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(54) Title: IMMUNOCONJUGATES THAT BIND PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA)

(57) Abstract: The invention relates to conjugated immunoconjugates that bind specifically to prostate specific membrane antigen (PSMA), in particular, conjugates comprising single human variable heavy chain domain antibodies and MMAE toxins as well as related methods for treatment of cancer.

Immunoconjugates that bind prostate specific membrane antigen (PSMA)

Field of the Invention

The invention relates to immunoconjugates that bind to prostate specific membrane antigen (PSMA), and the use of such immunoconjugates in the treatment of disease.

Introduction

Prostate cancer is the most commonly diagnosed non-skin-related malignancy in males in developed countries. It is estimated that one in six males will be diagnosed with prostate cancer.

Current treatments for prostate cancer include surgery, radiation, and adjuvant hormonal therapy. Although these therapies are relatively effective in the early stages of disease, the majority of patients initially diagnosed with localized prostate cancer ultimately relapse. Whilst chemotherapy is one of the most widely used approaches in combating advanced prostate cancer, its therapeutic efficacy is usually insufficient due to lack of specificity and associated toxicity. Lack of targeted delivery to prostate cancer cells is one of the primary obstacles in achieving feasible therapeutic effect. Consequently, there remains a critical need for strategies to increase the selectivity of anti-prostate cancer agents (Barve *et al.*, *J Control Release*. 2014 August 10; 0: 118–132).

The diagnosis of prostate cancer has greatly improved following the use of serum-based markers such as the prostate specific antigen (PSA). In addition, prostate tumour-associated antigens offer targets for tumour imaging, diagnosis, and targeted therapies. The prostate specific membrane antigen (PSMA), a prostate tumour associated marker, is such a target.

PSMA is a 750-residue type II transmembrane glycoprotein highly restricted to prostate secretory epithelial cell membranes. It is highly expressed in prostate cancer cells and in nonprostatic solid tumor neovasculature and expressed at lower levels in other tissues, including healthy prostate, kidney, liver, small intestine, and brain. PSMA expression increases with prostate disease progression and metastasis and its expression level has thus been correlated with tumour aggressiveness. Various immunohistological studies have demonstrated increased PSMA levels in virtually all cases of prostatic carcinoma compared to those levels in benign prostate epithelial cells. Intense PSMA staining is found in all stages of the disease, including prostatic intraepithelial neoplasia, late stage androgen-independent

prostate cancer and secondary prostate tumours localized to lymph nodes, bone, soft tissue, and lungs. PSMA is thus widely used as a biomarker for prostate cancer cells.

PSMA has a 3-part structure: a 19-amino-acid internal portion, a 24-amino-acid transmembrane portion, and a 707-amino-acid external portion. It forms a noncovalent homodimer that possesses glutamate carboxypeptidase activity based on its ability to process the neuropeptide N-acetylaspartylglutamate and glutamate-conjugated folate derivatives. PSMA is rapidly and efficiently internalized by an endocytic pathway and rapidly recycles back to the membrane.

Antibody-based therapeutics have emerged as important components of therapies for an increasing number of human malignancies in such fields as oncology, inflammatory and infectious diseases. In most cases, the basis of the therapeutic function is the high degree of specificity and affinity the antibody-based drug has for its target antigen. Arming monoclonal antibodies (mAbs) with drugs, toxins, or radionuclides is yet another strategy by which mAbs may induce a therapeutic effect. By combining the targeting specificity of an antibody with the tumour killing power of toxic effector molecules, immunoconjugates permit sensitive discrimination between target and normal tissue thereby resulting in fewer side effects than most conventional chemotherapeutic drugs.

Due to their size and other physical properties, however, mAbs have to be administered either intravenously (iv) or subcutaneously (sc) and therefore have a high systemic exposure. Thus, their route of delivery can often be suboptimal, resulting either in antibody binding to target antigen at non-disease locations (potentially compromising the healthy function of normal, non-disease tissue) or resulting in suboptimal PK/PD characteristics. Either outcome may result in a loss of efficacy and/or a compromised safety profile by virtue of the suboptimal route of administration.

The first PSMA-specific mAb reported, murine mAb 7E11, was subsequently developed and commercialized as a diagnostic agent for tumour imaging (ProstaScint, Cytogen, Princeton, N.J.). However, this antibody recognizes an intracellular epitope of PSMA exposed upon cell death which limits its usefulness as an imaging agent for the detection of PSMA. More recently, mAbs such as J591 that recognize the extracellular portion of PSMA have been identified.

The aim of the present invention is to address the need of alternative antibody-based treatments for use in the treatment of prostate cancer.

Summary of the invention

The invention provides isolated immunoconjugates or biinding molecules that bind to human PSMA and comprise one or more single V_H domain antibody that bind human PSMA conjugated to an Auristatin toxin. In one aspect, the invention provides an immunoconjugate of the formula A-(L-D)n wherein A is an antigen-binding moiety comprising a first human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA, optionally comprising a second human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA and optionally comprising a third human single heavy chain variable immunoglobulin (V_H) domain antibody, L is a linker, and D is an auristatin or a derivative thereof and n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In one embodiment, the immunoconjugate comprises two or more single human heavy chain variable (V_H) domain antibodies. In particular, the binding is to human PSMA in its native form. In preferred embodiments, the single V_H domain is generated from a heavy chain only antibody produced in a transgenic rodent expressing human V gene loci and immunised with a human PSMA antigen. Single domain antibodies used in the immunoconjugates of the invention bind a target in monovalent form. Single domain antibodies are smaller than conventional monoclonal antibody formats and the inventors have shown that such molecules facilitate high levels of specific tumor targeting, fast penetration and high accumulation in the tumor compared to a monoclonal antibody benchmark. As shown herein, single domain antibodies bind human PSMA with high affinity, are very stable and expressed to high level. Furthermore, single V_H domain antibodies are less immunogenic than murine antibodies and no humanization is required. These properties make the compounds of the invention particularly useful in different formats, for example conjugated to a toxin or half-life extending moiety. The compounds are thus useful in treating disease, in particular cancer.

Drawings

Figure 1. Family 1 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 1. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 2. Family 2 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 2. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 3. Family 3 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 3. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 4. Family 4 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 4. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 5. Family 5 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 5. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 6. Family 6 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 6. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 7. Family 7 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 7. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 8. Family 8 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 8. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 9. Family 9 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 9. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 10. Family 10 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 10. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 11. Family 11 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 11. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 12. Family 12 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 12. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 13. Family 13 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 13. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 14. Family 14 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 14. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 15. Family 15 sequences. This Figure shows the full length V_H sequence for single domain antibodies in family 15. Framework (FR) and complementarity determining regions (CDR) are labelled. CDR1, CDR2 and CDR3 are highlighted in bold.

Figure 16. Binding of purified anti-PSMA V_H in FMAT Mirrorball Assay. **16a** ●1.1, ▪3.1, ▲2.10, ▼2.1, **16b** ● 2.1, ▲ 2.13, ▼2.17 ◊2.15, ○2.12 Δ2.22 **16c** single domain antibodies tested as shown by symbols from top to bottom ● 1.8, ▪ 1.10, ▲1.11, ▼1.12, 1.13, ○1.14, 1.16, Δ 1.17, 1.18 d) biparatopic immunoconjugates tested as shown by symbols from top to bottom: L=$(G4S)_6$ 1.1-L-2.1; 1.16-L-2.1; 1.11-L-2.1, 1.18-L-2.1, 1.17-2.1, 1.1-2.17, 1.16-L-2.17, 1.11-L-2.17, 1.18-L-2.17, 1.17-L-2.17; e) 1.1-L-2.15, 1.16-L-2.15, 1.11-L-2.15, 1.18-L-2.15, 1.17-L-2.15, 1.1-L-2.22, 1.16-L-2.22, 1.11-L-2.22, 1.11-L-2.22, 1.18-L-2.22, 1.17-L-L2.22

Figure 17. pHrodoGreen internalisation of purified anti-PSMA single domain antibodies. sDAbs used (symbols in legend from top to bottom): 2.20, 12.1, 3.1, 3.2, 4.1, 5.1, 9.1, 14.1, 10.1, 7.1.

Figure 18. Killing of cynoPSMA and human PSMA CHO with anti-PSMA single domain antibodies **A.** 2.1 **B.** 1.1.

Figure 19. Killing of LNCap with anti-PSMA single domain antibodies. sDAbs used (symbols in legend from top to bottom): 1.1, 2.1, 7.1, 3.1, 12.1, 4.1.

Figure 20. shows in vitro cytotoxicity of monomeric MMAE-conjugated V_H (A and B), bivalent V_H (C and D) and biparatopic VH (E and F) on human cells stably expressing human PSMA protein and matched parental cells (PSMA negative) at a 48 hour incubation time point.

Figure 21. shows in vitro cytotoxicity of MMAE-conjugated V_H on human cells stably expressing human PSMA protein at a 72 hour incubation time point.

Figure 22. shows the HiPEG™ val-cit-PAB-MMAE reagent (MW = 2805 g/mol) used to prepare Humabody™ drug conjugates (HDCs).

Figure 23. shows the Maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonyl-monomethyl auristatin E (mc-val-cit-PAB-MMAE) conjugation reagent (MW = 1317 g/mol) used to produce the Pro_006 control antibody drug conjugates (ADC).

Figure 24. A & B show IC_{50} values in the PSMA-DU145 cytotoxicity assay (72h) for: Control ADC mAb-MMAE (■), 2.1 6G4S-1.1-MMAE biparatopic (▲), 2.1 6GS-1.1-MMAE-HLE half-life extended biparatopic(▼), HEL4-MMAE Monovalent (●) and HEL4-HLE-MMAE Monovalent half-life extended (I), for (A) DU145 cells expressing PSMA and (B) DU145 parental cells that have not been modified to express PSMA.

Detailed description

The present invention will now be further described. In the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any

feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, pathology, oncology, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art. The methods and techniques of the present disclosure are generally performed according to conventional methods well-known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, *e.g.*, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

The invention provides isolated immunoconjugates that bind to human PSMA and comprise one or more single V_H domain antibody that binds human PSMA conjugated to an Auristatin or derivative thereof. In one aspect, the invention relates to an immunoconjugate of the formula A-(L-D)n wherein A is an antigen-binding moiety comprising a first human single heavy chain variable immunoglobulin (VH) domain antibody capable of binding specifically to human PSMA, optionally comprising a second human single heavy chain variable immunoglobulin (VH) domain antibody capable of binding specifically to human PSMA and optionally comprising a third human single heavy chain variable immunoglobulin (VH) domain antibody,

L is a linker, andD is an auristatin or a derivative thereof and n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. The linker is a preferably specified herein. The invention also relates to pharmaceutical compositions comprising such immunoconjugates. Also provided are methods of using the immunoconjugates disclosed herein to target human PSMA either *in vitro* or *in vivo*, and methods of treating disease.

The PSMA binding immunoconjugates of the invention bind to wild type human PSMA (accession NO. Q04609). The sequence for the monomer is shown below (SEQ ID No.529).

1 MWNLLHETDS AVATARRPRW LCAGALVLAG GFFLLGFLFG WFIKSSNEAT NITPKHNMKA

61 FLDELKAENI KKFLYNFTQI PHLAGTEQNF QLAKQIQSQW KEFGLDSVEL AHYDVLLSYP

121 NKTHPNYISI INEDGNEIFN TSLFEPPPPG YENVSDIVPP FSAFSPQGMP EGDLVYVNYA
181 RTEDFFKLER DMKINCSGKI VIARYGKVFR GNKVKNAQLA GAKGVILYSD PADYFAPGVK
241 SYPDGWNLPG GGVQRGNILN LNGAGDPLTP GYPANEYAYR RGIAEAVGLP SIPVHPIGY
301 DAQKLLEKMG GSAPPDSSWR GSLKVPYNVG PGFTGNFSTQ KVKMHIHSTN EVTRIYNVIG
361 TLRGAVEPDR YVILGGHRDS WVFGGIDPQS GAAVVHEIVR SFGTLKKEGW RPRRTILFAS
421 WDAEEFGLLG STEWAEENSR LLQERGVAYI NADSSIEGNY TLRVDCTPLM YSLVHNLTKE
481 LKSPDEGFEG KSLYESWTKK SPSPEFSGMP RISKLGSGND FEVFFQRLGI ASGRARYTKN
541 WETNKFSGYP LYHSVYETYE LVEKFYDPMF KYHLTVAQVR GGMVFELANS IVLPFDCRDY
601 AVVLRKYADK IYSISMKHPQ EMKTYSVSFD SLFSAVKNFT EIASKFSERL QDFDKSNPIV
661 LRMMNDQLMF LERAFIDPLG LPDRPFYRHV IYAPSSHNKY AGESFPGIYD ALFDIESKVD
721 PSKAWGEVKR QIYVAAFTVQ AAAETLSEVA

In one embodiment, the PSMA binding immunoconjugates of the invention bind to wild type human PSMA and/or cyno PSMA. The terms "PSMA binding immunoconjugate", “PSMA binding protein” “anti-PSMA single domain antibody” or “anti-PSMA antibody” as used herein all refer to a molecule capable of binding to the human PSMA antigen. The binding reaction may be shown by standard methods (qualitative assays) including, for example, a binding assay, competition assay or a bioassay for determining the inhibition of PSMA binding to its receptor or any kind of binding assays, with reference to a negative control test in which an antibody of unrelated specificity. Suitable assays are shown in the examples.

