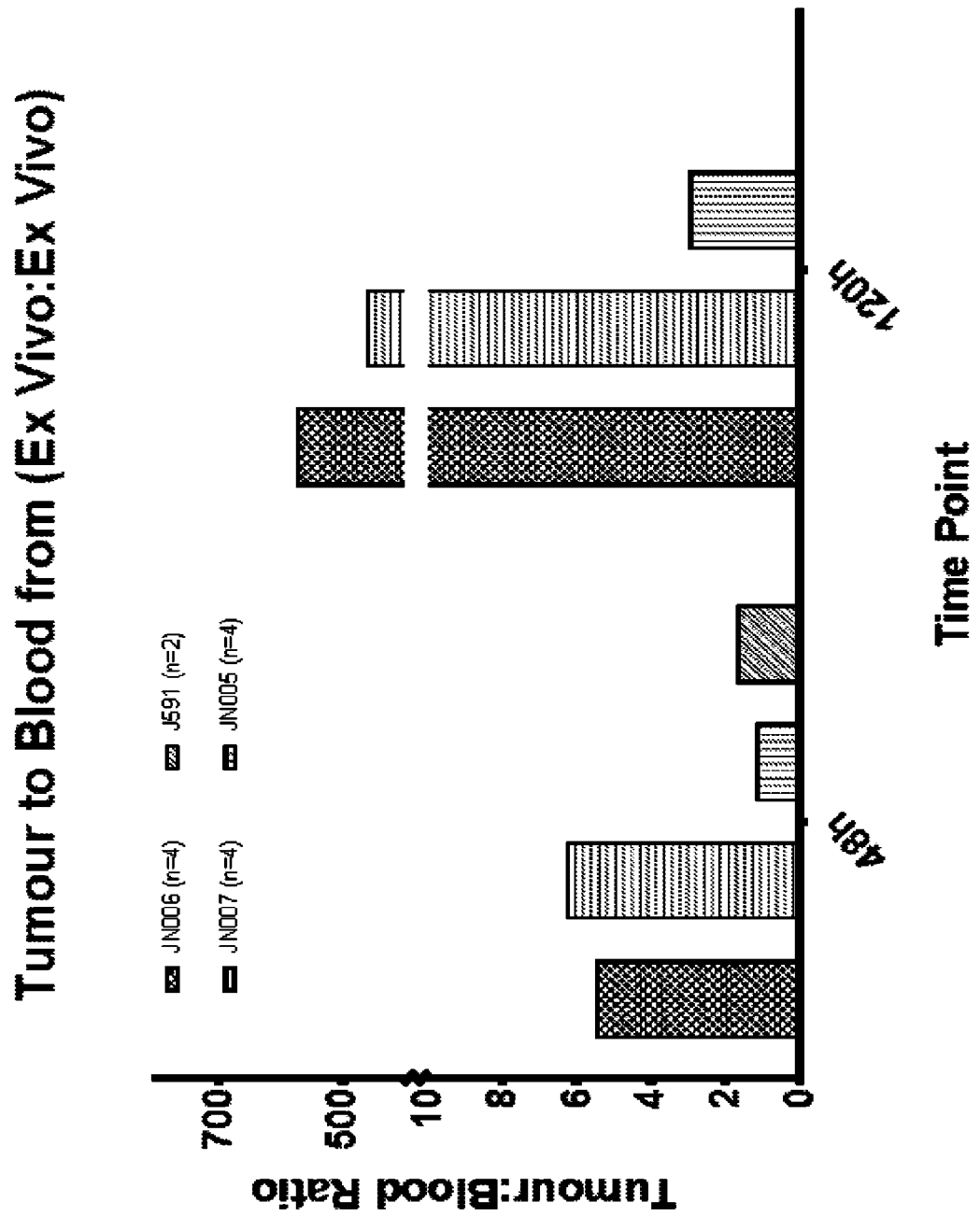


Figure 11

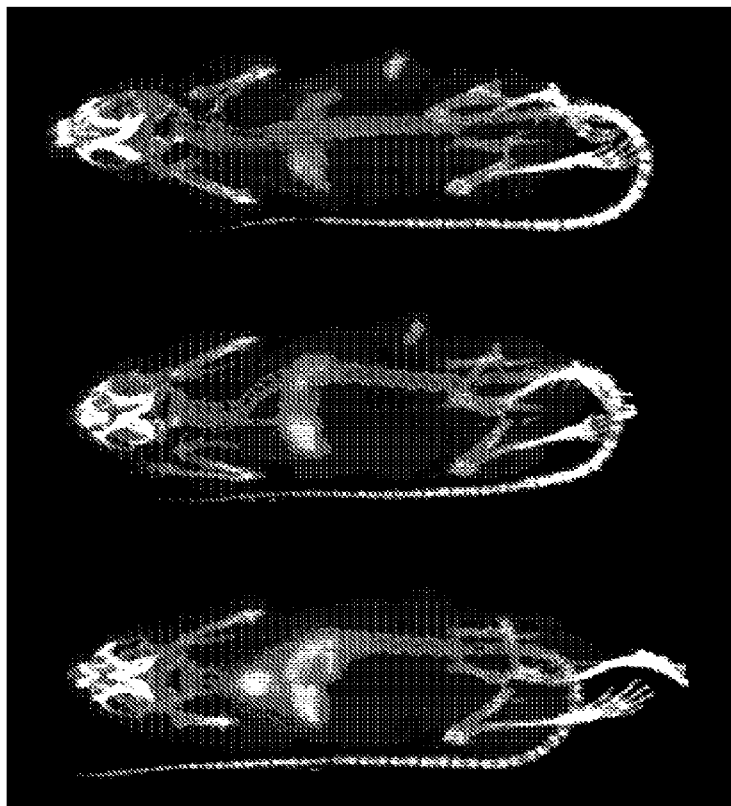