An antibody, fragment thereof or immunoconjugate of the invention, "which binds" or is “capable of binding” an antigen of interest, e.g. PSMA, is one that binds, i.e. targets, the PSMA antigen with sufficient affinity such that it is useful in therapy in targeting a cell or tissue expressing the antigen.

Single domain antibodies used in the immunoconjugates of the invention bind specifically to human PSMA. In other words, binding to the PSMA antigen is measurably different from a non-specific interaction. Preferably, the single domain antibodies used in the immunoconjugates of the invention bind to human PSMA and also bind to cyno PSMA. The term "specific binding" or "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide target as used herein can be exhibited, for example, by a molecule having a KD for the target of at least about 10^{-4} M, alternatively at least about 10^{-5} M, alternatively at least about 10^{-6} M, alternatively at least about 10^{-7} M, alternatively at least about 10^{-8} M, alternatively at least about 10^{-9} M, alternatively at least about 10^{-10} M, alternatively at least about 10^{-11} M, alternatively at least about 10^{-12} M, or greater. In one embodiment, the term "specific binding" refers to binding where a molecule binds to a particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope.

In one aspect, the immunoconjugate comprises a single V_H domain antibody capable of binding human PSMA. In another embodiment, the immunoconjugate comprises a first single V_H domain antibody capable of binding human PSMA and a second single V_H domain antibody capable of binding human PSMA. The single V_H domain antibodies are preferably covalently linked, for example via a peptide linker.

In one aspect, the invention relates to a MMA conjugated immunoconjugate comprising at least one HCAb comprising a V_H domain as described herein, capable of binding human PSMA

In one embodiment, the heavy chain only antibody comprises human variable regions. In one embodiment, the HCAb lacks the C_H1 domain. In one embodiment, the HCAb comprises murine C regions. In one embodiment, the immunoconjugate comprises at least one single V_H domain antibody.

In one aspect, the invention relates to multivalent/multiparatopic isolated immunoconjugates capable of binding to human PSMA comprising a heavy chain variable immunoglobulin domain (V_H) comprising a CDR3 sequence as shown in any of Figures 1 to 15 with reference to Tables 1 to 15 or a sequence with at least 60%, 70%, 80%, 90%, 95% or more sequence identity thereto. In one embodiment, the immunoconjugate comprises a set of CDR1, 2 and 3 sequences selected from the sets of CDR1, 2 and 3 sequences as shown for the any of the clones of any of Figures 1 to 15 with reference to Tables 1 to 15. In one embodiment, the immunoconjugate comprises a V_H domain with a set of CDR1, 2 and 3 sequences selected from the sets of CDR1, 2 and 3 sequences as shown for the any of the clones of any of Figures 1 to 15 with reference to Tables 1 to 15. The immunoconjugates of the invention may comprise more than one single V_H domain antibody, e.g. two, three or four more single heavy chain variable immunoglobulin domain (V_H) antibodies.

The terms “single domain antibody, variable single domain or immunoglobulin single variable domain (ISV)” are all well known in the art and describe the single variable fragment of an antibody that binds to a target antigen. These terms are used interchangeably herein. As explained below, preferred embodiments of the various aspects of the invention relate to single heavy chain variable domain antibodies/immunoglobulin heavy chain single variable domains which bind a PSMA antigen in the absence of light chain. Fragments of the single domain antibody, variable single domain or immunoglobulin single variable domain that bind to human PSMA are also within the scope of the invention. Single heavy chain variable domain antibodies (V_H) do not comprise an immunoglobulin light chain. Human heavy chain single variable (V_H) domain antibodies are particularly preferred. Human heavy chain single

variable V_H are commonly abbreviated as V_H domains. Single V_H domain antibodies are also termed Humabody® herein. Humabody® is a registered trademark of Crescendo Biologics Ltd.

Thus, in some preferred embodiments, the isolated binding agents/molecules of the invention comprise at least two single V_H domain antibodies wherein said domain is preferably a human heavy chain variable domain. Thus, in one aspect, the binding agents of the invention comprise at least two human immunoglobulin single variable heavy chain domain; they are devoid of V_L domains.

Each single V_H domain antibody comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. Thus, in one embodiment of the invention, the domain is a human variable heavy chain (V_H) domain with the following formula FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

The term "isolated" single domain antibody refers to a single domain antibody that is substantially free of other single domain antibodies, antibodies or antibody fragments having different antigenic specificities. Moreover, an isolated single domain antibody may be substantially free of other cellular material and/or chemicals.

In one embodiment, said first or second single V_H domain antibody comprises a CDR3 sequence as shown in any of Figures 1 to 15 with reference to Tables 1 to 15 or a sequence with at least 60%, 70%, 80%, 90%, 95% or more sequence identity thereto. In one embodiment, said first or second single V_H domain comprises a set of CDR1, 2 and 3 sequences selected from the sets of CDR1, 2 and 3 sequences as shown for the any of the clones of any of Figures 1 to 15 with reference to Tables 1 to 15. In one embodiment, the first or second single V_H domain antibody comprises a set of CDR1, 2 and 3 sequences selected from the sets of CDR1, 2 and 3 sequences as shown for the any of the clones of any of Figures 1 to 15 and Tables 1 to 15. In one embodiment, the immunoconjugate is a heavy-chain-only antibody (HCAb).

In one embodiment, the first or second single V_H domain antibody comprises a CDR3 sequence as shown in any of Figures 1 to 15 and Tables 1 to 15 or a sequence with at least 60%, 70%, 80%, 90%, 95% or more sequence identity thereto. In one embodiment, the first or second single V_H domain antibody comprises a set of CDRs1, 2 and 3 sequences selected from the sets of CDR1, 2 and 3 sequences as shown for any of the sdAbs of any of Figures 1 to 15 and 1 to 15. In another embodiment, the first or second single V_H domain antibody is

selected from any of the following single V_H domain antibodies 1.1 to 1.20, 2.1 to 2.25, 3.1 to 3.24, 4.1 to 4.4, 5.1-5.2, 6.1 to 6.7, 7.1 to 7.8, 8.1, 9.1, 10.1, 11.1, 12.1, 13.1, 14.1 or 15.1.

In one embodiment, said sequence homology or identity is at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%.

"Homology" generally refers to the percentage of amino acid residues in the candidate sequence that are identical with the residues of the polypeptide with which it is compared, after aligning the sequences and in some embodiments after introducing gaps, if necessary, to achieve the maximum percent homology, and not considering any conservative substitutions as part of the sequence identity. Thus, the percent homology between two amino acid sequences is equivalent to the percent identity between the two sequences. Neither N- or C-terminal extensions, tags or insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known.

The term "antibody", broadly refers to any immunoglobulin (Ig) molecule, or antigen binding portion thereof, comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative antibody formats are known in the art. In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, C_H1, C_H2 and C_H3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG 1, IgG2, IgG 3, IgG4, IgAl and IgA2) or subclass.

An antibody fragment is a portion of an antibody, for example as $F(ab')_2$, Fab, Fv, sFv and the like. Functional fragments of a full length antibody retain the target specificity of a full length antibody. Recombinant functional antibody fragments, such as Fab (Fragment,

antibody), scFv (single chain variable chain fragments) and single domain antibodies (dAbs) have therefore been used to develop therapeutics as an alternative to therapeutics based on mAbs. scFv fragments (~25kDa) consist of the two variable domains, V_H and V_L. Naturally, V_H and V_L domain are non-covalently associated via hydrophobic interaction and tend to dissociate. However, stable fragments can be engineered by linking the domains with a hydrophilic flexible linker to create a single chain Fv (scFv). The smallest antigen binding fragment is the single variable fragment, namely the V_H or V_L domain. Binding to a light chain/heavy chain partner respectively is not required for target binding. Such fragments are used in single domain antibodies. A single domain antibody (~12 to 15 kDa) therefore has either the V_H or V_L domain.

Thus, in some preferred embodiments of the invention, the immunoconjugate does not comprise a light chain. In some embodiments, the immunoconjugate does not comprise heavy chain domains C_H2 and C_H3. In some embodiments, the immunoconjugate does not comprise a hinge region and heavy chain domains C_H2 and C_H3. In some embodiments, the immunoconjugate does not comprise heavy chain domains C_H1, C_H2, and C_H3. In some embodiments the immunoconjugate does not comprise heavy chain domain C_H1, a hinge region heavy chain domain C_H2 and heavy chain domain C_H3. In some embodiments the immunoconjugate does not comprise a light chain, a heavy chain domain C_H1, a hinge region heavy chain domain C_H2 and heavy chain domain C_H3.

Each V_H domain comprises three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Modifications to the V_H framework may be made to improve binding properties. For example, the V_H domain may comprise C or N-terminal extensions. In one embodiment, the V_H domain comprises C-terminal extensions of from 1 to 10, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids. In one embodiment, the V_H domain comprises C-terminal extensions of from 1 to 12 amino acid residues, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acids of the C_H1 domain. In one embodiment, said extension comprises at least 1 alanine residue, for example a single alanine residue, a pair of alanine residues or a triplet of alanine residues. Such extended V_H domains are within the scope of the invention. Also within the scope of the invention are immunoconjugates that comprise V_H domains that comprise additional C or N-terminal residues, for example linker residues and / or His tags, e.g., hexa-His (HHHHHH, SEQ ID No. 530) or myc tags. Additional residues of the vector may also be present, for example in addition to tags. V_H domains used may have the additional residues LEGGGSEQKLISEEDLNHHHHHHGS (SEQ ID No. 531). In preferred embodiments of the invention the antigen-binding moiety comprises

a terminal His multimer, preferably a C-terminal His multimer, for conjugation of the antigen binding moiety to the linker (L) of the auristatin toxin (D). Attachment of each payload molecule requires the presence of 2 histines, thus using His multimers, multiple payloads may be attached to each antigen-binding moiety to achieve a DAR>1.

According to the various aspects and embodiments of the invention, the variable domain of the single domain antibodies is preferably a human variable domain (V_H). As used herein, a human V_H domain includes a fully human or substantially fully human V_H domain. As used herein, the term human V_H domain also includes V_H domains that are isolated from heavy chain only antibodies made by transgenic mice expressing fully human immunoglobulin heavy chain loci, in particular in response to an immunisation with an antigen of interest, for example as described in WO2016/062990 and in the examples. In one embodiment, a human V_H domain can also include a V_H domain that is derived from or based on a human V_H domain amino acid or nucleic acid sequence encoding such V_H domain. Thus, the term includes variable heavy chain regions derived from or encoded by human germline immunoglobulin sequences. A substantially human V_H domain or V_H domain that is derived from or based on a human V_H domain may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced in vitro, e.g. by random or site-specific mutagenesis, or introduced by somatic mutation in vivo). The term "human V_H domain" therefore also includes a substantially human V_H domain wherein one or more amino acid residue has been modified. For example, a substantially human V_H domain the V_H domain may include up to 10, for example 1, 2, 3, 4 or 5 amino acid modifications compared to a fully human sequence. However, the term "human V_H domain" or "substantially human V_H domain", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences. Preferably, the term "human V_H domain", as used herein, is also not intended to include camelized V_H domains, that is human V_H domains that have been specifically modified, for example *in vitro* by conventional mutagenesis methods to select predetermined positions in the V_H domains sequence and introduce one or more point mutation at the predetermined position to change one or more predetermined residue to a specific residue that can be found in a camelid V_{HH} domain.

As used herein, the term V_H or "variable domain" refers to immunoglobulin variable domains defined by Kabat *et al.*, Sequences of Immunological Interest, 5th ed., U.S. Dept. Health & Human Services, Washington, D.C. (1991). The numbering and positioning of CDR amino acid residues within the variable domains is in accordance with the well-known Kabat numbering convention.

More particularly, the invention provides an immunoconjugate comprising a single V_H domain antibody wherein said single V_H domain antibody binds to human PSMA with an affinity, a Kon-rate, a Koff rate, KD and/or KA, EC50 and IC50 values as further described herein, in particular in the examples. Assays suitable for measuring these values are also shown in the examples.

An immunoconjugate of the invention comprises a single V_H domain antibody having an amino acid sequence and preferred sequences and/or parts thereof, such as CDRs, as defined herein.

The term "CDR" refers to the complementarity-determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat is used herein. The terms "Kabat numbering", "Kabat definitions" and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.*, hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.*, (1971) Ann. NY Acad. Sci. 190:382-391 and Kabat, *et al.*, (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

The immunoconjugate may be monovalent, multivalent, for example bivalent, or multiparatopic, for example biparatopic. Thus, the immunoconjugate may have the following formula: A-(L-D)n wherein A is an antigen-binding moiety comprising a first human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA, optionally comprising a second human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA and optionally comprising a third human single heavy chain variable immunoglobulin (V_H) domain antibody, L is a linker, and D is an auristatin or a derivative thereof and n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

Each V_H comprises CDR and FR regions. Thus, A may comprise FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4 or a binding fragment thereof. In embodiments that relate to a biparatopic binding moiety of the immunoconjugate, A has the following formula: FR1(A)-CDR1(A)-FR2(A)-CDR2(A)-FR3(A)-CDR3(A)-FR4(A)- FR1(B)-CDR1(B)-FR2(B)-CDR2(BA)-FR3(B)-

CDR3(B)-FR4(B). The order of the immunoglobulin single variable domains A (first single V_H domain antibody) and B (second single V_H domain antibody) is not particularly limited, so that, within a polypeptide of the invention, immunoglobulin single variable domain A may be located N-terminally and immunoglobulin single variable domain B may be located C-terminally, or vice versa. The V_H domains may be connected via a linker, e.g. a (G4S)n linker.

In one embodiment, the immunoconjugate is biparatopic. In a biparatopic immunoconjugate, the two binding moieties bind to different epitopes on a target molecule. Preferred biparatopic immunoconjugates comprise two different single V_H domain antibodies that bind to the target protein PSMA, but on different sites. These sites may be overlapping. Complete or partial blocking can be assessed in epitope binning studies.

The term “epitope” or “antigenic determinant” refers to a site on the surface of an antigen (e.g., PSMA) to which an immunoglobulin, antibody or antibody fragment, including a VH single domain antibody specifically binds. Generally, an antigen has several or many different epitopes and reacts with many different antibodies. The term specifically includes linear epitopes and conformational epitopes. Epitopes within protein antigens can be formed both from contiguous amino acids (usually a linear epitope) or non-contiguous amino acids juxtaposed by tertiary folding of the protein (usually a conformational epitope). Epitopes formed from contiguous amino acids are typically, but not always, retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods for determining what epitopes are bound by a given antibody or antibody fragment (i.e., epitope mapping) are well known in the art and include, for example, immunoblotting and immunoprecipitation assays, wherein overlapping or contiguous peptides from are tested for reactivity with a given antibody or antibody fragment. An antibody binds "essentially the same epitope" as a reference antibody, when the two antibodies recognize identical or sterically overlapping epitopes. The most widely used and rapid methods for determining whether two epitopes bind to identical or sterically overlapping epitopes are competition assays, which can be conFigured in different formats, using either labelled antigen or labelled antibody.

In one embodiment, the immunoconjugate is bivalent. Bivalent immunoconjugates comprise two single heavy chain variable immunoglobulin (V_H) domain antibody moieties, e.g., two Humabody® V_H that bind to the target protein (PSMA) at the same site. In one embodiment, such molecules may comprise two identical single heavy chain variable immunoglobulin (V_H) domain antibody, e.g. two identical Humabody® V_H. In another embodiment, such molecules

may comprise two different single heavy chain variable immunoglobulin (V_H) domain antibodies, e.g. two different single heavy chain variable immunoglobulin (V_H) domain antibodies that are from the same family, such as two Humabody® V_H that are from the same Humabody® V_H family. In another embodiment, such molecules may comprise two different single heavy chain variable immunoglobulin (V_H) domain antibodies, e.g., two different Humabody® V_H, that are not from the same family but which bind to the same site on the target. Biparatopic and bivalent immunoconjugates of the present invention can be constructed using methods known in the art.

As described in more detail in the experimental part, single V_H domain antibodies that can be used in the multiparatopic or multivalent immunoconjugates of the invention were isolated and grouped into 15 families based on sequence homology in the CDR3 sequence. Through a process of optimization, a panel of variant single V_H domain antibodies with a CDR sequence derived from a parent CDR sequence were also generated to improve affinities to PSMA and/or improve potencies compared to the parent molecule. Each single V_H domain antibody has a set of CDR sequences (CDR1, 2 and 3) as shown in Figures 1 to 15. Epitope binning studies were conducted to assess epitope binding as demonstrated in example 11. For example, it was demonstrated that the single V_H domain antibodies in family 1 bind to a different epitope than those in family 2. Combinations of a family 1 single V_H domain antibody and a family 2 single V_H domain antibody are thus one embodiment of the biparatopic immunoconjugate of the invention. Family 1 single V_H domain antibody can be located at the C or N terminus. In a preferred embodiment, a family 1 single V_H domain antibody is located at the N terminus.

In some embodiments, the first or second single V_H domain antibody is a variant V_H single domain antibodies of a parent molecules, in particular of a parent V_H single domain antibody selected from sdAb 1.1, 2.1, 3.1, 4.1, 5.1, 6.1, 7.1, 8.1, 9.1, 10.1, 11.1, 12.1, 13.1, 14.1 or 15.1 having one or more amino acid substitutions, deletions, insertions or other modifications, and which retains a biological function of the single domain antibody. Thus, a variant V_H single domain antibody can be sequence engineered. Modifications may include one or more substitution, deletion or insertion of one or more codons encoding the single domain antibody or polypeptide that results in a change in the amino acid sequence as compared with the native sequence V_H single domain antibody or polypeptide. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, i.e., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by

systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence. A variant of a V_H single domain antibody described herein has at least 75%, 76%, 77%, 78%, 79%, 8 0%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence homology to the non-variant molecule, preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence homology.

In one embodiment, the modification is a conservative sequence modification. As used herein, the term "conservative sequence modifications" is intended to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence. Such conservative modifications include amino acid substitutions, additions and deletions. Modifications can be introduced into V_H and / or the antigen-binding moiety of the immunoconjugate of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are ones in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with 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, tryptophan), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, one or more amino acid residues within the CDR regions of a single domain antibody can be replaced with other amino acid residues from the same side chain family and the altered antibody can be tested for retained function (i.e., the functions set forth in (c) through (l) above) using the functional assays described herein.

In some embodiments, the V_H single domain antibody that is a variant of a single domain antibody selected from those shown in Tables 1 to 15 that comprises one or more sequence modification and has improvements in one or more of a property such as binding affinity, specificity, thermostability, expression level, effector function, glycosylation, reduced immunogenicity, or solubility as compared to the unmodified single domain antibody.

A skilled person will know that there are different ways to identify, obtain and optimise the a single V_H domain antibodies as described herein, including in vitro and in vivo expression libraries. This is further described in the examples. Optimisation techniques known in the art,

such as display (e.g., ribosome and/or phage display) and / or mutagenesis (e.g., error-prone mutagenesis) can be used. The invention therefore also comprises sequence optimised variants of the single domain antibodies described herein.

In one embodiment, modifications can be made to decrease the immunogenicity of the single domain antibody. For example, one approach is to revert one or more framework residues to the corresponding human germline sequence. More specifically, a single domain antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the single domain antibody is derived. Such residues can be identified by comparing the single domain antibody framework sequences to the germline sequences from which the single domain antibody is derived. To return one or more of the amino acid residues in the framework region sequences to their germline configuration, the somatic mutations can be "backmutated" to the germline sequence by, for example, site-directed mutagenesis or PCR-mediated mutagenesis.

Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T cell epitopes to thereby reduce the potential immunogenicity of the antibody. In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen.

In one aspect, the immunoconjugate comprises a single V_H domain antibody comprising a family 1 or a family-1 like sequence. In one embodiment, two V_H domains comprising a family 1 or a family-1 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, a first V_H domain comprising a family 1 or a family-1 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from family 1, 5, 6, 12 or 13 or family 1, 5, 6, 12 or 13-like sequence. In one embodiment, the first V_H domain comprises SEQ ID No:4 and the second V_H domain comprises SEQ ID NO:4. Further sequence information on the single domain antibodies used is provided below.

The immunoconjugates of the invention are of the formula A-(L-D)n wherein A is an Auristatin or derivative thereof. In one embodiment, D is MMAE, MMAF, or a derivative thereof.

Auristatins are synthetic analogues of the antineoplastic natural product Dolastatin. Auristatins inhibit cell division by blocking the polymerisation of tubulin and are used as toxic payloads in antibody-drug conjugates. The family of auristatins includes monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF). In preclinical models, auristatins have been found to be 100- to 1,000-fold more potent than traditionally-used chemotherapeutics.

MMAE & Vedotin

Monomethyl auristatin E (MMAE, desmethyl-auristatin E) is a synthetic antimitotic, antineoplastic agent The IUPAC name for MMAE is (S)-N-((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamido)butanamide.

Monomethyl auristatin E or MMAE is 100-1000 times more potent than doxorubicin, but its toxicity is such that cannot be used as a drug itself. However, it has been used as part of an antibody-drug conjugate or ADC, wherein MMAE is linked to a monoclonal antibody (mAb) that recognizes a specific marker expressed in cancer cells and directs MMAE to the cancer cell.

As MMAE is toxic, it has been used as a therapeutic only when conjugated to a monoclonal antibody (mAb) to target the MMAE to cancer cells. In the International Nonproprietary Names for MMAE-mAb-conjugates, the name “vedotin” denotes MMAE plus its linking structure to the antibody. The structure linking the targeting mAb to MMAE may comprise an attachment group (maleimide (mal) and caproic acid (cap)), a spacer (paraaminobenzoic acid) and a cathepsin-cleavable linker (amino acids valine (Val) and citrulline (Cit)).

The tether that connects MMAE to the monoclonal antibody is stable in extracellular fluid, but is cleaved by cathepsin once the antibody-drug-conjugate has bound to the targeted cancer cell antigen and entered the cancer cell, after which the ADC releases the toxic MMAE and activates the potent anti-mitotic mechanism. Antibody-drug conjugates enhance the antitumor effects of antibodies and reduce adverse systemic effects of highly potent cytotoxic agents.

MMAF & mafodotin

Monomethyl auristatin F (MMAF, desmethyl-auristatin F) is a synthetic antineoplastic agent. The IUPAC name for MMAF is (S)-2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamido)butanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-3-phenylpropanoic acid.

MMAF is the toxic payload used in some experimental anti-cancer antibody-drug conjugates such as vorsetuzumab mafodotin and SGN-CD19A. In International Nonproprietary Names for MMAF-antibody-conjugates, the name mafodotin refers to MMAF plus its attachment structure to the antibody. The attachment group may consist of maleimide and caproic acid.

Auristatins and their use as components of ADC are reviewed by Maderna and Leverett in "Recent Advances in the Development of New Auristatins: Structural Modifications and Application in Antibody Drug Conjugates"; Mol. Pharmaceutics, 2015, 12 (6), pp 1798–1812 Mendelsohn et al., "Investigation of Hydrophilic Auristatin Derivatives for Use in Antibody Drug Conjugates". Bioconjugate Chem., Article ASAP DOI: 10.1021/acs.bioconjchem.6b00530, Publication Date (Web): January 6, 2017 describe derivatives of the natural product dolastatin 10 containing pyridines and other basic amines, which were examined to assess more hydrophilic auristatin derivatives would be sufficiently potent for use in ADC. A pyridine derivative, monomethyl auristatin PYE, showed the greatest potency when tested in vivo.

The immunoconjugate, compositions and methods of the invention may feature an auristatin which is either monomethylauristatin E (MMAE) or monomethylauristatin F (MMAF) or a derivative thereof.

MMAE may be conjugated to the antigen-binding moiety via a valine-citrulline (vc) linker (vc-MMAE). MMAF is conjugated to the antigen-binding moiety via a maleimidocaproyl linker (mc-MMAF) using HiPEG™ technology (WO 2009/047500; Cong *et al.*, (2012) Bioconjugate Chem. 2012, 23, 248−263.

Thus, in one embodiment, D is MMAE or a derivative thereof conjugated to the antigen-binding moiety via a valine-citrulline (vc) linker (vc-MMAE). In another embodiment D is MMAF or a derivative thereof conjugated to the antigen-binding moiety via a maleimidocaproyl linker (mc-MMAF). In another embodiment L-D is vedotin or mafodotin. L-D may also comprise one of more H amino acid which links A and L-D.

In one embodiment, a first V_H domain comprising a family 1 or a family-1 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or 14 or a family 2, 3, 4, 7, 9, 10, 11 or 14-like sequence. In one embodiment, the first V_H domain comprises SEQ ID No:4 and the second V_H domain comprises SEQ ID NO:84.

A single V_H domain antibody of family 1 may include the sequence of the parent (1.1; SEQ ID NO. 4) or a part thereof, for example a CDR3 sequence, and sequences that are derived from the parent 1.1 through a process of optimization, for example sequences as shown as shown in Figure 1. CDR sequences and full length V_H sequences in family 1 are numbered according to Table 1 as shown below.