Figure 12

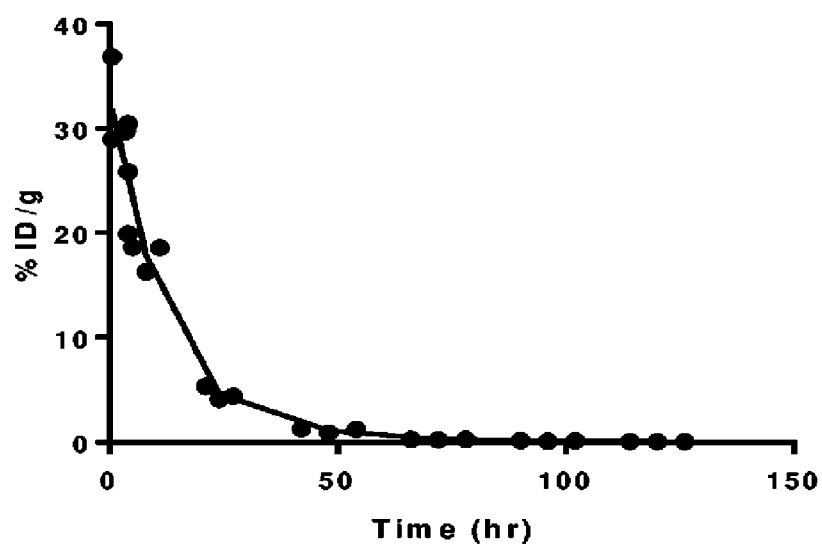
Blood PK

Figure 13