Name	CDR1	CDR2	CDR3	VH Full length sequence
1.1	SEQ ID NO. 1 SYAMS	SEQ ID NO. 2 SIGENDGT TDYADSV KG	SEQ ID NO. 3 DGVH	SEQ ID NO. 4 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYAMSWVRQAPGKGLEWVSSIGEND GTTDYADSVKGRFTISRDNSKSMLYLQM NSLRVEDTAVYYCVKDGVHWGQGTLVTV SS
1.2	SEQ ID NO. 5 SYAMS	SEQ ID NO. 6 SIGDNNNS TEYADSV KG	SEQ ID NO. 7 DGVH	SEQ ID NO. 8 EVQLVESGGGLVQPGGSLRLSCAASGFT FSSYAMSWVRQAPGKGLEWVSSIGDNN NSTEYADSVKGRFTISRDNSKSTLYLQMN SLSAEDTAVYYCVKDGVHWGQGTLVTVS S
1.3	SEQ ID NO. 9 SYAMS	SEQ ID NO. 10 IGDNNNST DYADSVK G	SEQ ID NO. 11 DGVH	SEQ ID NO. 12 EVQLVESGGGLVQPGGSLRLSCAASGFS FSSYAMSWVRQAPGKGLEWVSSIGDNN NSTDYADSVKGRFTISRDNSKSTLYLQMN SLRAEDTAVYYCVKDGVHWGQGTLVTVS S
1.4	SEQ ID NO. 13 SYAMS	SEQ ID NO. 14 IGDGTTYY ADSVKG	SEQ ID NO. 15 DGVH	SEQ ID NO. 16 EVQLVESGGGLVQPGGSLRLSCAASGFT FSSYAMSWVRQAPGKGLEWVSSIGDGT TYYADSVKGRFTISRDNSKSTLYLQMNSL RAEDTAVYYCAKDGVHWGQGTLVTVSS
1.5	SEQ ID NO. 17 TYAMS	SEQ ID NO. 18 SIGENDRT TYYVDSV KG	SEQ ID NO. 19 DGVH	SEQ ID NO. 20 EVQLVESGGGLVQPGGSLRLSCAASGFT FSTYAMSWVRQAPGKGLEWVSSIGEND RTTYYVDSVKGRFTISRDNSKSTLYLQMN SLRAEDTAVYYCAKDGVHWGQGTLVTVS S
1.6	SEQ ID NO. 21 SYAMS	SEQ ID NO. 22 SIGDNNRT TYYADSV KG	SEQ ID NO. 23 DGVH	SEQ ID NO. 24 QVQLVESGGGLVQPGGSLRLSCAASGFT FSSYAMSWVRQAPGKGLEWVSSIGDNN RTTYYADSVKGRFTISRDNSKSTLYLQMN SLRAEDTAVYYCAKDGVHWGQGTLVTVS

				S
1.7	SEQ ID NO. 25 SYAMS	SEQ ID NO. 26 SIGDGTTY YADSVKG	SEQ ID NO. 27 DGVH	SEQ ID NO. 28 EVQLVESGGGLVQPGGSLRLSCAASGFT FSSYAMSWVRQAPGKGLEWVSSIGDGT TYYADSVKGRFTISRDNSKSTLYLQMNSL RAEDTAVYYCAKDGVHWGQGTLVTVSS
1.8	SEQ ID NO. 29 SYAMS	SEQ ID NO. 30 SIGENDGT TDYADSV KG	SEQ ID NO. 31 DGVH	SEQ ID NO. 32 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYAMSWVRQAPGKGLEWVSSIGEND GTTDYADSVKGRFTISRDNSKNTLYLQM NSLRVEDTAVYYCVKDGVHWGQGTLVTV SS
1.9	SEQ ID NO. 33 SYALS	SEQ ID NO. 34 SIGENDGT TDYADSV KG	SEQ ID NO. 35 DGVH	SEQ ID NO. 36 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENDG TTDYADSVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.10	SEQ ID NO. 37 SYALS	SEQ ID NO. 38 SIGENNAT TDYADFV KG	SEQ ID NO. 39 DGVH	SEQ ID NO. 40 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNA TTDYADFVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.11	SEQ ID NO. 41 SYALS	SEQ ID NO. 42 SIGENNDT TDYADNV KG	SEQ ID NO. 43 DGVH	SEQ ID NO. 44 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENND TTDYADNVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.12	SEQ ID NO. 45 SYALS	SEQ ID NO. 46 SIGENNAT TDYADAV KG	SEQ ID NO. 47 DGVH	SEQ ID NO. 48 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNA TTDYADAVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.13	SEQ ID NO. 49 SYALS	SEQ ID NO. 50 SIGENNHT TDYAADV KG	SEQ ID NO. 51 DGVH	SEQ ID NO. 52 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNH TTDYAADVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.14	SEQ ID NO. 53 SYALS	SEQ ID NO. 54 SIGENNAT TDYADVV KG	SEQ ID NO. 55 DGVH	SEQ ID NO. 56 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNA TTDYADVVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.15	SEQ ID NO. 57 SYALS	SEQ ID NO. 58 SIGENNHT TDYAAFVK G	SEQ ID NO. 59 DGVH	SEQ ID NO. 60 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNH TTDYAAFVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.16	SEQ ID NO. 61 SYALS	SEQ ID NO. 62 SIGENNHT TDYADTV KG	SEQ ID NO. 63 DGVH	SEQ ID NO. 64 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNH TTDYADTVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.17	SEQ ID NO. 65	SEQ ID NO. 66	SEQ ID NO. 67	SEQ ID NO. 68 EVQLLESGGGLVQPGGSLRLSCAASGFS

	SYALS	SIGENNDT TDYADAV KG	DGVH	FSSYALSWVRQAPGKGLEWVSSIGENND TTDYADAVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.18	SEQ ID NO. 69 SYALS	SEQ ID NO. 70 SIGENNAT TDYAASV KG	SEQ ID NO. 71 DGVH	SEQ ID NO. 72 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNA TTDYAASVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.19	SEQ ID NO. 73 SYALS	SEQ ID NO. 74 SIGENNDT TDYAAYV KG	SEQ ID NO. 75 DGVH	SEQ ID NO. 76 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENND TTDYAAYVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.20	SEQ ID NO. 77 SYALS	SEQ ID NO. 78 SIGENNHT TDYAATVK G	SEQ ID NO. 79 DGVH	SEQ ID NO. 80 EVQLLESGGGLVQPGGSLRLSCAASGFS FSSYALSWVRQAPGKGLEWVSSIGENNH TTDYAATVKGRFTISRDNSKNTLYLQMNS LRVEDTAVYYCVKDGVHWGQGTLVTVSS

Table 1. This shows SEQ ID NOs. of family 1 CDR sequences and of family 1 full length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 1. Family 1-like sequences are variants that have certain percentage sequence identity with family 1 sequences as set out herein.

In one aspect, the V_H domain comprises a CDR3 sequence comprising SEQ ID NO. 3 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 3.

In one embodiment, the V_H domain comprises a CDR3 sequence comprising or consisting of an amino acid sequence selected from SEQ ID NO. 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 51, 55, 59, 63, 67, 71, 75 or 79.

In one embodiment, the V_H domain comprises hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises the amino acid sequence SEQ ID NO. 1 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprising the amino acid sequence SEQ ID NO. 2 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprising the amino acid sequence SEQ ID NO. 3 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. For example, the CDR may be a CDR selected from those shown in Figure 1.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 1 or a sequence with at least at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%,

78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 2 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 3 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the CDR sequences of the V_H domain are as shown for single V_H domain antibodies 1.1 to 1.20 as in Figure 1 or combinations thereof. In one embodiment, CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, 57, 61, 65, 69, 73 or 77, CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, 62, 66, 70, 74 or 78 and CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, 47, 51, 55, 59, 63, 67, 71, 75 or 79. IN one embodiment, CDR is SEQ ID NO. 33.

In one aspect, the single V_H domain antibody has combinations of CDR1, CDR2 and CDR3 as shown for clones 1.1 to 1.20 in Figure 1. Thus, in one embodiment, the single V_H domain antibody comprises CDR1, 2 and 3 sequences wherein CDR1 is SEQ ID NO. 1, CDR2 is SEQ ID NO. 2 and CDR3 is SEQ ID NO. 3. In another embodiment, CDR1 is SEQ ID NO. 5, CDR2 is SEQ ID NO. 6 and CDR3 is SEQ ID NO. 7. In another embodiment, CDR1 is SEQ ID NO. 9, CDR2 is SEQ ID NO. 10 and CDR3 is SEQ ID NO. 11. In another embodiment, CDR1 is SEQ ID NO. 13, CDR2 is SEQ ID NO. 14 and CDR3 is SEQ ID NO. 15. In another embodiment, CDR1 is SEQ ID NO. 17, CDR2 is SEQ ID NO. 18 and CDR3 is SEQ ID NO. 19. In another embodiment, CDR1 is SEQ ID NO. 21, CDR2 is SEQ ID NO. 22 and CDR3 is SEQ ID NO. 23. In another embodiment, CDR1 is SEQ ID NO. 25, CDR2 is SEQ ID NO. 26 and CDR3 is SEQ ID NO. 27. In another embodiment, CDR1 is SEQ ID NO. 29, CDR2 is SEQ ID NO. 30 and CDR3 is SEQ ID NO. 31. In another embodiment, CDR1 is SEQ ID NO. 33, CDR2 is SEQ ID NO. 34 and CDR3 is SEQ ID NO. 35. In another embodiment, CDR1 is SEQ ID NO. 37, CDR2 is SEQ ID NO. 38 and CDR3 is SEQ ID NO. 39. In another embodiment, CDR1 is SEQ ID NO. 41, CDR2 is SEQ ID NO. 42 and CDR3 is SEQ ID NO. 43. In another embodiment, CDR1 is SEQ ID NO. 45, CDR2 is SEQ ID NO. 46 and CDR3 is SEQ ID NO. 47. In another embodiment, CDR1 is SEQ ID NO. 49, CDR2 is SEQ ID NO. 50 and CDR3 is SEQ ID NO. 51. In another embodiment, CDR1 is SEQ ID NO. 53, CDR2 is

SEQ ID NO. 54 and CDR3 is SEQ ID NO. 55. In another embodiment, CDR1 is SEQ ID NO. 57, CDR2 is SEQ ID NO. 58 and CDR3 is SEQ ID NO. 59. In another embodiment, CDR1 is SEQ ID NO. 61, CDR2 is SEQ ID NO. 62 and CDR3 is SEQ ID NO. 63. In another embodiment, CDR1 is SEQ ID NO. 65, CDR2 is SEQ ID NO. 66 and CDR3 is SEQ ID NO. 67. In another embodiment, CDR1 is SEQ ID NO. 69, CDR2 is SEQ ID NO. 70 and CDR3 is SEQ ID NO. 71. In another embodiment, CDR1 is SEQ ID NO. 73, CDR2 is SEQ ID NO. 74 and CDR3 is SEQ ID NO. 75. In another embodiment, CDR1 is SEQ ID NO. 77, CDR2 is SEQ ID NO. 78 and CDR3 is SEQ ID NO. 79.

In one embodiment, the single V_H domain antibody has a V_H domain that comprises or consists of SEQ ID NO. 4 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto. CDR sequences of such sequences are shown in Figure 1. For example, the V_H domain comprises or consists of SEQ ID NO. 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72 76 or 80. In another embodiment, the V_H domain is selected from one of the sequences above, for example SEQ ID NO. 4, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences. In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 4 or a sequence which comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions in the framework regions as compared to SEQ ID NO. 4. In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 32.

Thus, in one embodiment, the invention relates to an immunoconjugate comprising at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain and wherein said V_H domain comprises or consists of SEQ ID NO. 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76 or 80 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the single V_H domain antibody comprises a V_H domain as shown in SEQ ID NO. 4 or a variant thereof wherein in said variant, residue 33 is T, 36 is L, residue 57 is D, residue 59 is N, R, A, D, H, residue 63 D, Y, residue 65 is V, residue 66 is A, and/or residue 67 is F, N, A, D, V, T, S, Y.

In one embodiment, the V_H domain is as shown in SEQ ID NO. 4 or a variant thereof wherein said variant includes the following changes compared to SEQ ID NO. 4

- S77→N and M78→T (as shown for 1.8)
- M34→L, D55→N, G56→H, D62→A, S63→T and optionally S77→N and M78→T (as shown for 1.20)
- M34→L, D55→N, G56→A, S63→F and optionally S77→N and M78→T (as shown for 1.10)
- M34→L, D55→N, G56→D, S63→F and optionally S77→N and M78→T (as shown for 1.11)
- M34→L, D55→N, G56→A, S63→A and optionally S77→N and M78→T (as shown for 1.12)
- M34→L, D55→N, G56→H, D62→A, S63→D and optionally S77→N and M78→T (as shown for 1.13)
- M34→L, D55→N, G56→A, S63→V and optionally S77→N and M78→T (as shown for 1.14)
- M34→L, D55→N, G56→H, D62→A, S63→F and optionally S77→N and M78→T (as shown for 1.15)
- M34→L, D55→N, G56→H, S63→T and optionally S77→N and M78→T (as shown for 1.16)
- M34→L, D55→N, G56→H, S63→T and optionally S77→N and M78→T (as shown for 1.17)
- M34→L, D55→N, G56→D, D62→A, S63→S and optionally S77→N and M78→T (as shown for 1.18)
- M34→L, D55→N, G56→A, D62→A, S63→Y and optionally S77→N and M78→T (as shown for 1.19).