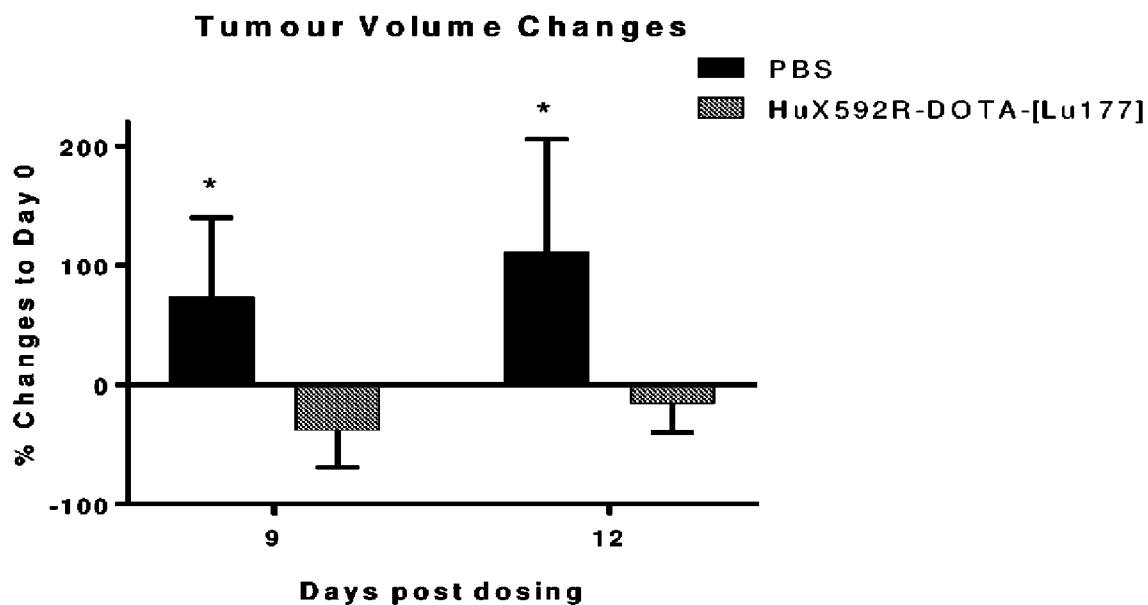


Figure 14

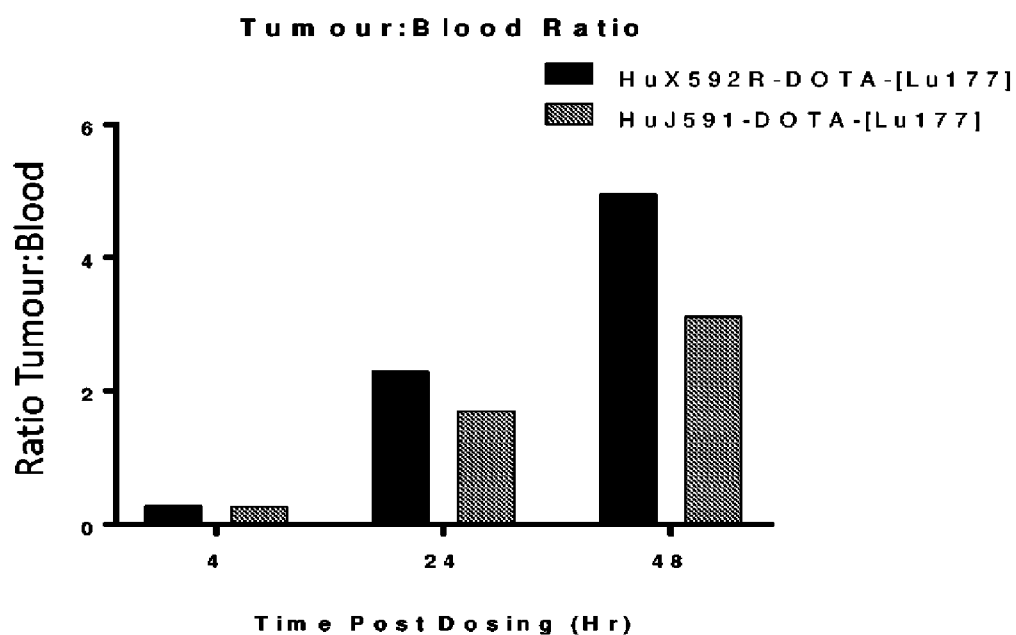