In one embodiment, additional changes may be included. In another embodiment, the variants listed above do not include additional changes.

The single V_H domain antibody in family 1 has KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples. The term "KD" as used in this application refers to the "equilibrium dissociation constant" and refers to the value obtained in a titration measurement at equilibrium, or by dividing the dissociation rate constant (Koff) by the association rate constant (Kon). “KA” as used in this application refers to the affinity constant. The association rate constant, the dissociation rate constant and the equilibrium dissociation constant are used to represent the binding affinity of an antibody to an antigen.

Methods for determining association and dissociation rate constants are well known in the art. Using fluorescence-based techniques offers high sensitivity and the ability to examine samples in physiological buffers at equilibrium. Other experimental approaches and instruments such as a BIAcore® (biomolecular interaction analysis) assay and assays described in the examples can be used to test the a single V_H domain antibodies used in the immunoconjugates of the invention.

In one aspect, the immunoconjugate of the invention comprises one or more human V_H domain comprising a family 2 or family-2 like sequence. Thus, in one embodiment, the immunoconjugate comprises or consists of at least two single V_H domain antibody capable of binding PSMA, preferably human PSMA, of family 2. In another embodiment, one V_H domain comprising a family 2 or a family-2 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.

In one embodiment, one V_H domain comprising a family 2 or a family-2 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence.

The family 2 single V_H domain antibody may include sequences that are derived from the parent (2.1; SEQ ID NO. 84) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 2.1 through a process of optimization, for example as shown in Figure 2. CDR sequences and full length sequences of clones in family 2 are numbered according to Table 2 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
2.1	SEQ ID NO. 81 GYGMH	SEQ ID NO. 82 YISYD GSNKY YADSV KG	SEQ ID NO. 83 DPAWGLRL GESSSYDF DI	SEQ ID NO. 84 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAYISYDGSNKY YADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.2	SEQ ID NO. 85 GYGMH	SEQ ID NO. 86 YISYD	SEQ ID NO. 87 DPAWGLRL	SEQ ID NO. 88 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAYISYDGSNKY

		GSNKY YADSV KG	GESSSYDF DI	YADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.3	SEQ ID NO. 89 GYGMH	SEQ ID NO. 90 HISYD GSNRY YAESV KG	SEQ ID NO. 91 DPAWGLRL GELSSYDF DI	SEQ ID NO. 92 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAHISYDGSNRY YAESVKGRFTISRENSKNTLSLQMNSLRAED TAVYYCAKDPAWGLRLGELSSYDFDIWGQG TMVTVSS
2.4	SEQ ID NO. 93 GYGMH	SEQ ID NO. 94 VISYD GSNRY YADSV KG	SEQ ID NO. 95 DPAWGLRL GELSSYDF EI	SEQ ID NO. 96 QVTLKESGGGVVQPGRSLKLSCAASGFSFS GYGMHWVRQAPGKGLEWVAVISYDGSNRY YADSVKGRFTISRDNSKNTLSLQMNSLRAE DTAVYYCARDPAWGLRLGELSSYDFEIWGQ GTMVTVSS
25	SEQ ID NO. 97 GYGMH	SEQ ID NO. 98 VISYD GSNRY YADSV KG	SEQ ID NO. 99 DPAWGLRL GELSSYDF EI	SEQ ID NO. 100 QVQLVESGGGVVQPGRSLRLSCAASGFSF SGYGMHWVRQAPGKGLEWVAVISYDGSNR YYADSVKGRFTISRDNSKNTLSLQMNSLRA EDTAVYYCAKDPAWGLRLGELSSYDFEIWG QGTMVTVSS
2.6	SEQ ID NO. 101 GYGMH	SEQ ID NO. 102 VISYD GSNKY YADSV KG	SEQ ID NO. 103 DPAWGLRL GELSSYKF EI	SEQ ID NO. 104 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAVISYDGSNKY YADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGELSSYKFEIWGQ GTMVTVSS
2.7	SEQ ID NO. 105 GYGMH	SEQ ID NO. 106 LISYD GSNKY YADSV KG	SEQ ID NO. 107 DPAWGLRL GEQSSYAF DI	SEQ ID NO. 108 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVALISYDGSNKY YADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGEQSSYAFDIWG QGTMVTVSS
2.8	SEQ ID NO. 109	SEQ ID NO.	SEQ ID NO. 111	SEQ ID NO. 112 QVQLVESGGGVVQPGRSLRLSCAASGFSF

	GYGMH	110 VISYD GSNKY YADSV KG	DPAWGLRL GEQSSYAF E	SGYGMHWVRQAPGKGLEWVSVISYDGSNK YYADSVKGRFTISRDNSKNTLYLQMNSLRTE DTAVYYCAKDPAWGLRLGEQSSYAFEIWG QGTMVTVSS
2.9	SEQ ID NO. 113 GYGMH	SEQ ID NO. 114 VISYD GSNKY YADSV KG	SEQ ID NO. 115 DPAWGLRL GEQSSYAF EI	SEQ ID NO. 116 EVQLLESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAVISYDGSNKY YADSVKGRFTISRDNSKNTLYLQMNSLRVE DTAVYYCAKDPAWGLRLGEQSSYAFEIRGQ GTTVTVSS
2.10	SEQ ID NO. 117 GYGMH	SEQ ID NO. 118 YISYD GSNRY YADSV KG	SEQ ID NO. 119 DPAWGLRL GESSSYDF DI	SEQ ID NO. 120 EVQLVESGGGVVQPGRSLRLSCAASGFTFS GYGMHWVRQAPGKGLEWVAYISYDGSNRY YADSVKGRFTISRDNSKKTLSLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.11	SEQ ID NO. 121 GYGLH	SEQ ID NO. 122 YISYD ESNKY YAPSV KG	SEQ ID NO. 123 DPAWGLRL GESSSYDF DI	SEQ ID NO. 124 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGLHWVRQAPGKGLEWVAYISYDESNKY YAPSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.12	SEQ ID NO. 125 GYGMH	SEQ ID NO. 126 YISYD KSNKY YADKV KG	SEQ ID NO. 127 DPAWGLRL GESSSYDF DI	SEQ ID NO. 128 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAYISYDKSNKY YADKVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.13	SEQ ID NO. 129 GYGLH	SEQ ID NO. 130 YISYD	SEQ ID NO. 131 DPAWGLRL GESSSYDF	SEQ ID NO. 132 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGLHWVRQAPGKGLEWVAYISYDASNKY YADNVKGRFTISRDNSKNTLYLQMNSLRAE

		ASNKY YADNV KG	DI	DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.14	SEQ ID NO. 133 GYGVH	SEQ ID NO. 134 YISYD ASNKY YADNV KG	SEQ ID NO. 135 DPAWGLRL GESSSYDF DI	SEQ ID NO. 136 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGVHWVRQAPGKGLEWVAYISYDASNKY YADNVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.15	SEQ ID NO. 137 GYGLH	SEQ ID NO. 138 YISYD KSNKY YADKV KG	SEQ ID NO. 139 DPAWGLRL GESSSYDF DI	SEQ ID NO. 140 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGLHWVRQAPGKGLEWVAYISYDKSNKY YADKVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.16	SEQ ID NO. 141 GYGAH	SEQ ID NO. 142 YISYD KSNKY YADKV KG	SEQ ID NO. 143 DPAWGLRL GESSSYDF DI	SEQ ID NO. 144 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGAHWVRQAPGKGLEWVAYISYDKSNKY YADKVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.17	SEQ ID NO. 145 GYGMH	SEQ ID NO. 146 YISYD ASNKY YADNV KG	SEQ ID NO. 147 DPAWGLRL GESSSYDF DI	SEQ ID NO. 148 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAYISYDASNKY YADNVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.18	SEQ ID NO. 149 GYGQH	SEQ ID NO. 150 YISYD ASNKY YADNV	SEQ ID NO. 151 DPAWGLRL GESSSYDF D	SEQ ID NO. 152 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGQHWVRQAPGKGLEWVAYISYDASNKY YADNVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS

		KG		
2.19	SEQ ID NO. 153 GYGFH	SEQ ID NO. 154 YISYD ASNKY YADNV KG	SEQ ID NO. 155 DPAWGLRL GESSSYDF DI	SEQ ID NO. 156 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGFHWVRQAPGKGLEWVAYISYDASNKY YADNVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFDIWG QGTMVTVSS
2.20	SEQ ID NO. 157 GYGMH	SEQ ID NO. 158 IISYDG SNRYY ADSVK G	SEQ ID NO. 159 DPAWGLRL GESSSYDF EI	SEQ ID NO. 160 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAIISYDGSNRY YADSVKGRFTISRDNSKNTLSLQMNSLRAE DTAVYYCAKDPAWGLRLGESSSYDFEIWGQ GTMVTVSS
2.21	SEQ ID NO. 161 GYGMH	SEQ ID NO. 162 VISYD GSNRY YADSV KG	SEQ ID NO. 163 DPAWGLRL GKLSSYDF EI	SEQ ID NO. 164 QVQLVESGGGVVQPGRSLKLSCAASGFSFS GYGMHWVRQAPGKGLEWVAVISYDGSNRY YADSVKGRFTISRDNSKNTLSLQMNSLRAE DTAVYYCAKDPAWGLRLGKLSSYDFEIWGQ GTMVTVSS
2.22	SEQ ID NO. 165 GYGTH	SEQ ID NO. 166 YISYD GSNKY YAAPV KG	SEQ ID NO. 167 DAAWGLRL GESSSYDF DI	SEQ ID NO. 168 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGTHWVRQAPGKGLEWVAYISYDGSNKY YAAPVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDAAWGLRLGESSSYDFDIWG QGTMVTVSS
2.23	SEQ ID NO. 169 GYGTH	SEQ ID NO. 170 YISYD ESNKY YASSV KG	SEQ ID NO. 171 DRAWGLRL GESSSYDF DI	SEQ ID NO. 172 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGTHWVRQAPGKGLEWVAYISYDESNKY YASSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDRAWGLRLGESSSYDFDIWG QGTMVTVSS
2.24	SEQ ID	SEQ ID	SEQ ID NO.	SEQ ID NO. 176

	NO. 173 GYGMH	NO. 174 YISYD ESNKY YARLV KG	175 DTAWGLRL GESSSYDF DI	EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGMHWVRQAPGKGLEWVAYISYDESNKY YARLVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCAKDTAWGLRLGESSSYDFDIWGQ GTMVTVSS
2.25	SEQ ID NO. 177 GYGLH	SEQ ID NO. 178 YISYDL SNKYY ARGVK G	SEQ ID NO. 179 DVAWGLRL GESSSYDF DI	SEQ ID NO. 180 EVQLVESGGGVVQPGRSLRLSCAASGFSFS GYGLHWVRQAPGKGLEWVAYISYDLSNKY YARGVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDVAWGLRLGESSSYDFDIWG QGTMVTVSS

Table 2. This shows SEQ ID NOs of family 2 CDR sequences and of family 2 full length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 2. Family 2-like sequences are variants that have certain percentage sequence identity with Family 2 sequences as set out herein.

In one aspect, the V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 83 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 83. In one embodiment, the V_H domain comprises a CDR3 selected from SEQ ID NO. 83, 87, 91, 95, 99, 103, 107, 111, 115, 119, 123, 127, 131, 135, 139, 143, 147, 151, 155, 159, 163, 167, 171, 175 or 179.

In one embodiment, the V_H domain comprises at least one antigen binding site comprising CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 75 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 2 family 2-like sequence comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 81 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 82 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 83 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 81 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 82 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 83 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the CDR sequences of the V_H domain are as shown for sdAbs 2.1 to 2.25 as in Figure 2 or combinations thereof. In one embodiment, CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 81, 85, 89, 93, 97, 101, 105, 109, 113, 117, 121, 125, 129, 133, 137, 141, 145, 149, 153, 157, 161, 165, 169, 173 or 177, CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 122, 126, 130, 134, 138, 142, 146, 150, 154, 158, 162, 166, 170, 174 or 178 and CDR3 comprises or consists of the amino acid sequence SEQ ID NO. SEQ ID NO. 783, 87, 91, 95, 99, 103, 107, 111, 115, 119, 123, 127, 131, 135, 139, 143, 147, 151, 155, 159,163, 167, 171, 175 or 179.

In one aspect, the single V_H domain antibody has combinations of CDR1, CDR2 and CDR3 as shown for 2.1 to 2.25 in Figure 2. In another embodiment, CDR1 is SEQ ID NO. 81, CDR2 is SEQ ID NO. 82 and CDR3 is SEQ ID NO. 83. In another embodiment, CDR1 is SEQ ID NO. 85, CDR2 is SEQ ID NO. 86 and CDR3 is SEQ ID NO. 87. In another embodiment, CDR1 is SEQ ID NO. 89, CDR2 is SEQ ID NO. 90 and CDR3 is SEQ ID NO. 91. In another embodiment, CDR1 is SEQ ID NO. 93, CDR2 is SEQ ID NO. 94 and CDR3 is SEQ ID NO. 95. In another embodiment, CDR1 is SEQ ID NO. 97, CDR2 is SEQ ID NO. 98 and CDR3 is SEQ ID NO. 99. In another embodiment, CDR1 is SEQ ID NO. 101, CDR2 is SEQ ID NO. 102 and CDR3 is SEQ ID NO. 103. In another embodiment, CDR1 is SEQ ID NO. 104, CDR2 is SEQ ID NO. 105 and CDR3 is SEQ ID NO. 106. In another embodiment, CDR1 is SEQ ID NO. 108, CDR2 is SEQ ID NO. 109 and CDR3 is SEQ ID NO. 110. In another embodiment, CDR1 is SEQ ID NO. 112, CDR2 is SEQ ID NO. 113 and CDR3 is SEQ ID NO. 115. In another embodiment, CDR1 is SEQ ID NO. 117, CDR2 is SEQ ID NO. 118 and CDR3 is SEQ ID NO. 119. In another embodiment, CDR1 is SEQ ID NO. 121, CDR2 is SEQ

ID NO. 122 and CDR3 is SEQ ID NO. 123. In another embodiment, CDR1 is SEQ ID NO. 125, CDR2 is SEQ ID NO. 127 and CDR3 is SEQ ID NO. 127. In another embodiment, CDR1 is SEQ ID NO. 129, CDR2 is SEQ ID NO. 130 and CDR3 is SEQ ID NO. 131. In another embodiment, CDR1 is SEQ ID NO. 133, CDR2 is SEQ ID NO. 134 and CDR3 is SEQ ID NO. 135. In another embodiment, CDR1 is SEQ ID NO. 137, CDR2 is SEQ ID NO. 138 and CDR3 is SEQ ID NO. 139. In another embodiment, CDR1 is SEQ ID NO. 140, CDR2 is SEQ ID NO. 141 and CDR3 is SEQ ID NO. 142. In another embodiment, CDR1 is SEQ ID NO. 144, CDR2 is SEQ ID NO. 145 and CDR3 is SEQ ID NO. 146. In another embodiment, CDR1 is SEQ ID NO. 148, CDR2 is SEQ ID NO. 149 and CDR3 is SEQ ID NO. 150. In another embodiment, CDR1 is SEQ ID NO. 152, CDR2 is SEQ ID NO. 153 and CDR3 is SEQ ID NO. 154. In another embodiment, CDR1 is SEQ ID NO. 157, CDR2 is SEQ ID NO. 158 and CDR3 is SEQ ID NO. 159. In another embodiment, CDR1 is SEQ ID NO. 161, CDR2 is SEQ ID NO. 162 and CDR3 is SEQ ID NO. 163. In another embodiment, CDR1 is SEQ ID NO. 165, CDR2 is SEQ ID NO. 166 and CDR3 is SEQ ID NO. 167. In another embodiment CDR1 is SEQ ID NO. 169, CDR2 is SEQ ID NO. 170 and CDR3 is SEQ ID NO. 171. In another embodiment, CDR1 is SEQ ID NO. 173, CDR2 is SEQ ID NO. 174 and CDR3 is SEQ ID NO. 175. In another embodiment, CDR1 is SEQ ID NO. 177, CDR2 is SEQ ID NO. 178 and CDR3 is SEQ ID NO. 179.

In one embodiment, the single V_H domain antibody comprises or consists of SEQ ID NO. 84 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95% 96%, 97%, 98% or 99% homology thereto. In one embodiment, homology is at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. CDR sequences of such sequences are shown in Figure 2. For example, the single V_H domain antibody comprises or consists of SEQ ID NO. 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 124, 128, 132, 136, 140, 144, 148, 152, 156, 160, 164, 168, 172, 176 or 180.

In another embodiment, the V_H domain is selected from one of the sequences above, for example SEQ ID NO. 84, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

In one embodiment, the single V_H domain antibody comprises SEQ ID NO. 84 or a variant thereof wherein the variant has the following amino acid substitutions compared to SEQ ID

NO. 76: residue 34 is L, V, M, Q, T, F, residue 50 is H, V, L, I, residue 55 is E, K, A, L, residue 58 is R, residue 62 is E, P, R, S, A, residue 63 is E, residue 64 is N, K, P, L, G, S, residue 79 is K, residue is L, Q, residue 84 is K, A, residue is D.

In one embodiment, the single V_H domain antibody comprises or consists of a V_H as shown in SEQ ID NO. 4 or a variant thereof wherein said variant includes the following changes compared to SEQ ID NO. 84:

1) M34→L34, G55→A55 and S63→N63 (as shown for 2.13),
2) G55→A55, G55→A55 and S63→N63 (as shown for 2.17),
3) M34→L34, G55→K55 and S63→K63 (as shown for 2.15),
4) G55→K55, and S63→K63 (as shown for 2.15) or
5) M34→L34, G55→E55 and D62→S62 (as shown for 2.11).

In one embodiment, additional changes may be included. In another embodiment, the variants listed above do not include additional changes. In one embodiment, the variant does not include a combination of the following changes: G55→A55, S63→N63, D99→N99 together with P100→T100; G34→L34, G55→K55 together with S63→K63; G55→T55, S63→R63, D99→G99 together with P100→R100.

The family 2 or family 2-like single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the immunoconjugate capable of binding human PSMA comprises a human V_H domain comprising a family 3 or family-3 like sequence.

In one embodiment, two V_H domains comprising a family 3 or a family-3 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 3 or a family-3 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.

In one embodiment, one V_H domain comprising a family 3 or a family-3 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence.

A family 3 or family 3-like single V_H domain antibody includes the parent sequence and sequences of that are derived from the parent (3.1; SEQ ID NO. 184) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 3.1 through a process of optimization, for example as shown in Figure 3. CDR sequences and full-length sequences of clones in family 3 are numbered according to Table 3 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
3.1	SEQ ID NO. 181 SYGMH	SEQ ID NO. 182 FMTYD GSNRY YADSV KG	SEQ ID NO. 183 DRIVGGRV PDAFDI	SEQ ID NO. 184 EVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMHWVRQAPGKGLEWVAFMTYDGSNR YYADSVKGRFTISRDNSKNTLYLQMNSLRD EDTALYYCARDRIVGGRVPDAFDIWGQGTM VTVSS
3.2	SEQ ID NO. 185 SYGMN	SEQ ID NO. 186 FISYD GSNKY YADSV KG	SEQ ID NO. 187 DRIVGARV PDAFDI	SEQ ID NO. 188 EVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFISYDGSNKY YADSVKGRFTISKDNSKNTLYLQMNSLRAE DTAVYYCAKDRIVGARVPDAFDIWGQGTMV TVSS
3.3	SEQ ID NO. 189 SYGMN	SEQ ID NO. 190 FISYD GSNRY YADSV KG	SEQ ID NO. 191 DRIVGARV PDAFDI	SEQ ID NO. 192 EVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLEWVAFISYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDRIVGARVPDAFDIWGQGTMV TVSS
3.4	SEQ ID NO. 193 SYGMN	SEQ ID NO. 194 FITYD GSNRY YADSV KG	SEQ ID NO. 195 DRIVGARV PDAYDI	SEQ ID NO. 196 EVQLVESGGGAVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.5	SEQ ID NO. 197 SYGMN	SEQ ID NO. 198 FITYD GSNRY YADSV KG	SEQ ID NO. 199 DRIVGARV PDAYDI	SEQ ID NO. 200 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS

3.6	SEQ ID NO. 201 SYGMN	SEQ ID NO. 202 FITYD GSNRY YADSV KG	SEQ ID NO. 203 DRIVGARV PDAYDI	SEQ ID NO. 204 EVQLLESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.7	SEQ ID NO. 205 SYGMN	SEQ ID NO. 206 FITYD GSNRY YADSV KG	SEQ ID NO. 207 DRIVGARV PDAYDI	SEQ ID NO. 208 QVQLVESGGGLVQPGGSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLHLQMDSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.8	SEQ ID NO. 209 SYGMN	SEQ ID NO. 210 FITYD GSNRY YADSV KG	SEQ ID NO. 211 DRIVGARV PDAYDI	SEQ ID NO. 212 EVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTLV TVSS
3.9	SEQ ID NO. 213 SYGMN	SEQ ID NO. 214 FISYD GSNRY YADSV KG	SEQ ID NO. 215 DRIVGARV PDAYDI	SEQ ID NO. 216 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLEWVAFISYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.10	SEQ ID NO. 217 SYGMN	SEQ ID NO. 218 FITYD GSNRY YADSV KG	SEQ ID NO. 219 DRIVGARV PDAYDI	SEQ ID NO. 220 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLHLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.11	SEQ ID NO. 221 SYGMN	SEQ ID NO. 222 FITYD GSNRY YADSV KG	SEQ ID NO. 223 DRIVGARV PDAYDI	SEQ ID NO. 224 EVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLHLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.12	SEQ ID NO. 225	SEQ ID NO.	SEQ ID NO. 227	SEQ ID NO. 228 EVQLVESGGGVVQPGRSLRLSCAASGFPLI

	SYGMN	226 FITYD GSNRY YADSV KG	DRIVGARV PDAYDI	SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.13	SEQ ID NO. 229 SYGMN	SEQ ID NO. 230 FITYD GSNRY YADSV KG	SEQ ID NO. 231 DRIVGARV PDAYDI	SEQ ID NO. 232 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.14	SEQ ID NO. 233 SYGMN	SEQ ID NO. 234 FITYD GSNRY YADSV KG	SEQ ID NO. 235 DRIVGARV PDAYDI	SEQ ID NO. 236 EVQLVESGGGVVRPGGSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLHLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.15	SEQ ID NO. 237 SYGMN	SEQ ID NO. 238 FITYD GSNRY YADSV KG	SEQ ID NO. 239 DRIVGARV PDAYDI	SEQ ID NO. 240 EVQLVESGGGLVQPGGSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLHLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.16	SEQ ID NO. 241 SYGMN	SEQ ID NO. 242 FITYD GSNRY YADSV KG	SEQ ID NO. 243 DRIVGARV PDAYDI	SEQ ID NO. 244 EVQLLESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLHLQMNSLRPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.17	SEQ ID NO. 245 SYGMN	SEQ ID NO. 246 FITYD GSNRY YADSV KG	SEQ ID NO. 247 DRIVGARV PDAYDI	SEQ ID NO. 248 EVQLLESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLKPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.18	SEQ ID NO. 249 SYGMN	SEQ ID NO. 250 FITYD GSNRY YADSV KG	SEQ ID NO. 251 DRIVGARV PDAYDI	SEQ ID NO. 252 EVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLDWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLKPE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS
3.19	SEQ ID NO. 253 SYGMH	SEQ ID NO. 254	SEQ ID NO. 255 DRIVGGRV	SEQ ID NO. 256 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMHWVRQAPGKGLEWVAFMTYDGSNR

		FMTYD GSNRY YADAV KG	PDAFDI	YYADAVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCARDRIVGGRVPDAFDIWGQGTM VTVSS
3.20	SEQ ID NO. 257 SYGMH	SEQ ID NO. 258 FQTYD GSNRY YADAV KG	SEQ ID NO. 259 DRIVGGRV PDAFDI	SEQ ID NO. 260 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMHWVRQAPGKGLEWVAFQTYDGSNR YYADAVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCARDRIVGGRVPDAFDIWGQGTM VTVSS
3.21	SEQ ID NO. 261 SYGMH	SEQ ID NO. 262 FQTYD GSNRY YADSV KG	SEQ ID NO. 263 DRIVGGRV PDAFDI	SEQ ID NO. 264 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMHWVRQAPGKGLEWVAFQTYDGSNR YYADSVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCARDRIVGGRVPDAFDIWGQGTM VTVSS
3.22	SEQ ID NO. 265 SYGMH	SEQ ID NO. 266 FQTYD ASNRY YADSV KG	SEQ ID NO. 267 DRIVGGRV PDAFDI	SEQ ID NO. 268 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMHWVRQAPGKGLEWVAFQTYDASNR YYADSVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCARDRIVGGRVPDAFDIWGQGTM VTVSS
3.23	SEQ ID NO. 269 SYGMH	SEQ ID NO. 270 FQTYD ASNRY YADAV KG	SEQ ID NO. 271 DRIVGGRV PDAFDI	SEQ ID NO. 272 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMHWVRQAPGKGLEWVAFQTYDASNR YYADAVKGRFTISRDNSKNTLYLQMNSLRA EDTAVYYCARDRIVGGRVPDAFDIWGQGTM VTVSS
3.24	SEQ ID NO. 273 SYGMN	SEQ ID NO. 274 FITYD GSNRY YADSV KG	SEQ ID NO. 275 DRIVGARV PDAYDI	SEQ ID NO. 276 QVQLVESGGGVVQPGRSLRLSCAASGFPLI SYGMNWVRQAPGKGLEWVAFITYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRAE DTAVYYCAKDRIVGARVPDAYDIWGQGTMV TVSS

Table 3. This shows SEQ ID NOs of family 3 CDR sequences and of family 3 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 3. Family 3-like sequences are variants that have certain percentage sequence identity with Family 3 sequences as set out herein.