Figure 15

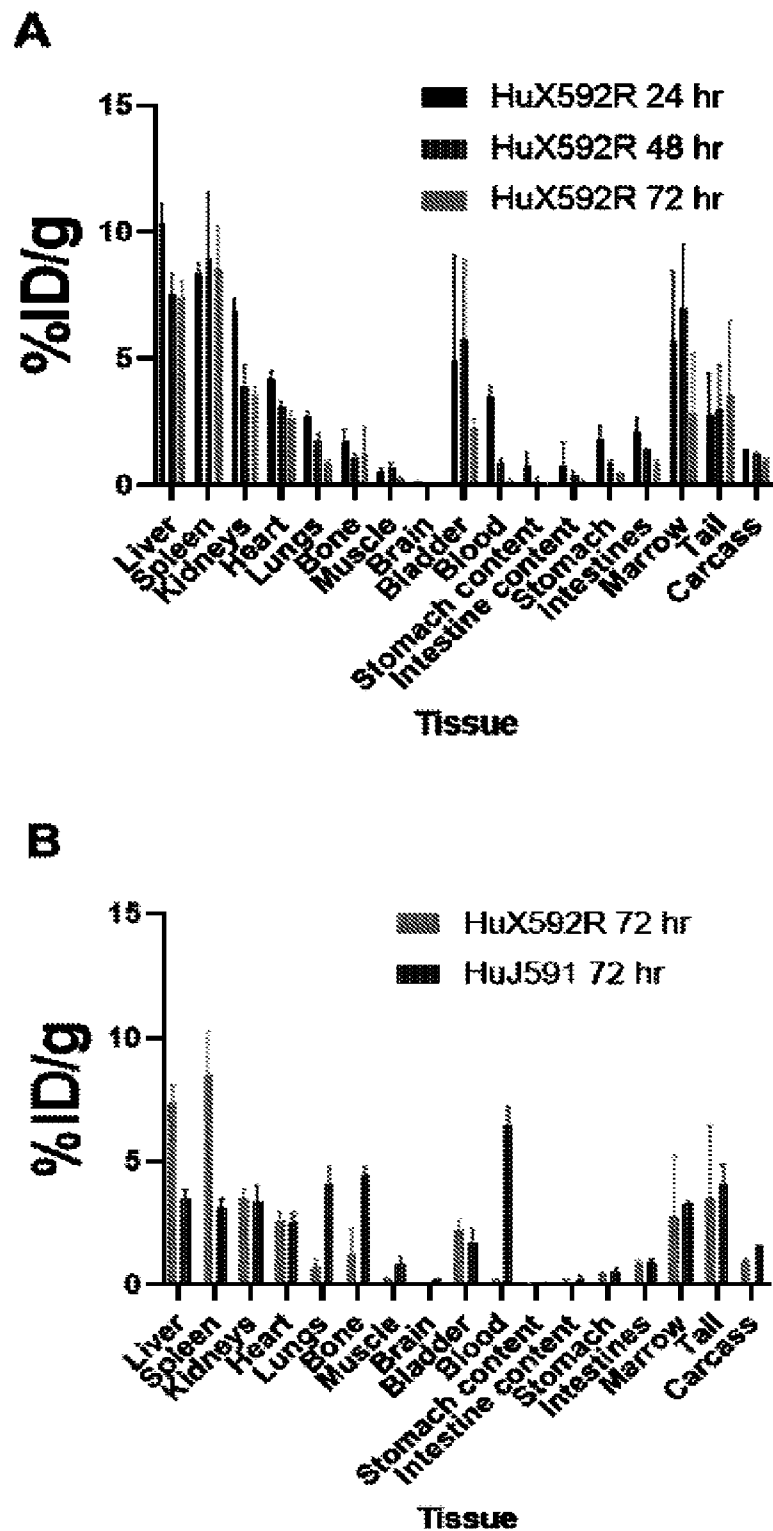
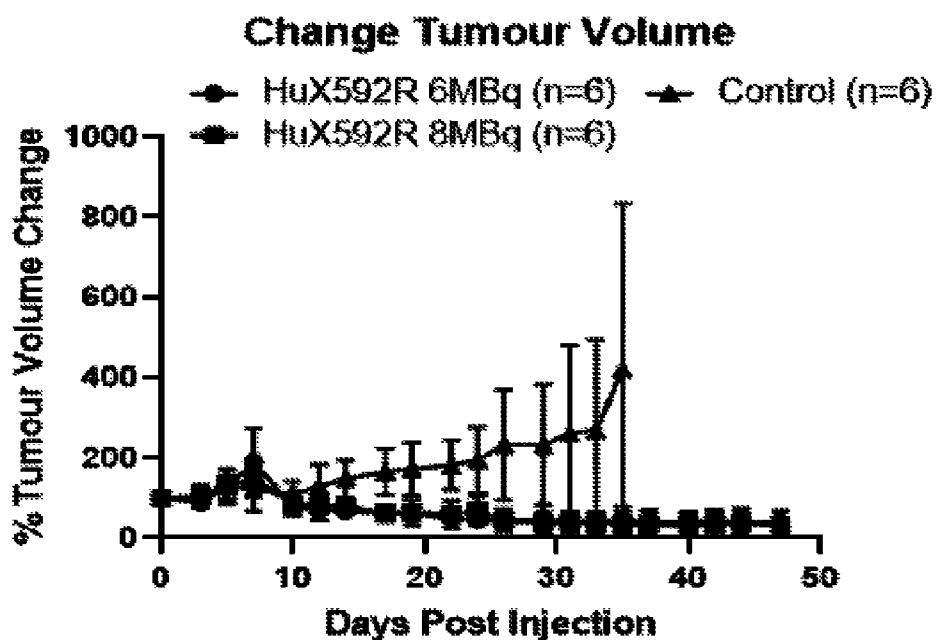