In one aspect, the invention relates to an immunoconjugate comprising a human V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 183 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 183.

In one embodiment, the V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 183 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 183. In one embodiment, homology is at least 90%.

In one embodiment, the single V_H domain antibody comprises the amino acid sequence SEQ ID NO. 183 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 3 or family 3-like sequence comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 181 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 182 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 183 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 181 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 182 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 183 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the CDR sequences of the V_H domain are as shown for sdAbs 3.1 to 3.24 as in Figure 3 or combinations thereof. In one embodiment, CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 181, 185, 189, 193, 197, 201, 205, 209, 213, 217, 221, 225, 229, 233, 237, 241, 245, 249, 253, 257, 261, 265, 269 or 273, CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 182, 186, 190, 194, 198, 202, 206, 210, 214, 218, 222, 226, 230, 234, 238, 242, 246, 250, 254, 258, 262, 266, 270 or 274 and CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 183, 187, 191,

195, 199, 203, 207, 211, 215, 219, 223, 227, 231, 235, 239, 243 or 247, 251, 255, 259, 263, 267, 271 or 275.

In one aspect, the invention relates to a single V_H domain antibody which has combinations of CDR1, CDR2 and CDR3 as shown for 3.1 to 3.24 in Figure 3. In one embodiment, CDR1 is SEQ ID NO. 181, CDR2 is SEQ ID NO. 182 and CDR3 is SEQ ID NO. 183. In one embodiment, CDR1 is SEQ ID NO. 185, CDR2 is SEQ ID NO. 186 and CDR3 is SEQ ID NO. 187. In one embodiment, CDR1 is SEQ ID NO. 189, CDR2 is SEQ ID NO. 190 and CDR3 is SEQ ID NO. 191. In one embodiment, CDR1 is SEQ ID NO. 193, CDR2 is SEQ ID NO. 194 and CDR3 is SEQ ID NO. 195. In one embodiment, CDR1 is SEQ ID NO. 197, CDR2 is SEQ ID NO. 198 and CDR3 is SEQ ID NO. 199. In one embodiment, CDR1 is SEQ ID NO. 201, CDR2 is SEQ ID NO. 202 and CDR3 is SEQ ID NO. 203. In one embodiment, CDR1 is SEQ ID NO. 205, CDR2 is SEQ ID NO. 206 and CDR3 is SEQ ID NO. 207. In one embodiment, CDR1 is SEQ ID NO. 209, CDR2 is SEQ ID NO. 210 and CDR3 is SEQ ID NO. 211. In one embodiment, CDR1 is SEQ ID NO. 213, CDR2 is SEQ ID NO. 214 and CDR3 is SEQ ID NO. 215. In one embodiment, CDR1 is SEQ ID NO. 217, CDR2 is SEQ ID NO. 218 and CDR3 is SEQ ID NO. 219. In one embodiment, CDR1 is SEQ ID NO. 221, CDR2 is SEQ ID NO. 222 and CDR3 is SEQ ID NO. 223. In one embodiment, CDR1 is SEQ ID NO. 225, CDR2 is SEQ ID NO. 226 and CDR3 is SEQ ID NO. 227. In one embodiment, CDR1 is SEQ ID NO. 229, CDR2 is SEQ ID NO. 230 and CDR3 is SEQ ID NO. 231. In one embodiment, CDR1 is SEQ ID NO. 233, CDR2 is SEQ ID NO. 234 and CDR3 is SEQ ID NO. 235. In one embodiment, CDR1 is SEQ ID NO. 237, CDR2 is SEQ ID NO. 238 and CDR3 is SEQ ID NO. 239. In one embodiment, CDR1 is SEQ ID NO. 241, CDR2 is SEQ ID NO. 242 and CDR3 is SEQ ID NO. 243. In one embodiment, CDR1 is SEQ ID NO. 245, CDR2 is SEQ ID NO. 246 and CDR3 is SEQ ID NO. 247. In one embodiment, CDR1 is SEQ ID NO. 249, CDR2 is SEQ ID NO. 250 and CDR3 is SEQ ID NO. 251. In one embodiment, CDR1 is SEQ ID NO. 253, CDR2 is SEQ ID NO. 254 and CDR3 is SEQ ID NO. 255. In one embodiment, CDR1 is SEQ ID NO. 257, CDR2 is SEQ ID NO. 258 and CDR3 is SEQ ID NO. 259. In one embodiment, CDR1 is SEQ ID NO. 261, CDR2 is SEQ ID NO. 262 and CDR3 is SEQ ID NO. 263. In one embodiment, CDR1 is SEQ ID NO. 265, CDR2 is SEQ ID NO. 266 and CDR3 is SEQ ID NO. 267. In one embodiment, CDR1 is SEQ ID NO. 269, CDR2 is SEQ ID NO. 270 and CDR3 is SEQ ID NO. 271. In one embodiment, CDR1 is SEQ ID NO. 273, CDR2 is SEQ ID NO. 274 and CDR3 is SEQ ID NO. 275.

In one embodiment, the single V_H domain antibody comprises or consists of SEQ ID NO. 180 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology

thereto. In one embodiment, homology is at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. CDR sequences of such sequences are shown in Figure 3. For example, the V_H domain comprises or consists of SEQ ID NO. 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228, 232, 236, 240, 244, 248, 252, 256, 260, 264, 268, 272 or 276.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 184, 188, 192, 196, 200, 204, 208, 212, 216, 220, 224, 228, 232, 236, 240, 244, 248, 252, 256, 260, 264, 268, 272 or 276, or a sequence with at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. CDR sequences of such sequences are listed below.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 3 or family 3-like single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the immunoconjugate capable of binding human PSMA comprises a human V_H domain comprising a family 4 or family 4-like sequence.

In one embodiment, two V_H domains comprising a family 4 or a family-4 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 4 or a family-4 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.
In one embodiment, one V_H domain comprising a family 4 or a family-4 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence.

Family 4 single V_H domain antibodies include the parent sequence and sequences that are derived from the parent (4.1, SEQ ID NO. 279) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 4.1 through a process of optimization, for example as shown in Figure 4. CDR sequences and full length sequences in family 4 are numbered according to Table 4 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
4.1	SEQ ID NO. 277 SYGMH	SEQ ID NO. 278 VISYDG SNRYY ADSVK G	SEQ ID NO. 279 ERIFGVLTP DDFDI	SEQ ID NO. 280 QVQLVESGGGVVQPGRSLRLSCVASGFPFI SYGMHWVRQAPGKGREWVAVISYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKERIFGVLTPDDFDIWGQGTTVT VSS
4.2	SEQ ID NO. 281 SYGMH	SEQ ID NO. 282 VISYDG SNRYY ADSVK G	SEQ ID NO. 283 ERIFGVLTP DDFDI	SEQ ID NO. 284 QVQLVESGGGVVQPGRSLRLSCAASGFPFI SYGMHWVRQAPGKGLEWVAVISYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKERIFGVLTPDDFDIWGQGTTVT VSS
4.3	SEQ ID NO. 285 SYGMH	SEQ ID NO. 286 VISYDG ANRYY ADSVK G	SEQ ID NO. 287 ERIFGVLTP DDFEI	SEQ ID NO. 288 EVQLLESGGGVVQPGRSLRLSCAASGFPFI SYGMHWVRQAPGKGLEWVAVISYDGANRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKERIFGVLTPDDFEIWGQGTTVT VSS
4.4	SEQ ID NO. 289 SYGMH	SEQ ID NO. 290 VISYDG SNRYY ADSVK G	SEQ ID NO. 291 ERIFGALTP DDFDI	SEQ ID NO. 292 EVQLVESGGGVVQPGRSLRLSCAASGFTFT SYGMHWVRQAPGKGLEWVAVISYDGSNRY YADSVKGRFTISRDNSKNTLYLQMNSLRPE DTAVYYCAKERIFGALTPDDFDIWGQGTTVT VSS

Table 4. This shows SEQ ID NOs of family 4 CDR sequences and of family 4 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 4. Family 4-like sequences are variants that have certain percentage sequence identity with Family 4 sequences as set out herein.

In one aspect, the invention relates to an immunoconjugate comprising a human V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 279 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 279. In one embodiment, the V_H domain comprises a CDR3 selected from SEQ ID NOs. 279, 282, 287 or 291.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 279 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 4 or family 4-like sequence comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain and wherein said PSMA V_H domain comprises at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 277 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 278 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 279 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 277 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 278 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 279 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the CDR sequences of the V_H domain are as shown for clones 4.1 to 4.4 as in Figure 4 or combinations thereof. In one embodiment, CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 277, 281, 285 or 289; CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 278, 282, 286 or 290 and CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 279, 283, 287 or 291.

In one aspect, the single V_H domain antibody has combinations of CDR1, CDR2 and CDR3 as shown for 4.1 to 4.4 in Figure 4. Thus, in one embodiment, CDR1 is SEQ ID NO. 277, CDR2 is SEQ ID NO. 278 and CDR3 is SEQ ID NO. 279. Thus, CDR1 is SEQ ID NO. 281, CDR2 is SEQ ID NO. 282 and CDR3 is SEQ ID NO. 283. In one embodiment, CDR1 is SEQ

ID NO. 285, CDR2 is SEQ ID NO. 286 and CDR3 is SEQ ID NO. 287. In one embodiment, CDR1 is SEQ ID NO. 289, CDR2 is SEQ ID NO. 290 and CDR3 is SEQ ID NO. 291.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 280 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% homology thereto. In one embodiment, homology is at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. CDR sequences of such sequences are shown in Figure 4. For example, the V_H domain comprises or consists of SEQ ID NO. 280, 284, 288 or 290.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 280, 284, 288 or 290, or a sequence with at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. CDR sequences of such sequences are listed below.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 4 or family 4-like single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 5 or family-5 like sequence.

In one embodiment, the immunoconjugate comprises or consists of at least one immunoglobulin single domain antibody capable of binding PSMA, preferably human PSMA, wherein said domain is a human V_H domain and wherein said PSMA single V_H domain comprises a family 5 or family-5 sequence. In one embodiment, two V_H domains comprising a family 5 or a family-5 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 5 or a family-5 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second

V_H domain may be selected from family 1, 5, 6, 12 or 13 or family 1, 5, 6, 12 or 13-like sequence.

In one embodiment, one V_H domain comprising a family 5 or a family-5 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or 14 or a family 2, 3, 4, 7, 9, 10, 11 or 14-like sequence.

A single V_H domain antibody of family 5 includes sequences that are derived from the parent (5.1; SEQ ID NO. 292) or a part thereof, for example a CDR3 sequence, and to V_H sequences or parts thereof that are derived from the parent 5.1 through a process of optimization, for example as shown in Figure 5. CDR sequences and full length sequences in family 5 are numbered according to Table 5 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
5.1	SEQ ID NO. 293 NYGMH	SEQ ID NO. 294 IISYDG NTKYYT DSVKG	SEQ ID NO. 295 GLWPSDV	SEQ ID NO. 296 QVQLVESGGGVVQPGRSLRLSCAASGFTF NNYGMHWVRQAPGKGLEWVAIISYDGNTK YYTDSVKGRFTISRDNSKNTLYLQMNSLRV EDTAVYYCAKGLWPSDVWGQGTTVTVSS
5.2	SEQ ID NO. 297 NYGMH	SEQ ID NO. 298 IISYDG NSKYYT DSVKG	SEQ ID NO. 299 GLWPSDV	SEQ ID NO. 300 EVQLVESGGGVVQPGRSLRLSCAASGFTF NNYGMHWVRQAPGKGLEWVAIISYDGNSK YYTDSVKGRFTISRDNSKNTLYLQMNSLRV EDTAVYYCAKGLWPSDVWGQGTTVTVSS

Table 5. This shows SEQ ID NOs of family 5 CDR sequences and of family 5 length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 5. Family 5-like sequences are variants that have certain percentage sequence identity with Family 5-like sequences as set out herein.

In one aspect, the human V_H domain comprises a CDR3 sequence comprising SEQ ID NO. 295 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 295. In one embodiment, the V_H domain comprises a CDR3 selected from SEQ ID NO. 295 and 299.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence of SEQ ID NO. 295 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 5 or family-5 sequence comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 293 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 294 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 295 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 293 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 294 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 295 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the CDR sequences of the V_H domain are as shown for clones 5.1 and 5.2 as in Figure 5 or combinations thereof. In one embodiment, CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 293 or 297, CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 294 or 2984 and CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 295 or 299.

In one aspect, the invention relates to a V_H domain which has combinations of CDR1, CDR2 and CDR3 as shown for 5.1 to 5.2 in Figure 5. Thus, in one embodiment, CDR1 is SEQ ID NO. 293, CDR2 is SEQ ID NO. 294 and CDR3 is SEQ ID NO. 295. In one embodiment, CDR1 is SEQ ID NO. 297, CDR2 is SEQ ID NO. 298 and CDR3 is SEQ ID NO. 299.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 296 or 300 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, homology is at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. CDR sequences of such sequences are shown in Figure 5. For example, the V_H domain comprises or consists of SEQ ID NO. 296 or 300.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 5 or family-5 single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 6 or family 6-like sequence.