Figure 16

A



B

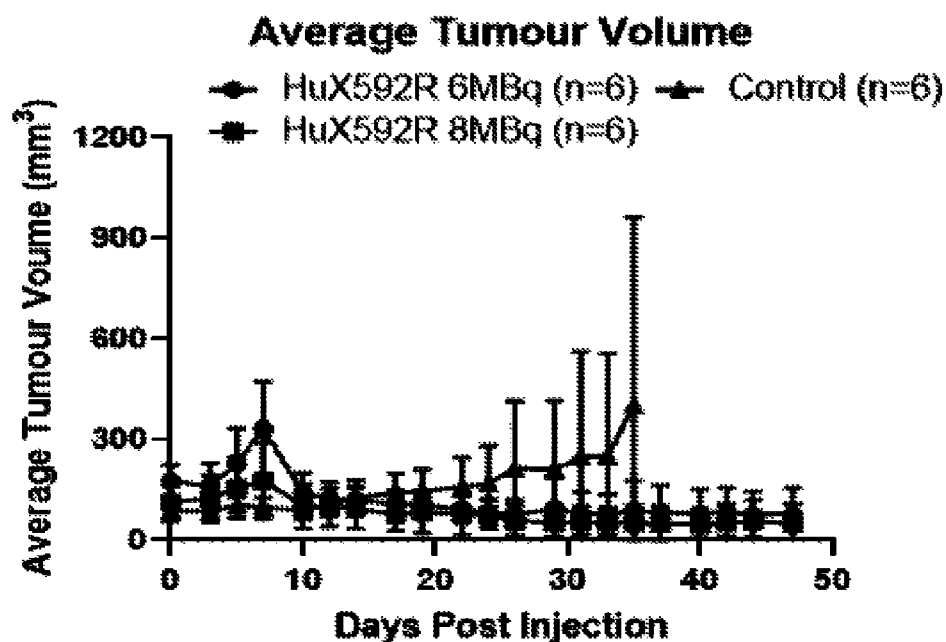


Figure 17