In one aspect, the immunoconjugate capable of binding human PSMA comprises a human V_H domain comprising a family 6 or family 6-like sequence. In one embodiment, two V_H domains comprising a family 6 or a family-6 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 6 or a family-6 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from family 1, 5, 6, 12 or 13 or family 1, 5, 6, 12 or 13-like sequence.

In one embodiment, one V_H domain comprising a family 6 or a family-6 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or 14 or a family 2, 3, 4, 7, 9, 10, 11 or 14-like sequence.

A family 6 single V_H domain antibody includes the parent (6.1; SEQ ID NO. 304) or a part thereof, for example a CDR3 sequence, and to V_H sequences or parts thereof that are derived from the parent 6.1 through a process of optimization, for example as shown in Figure 3. CDR sequences and full length sequences in family 6 are numbered according to Table 6 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
6.1	SEQ ID NO. 301 NSGYY WS	SEQ ID NO. 302 FIYYNG SIHYNP SLKS	SEQ ID NO. 303 DGDDYGDY	SEQ ID NO. 304 QVQLQESGPGLVKPSQTLSLTCTVSGGSIS NSGYYWSWVRQHPGKDLEWIGFIYYNGSI HYNPSLKSRVIISVDTSKNQFSLKMNSVTAA DTAVYYCARDGDDYGDYLRGQGTLVTVSS
6.2	SEQ ID NO. 305 NSGYY WS	SEQ ID NO. 306 FIYYNG SIHYNP SLKS	SEQ ID NO. 307 DGDDYGDY	SEQ ID NO. 308 QVQLQESGPGLVKPSQTLSLTCTVSGGSIS NSGYYWSWIRQHPGKGLEWIGFIYYNGSIH YNPSLKSRVIISVDTSKNQFSLKMSSVTAAD TAVYYCARDGDDYGDYLRGQGTLVTVSS
6.3	SEQ ID NO. 309 NSGYY WS	SEQ ID NO. 310 FIYYNG SIHYNP SLKS	SEQ ID NO. 311 DGDDYGDY	SEQ ID NO. 312 QVQLQESGPGLVKPSQTLSLTCTVSGGSIS NSGYYWSWVRQHPGKGLEWIGFIYYNGSI HYNPSLKSRVIISVDTSKNQFSLKLNSVTAA DTAVYYCARDGDDYGDYLRGQGTLVTVSS
6.4	SEQ ID NO. 313 NSGYY WS	SEQ ID NO. 314 FIYYNG SIHYNP SLKS	SEQ ID NO. 315 DGDDYGDY	SEQ ID NO. 316 QVQLQESGPGLVKPSQTLSLTCTVSGGSIS NSGYYWSWIRQHPGKGLEWIGFIYYNGSIH YNPSLKSRVIISVDTSKNQFSLKLSSVTAAD TAVYYCARDGDDYGDYLRGQGTLVTVSS
6.5	SEQ ID NO. 317 NSGYY WS	SEQ ID NO. 318 FIYYNG SIHYNP SLKS	SEQ ID NO. 319 DGDDYGDY	SEQ ID NO. 320 QVQLQESGPGLVKPSQTLSLTCTVSGGSIS NSGYYWSWVRQHPGKGLEWIGFIYYNGSI HYNPSLKSRVTISVDTSKNQFSLKMSSVTA ADTAVYYCARDGDDYGDYLRGQGTLVTVS

				S
6.6	SEQ ID NO. 321 NSGYY WS	SEQ ID NO. 322 FIYYNG SIHYNP SLKS	SEQ ID NO. 323 DGDDYGDY	SEQ ID NO. 324 QVQLQESGPGLVKPSQTLSLTCTVSGGSIS NSGYYWSWVRQHPGKGLEWIGFIYYNGSI HYNPSLKSRVTISVDTSKNQFSLKLNSVTAA DTAVYYCARDGDDYGDYLRGQGTLVTVSS
6.7	SEQ ID NO. 325 NSGYY WS	SEQ ID NO. 326 FIYYNG SIHYNP SLKS	SEQ ID NO. 327 DGDDYGDY	SEQ ID NO. 328 QVQLQESGPGLVKPSQTLSLTCTVSGGSIS NSGYYWSWVRQHPGKGLEWIGFIYYNGSI HYNPSLKSRVTISVDTSKNQFSLKLSSVTAA DTAVYYCARDGDDYGDYLRGQGTLVTVSS

Table 6. This shows SEQ ID NOs of family 6 CDR sequences and of family 6 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 6. Family 6-like sequences are variants that have certain percentage sequence identity with Family 6 sequences as set out herein.

In one aspect, the invention relates to an immunoconjugate comprising a family 6 or family 6-like human V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 303 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 303. In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 selected from SEQ ID NO. 303, 307, 311, 315, 319, 323 or 327.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 303 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the immunoconjugate comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 301 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 302 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 303 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 301 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO.302 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 303 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the CDR sequences of the V_H domain are as shown for clones 6.1 to 6.7 as in Figure 6 or combinations thereof. In one embodiment, CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 301, 305, 309, 313, 317, 321, 325, CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 302, 306, 310, 314, 318, 322, 326 and CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 303, 307, 311, 315, 319, 323, 327.

In one aspect, single V_H domain antibody which has combinations of CDR1, CDR2 and CDR3 as shown for 6.1 to 6.7 in Figure 6. Thus, in one embodiment, CDR1 is SEQ ID NO. 301, CDR2 is SEQ ID NO. 302 and CDR3 is SEQ ID NO. 303. Thus, in one embodiment, CDR1 is SEQ ID NO. 305, CDR2 is SEQ ID NO. 306 and CDR3 is SEQ ID NO. 307. In one embodiment, CDR1 is SEQ ID NO. 309, CDR2 is SEQ ID NO. 310 and CDR3 is SEQ ID NO. 311. In one embodiment, CDR1 is SEQ ID NO. 313, CDR2 is SEQ ID NO. 314 and CDR3 is SEQ ID NO. 315. In one embodiment, CDR1 is SEQ ID NO. 317, CDR2 is SEQ ID NO. 318 and CDR3 is SEQ ID NO. 319. In one embodiment, CDR1 is SEQ ID NO. 321, CDR2 is SEQ ID NO. 322 and CDR3 is SEQ ID NO. 323. In one embodiment, CDR1 is SEQ ID NO. 325, CDR2 is SEQ ID NO. 326 and CDR3 is SEQ ID NO. 327.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 304 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%homology thereto. In one embodiment, homology is at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. CDR sequences of such sequences are shown in Figure 6. For example, the V_H domain comprises or consists of SEQ ID NO. 304, 308, 312, 316, 320, 324 or 328.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 304, 308, 312, 316, 320, 324 or 328 or a sequence with at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 6 or family 6-like immunoconjugates have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 7 or family 7-like sequence.

In one aspect, the immunoconjugate capable of binding human PSMA comprises a human V_H domain comprising a family 7 or family 7-like sequence. In one embodiment, two V_H domains comprising a family 7 or a family-7 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 7 or a family-7 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.

In one embodiment, one V_H domain comprising a family 7 or a family-7 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence.

A family 7 or family 7-like sequence includes the parent sequence and sequences of clones that are derived from the parent (7.1) or a part thereof, for example a CDR3 sequence, and to V_H sequences or parts thereof that are derived from the parent 7.1 through a process of

optimization, for example as shown in Figure 7. CDR sequences and full length sequences in family 7 are numbered according to Table 7 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
7.1	SEQ ID NO. 329 SYWMY	SEQ ID NO. 330 NINHDGSEKYYV DSVKG	SEQ ID NO. 331 DSLIVGERGY	SEQ ID NO. 332 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHDGSEKYY VDSVKGRFTISRDNAK NSLYLQMNSLRAEDTA VYYCARDSLIVGERGY WGQGTLVTVSS
7.2	SEQ ID NO. 333 SYWMY	SEQ ID NO. 334 NINHDGSEKYYV DSVKG	SEQ ID NO. 335 DNLIVGERGY	SEQ ID NO. 336 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHDGSEKYY VDSVKGRFTISRDNAK NSLYLQMNSLRAEDTA VYYCARDNLIVGERGY WGQGTLVTVSS
7.3	SEQ ID NO. 337 SYWMY	SEQ ID NO. 338 NINHGGSEKYYV DSVKG	SEQ ID NO. 339 DSLIVGERGY	SEQ ID NO. 340 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHGGSEKYY VDSVKGRFTISRDNAK NSLYLQMNSLRAEDTA VYYCARDSLIVGERGY WGQGTLVTVSS
7.4	SEQ ID NO. 341 SYWMY	SEQ ID NO. 342 NINHQGSEKYYV DSVKG	SEQ ID NO. 343 DSLIVGERGY	SEQ ID NO. 344 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHQGSEKYY VDSVKGRFTISRDNAK NSLYLQMNSLRAEDTA VYYCARDSLIVGERGY WGQGTLVTVSS
7.5	SEQ ID NO. 345 SYWMY	SEQ ID NO. 346 NINHPGSEKYYV DSVKG	SEQ ID NO. 347 DSLIVGERGY	SEQ ID NO. 348 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHPGSEKYYV

				DSVKGRFTISRDNAKN SLYLQMNSLRAEDTAV YYCARDSLIVGERGY WGQGTLVTVSS
7.6	SEQ ID NO. 349 SYWMY	SEQ ID NO. 350 NINHEGSEKYYV DSVKG	SEQ ID NO. 351 DSLIVGERGY	SEQ ID NO. 352 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHEGSEKYYV DSVKGRFTISRDNAKN SLYLQMNSLRAEDTAV YYCARDSLIVGERGY WGQGTLVTVSS
7.7	SEQ ID NO. 353 SYWMY	SEQ ID NO. 354 NINHIGSEKYYVD SVKG	SEQ ID NO. 355 DSLIVGERGY	SEQ ID NO. 356 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHIGSEKYYV DSVKGRFTISRDNAKN SLYLQMNSLRAEDTAV YYCARDSLIVGERGY WGQGTLVTVSS
7.8	SEQ ID NO. 357 SYWMY	SEQ ID NO. 358 NINHDGSEKYYV DSVKG	SEQ ID NO. 359 DTLIVGERGY	SEQ ID NO. 360 EVQLVESGGGLVQPG GSLRLSCAASGFTFSS YWMYWVRQAPGKGL EWVANINHDGSEKYY VDSVKGRFTISRDNAK NSLYLQMNSLRAEDTA VYYCARDTLIVGERGY WGQGTLVTVSS

Table 7. This shows SEQ ID NOs of family 7 CDR sequences and of family 7 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 7. Family 7-like sequences are variants that have certain percentage sequence identity with Family 7 sequences as set out herein.

In one aspect, the invention relates to a family 7 or family 7-like immunoconjugate comprising a human V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 331 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 331.

In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 selected from SEQ ID NO. 331, 335, 339, 343, 347, 351, 355 or 359.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 331 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 7 or family 7-like sequence comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprises at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 329 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 330 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 331 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 329 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 330 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 331 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In one embodiment, the CDR sequences of the V_H domain are as shown for clones 7.1 to 7.8 as in Figure 7 or combinations thereof. In one embodiment, CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 329, 333, 337, 341, 345, 349, 353 or 357, CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 330, 334, 338, 342, 346, 350, 354 or 358 and CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 331, 335, 339, 343, 347, 351, 355 or 359.

In one aspect, the single V_H domain antibody has combinations of CDR1, CDR2 and CDR3 as shown for 7.1 to 7.8 in Figure 7. Thus, in one embodiment, CDR1 is SEQ ID NO. 329, CDR2 is SEQ ID NO. 330 and CDR3 is SEQ ID NO. 331. Thus, in one embodiment, CDR1 is SEQ ID NO. 333, CDR2 is SEQ ID NO. 334 and CDR3 is SEQ ID NO. 335. In one

embodiment, CDR1 is SEQ ID NO. 337, CDR2 is SEQ ID NO. 338 and CDR3 is SEQ ID NO. 339. In one embodiment, n CDR1 is SEQ ID NO. 341, CDR2 is SEQ ID NO. 342 and CDR3 is SEQ ID NO. 343. In one embodiment, CDR1 is SEQ ID NO. 345, CDR2 is SEQ ID NO. 346 and CDR3 is SEQ ID NO. 347. In one embodiment, CDR1 is SEQ ID NO. 349, CDR2 is SEQ ID NO. 350 and CDR3 is SEQ ID NO.351. In one embodiment, CDR1 is SEQ ID NO. 353, CDR2 is SEQ ID NO. 354 and CDR3 is SEQ ID NO. 355. In one embodiment, CDR1 is SEQ ID NO. 357, CDR2 is SEQ ID NO. 358 and CDR3 is SEQ ID NO. 359.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 332 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, homology is at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. CDR sequences of such sequences are shown in Figure 2. For example, the V_H domain comprises or consists of SEQ ID NO. 332, 336, 340, 344, 348, 352, 356 or 360.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 332, 336, 340, 344, 348, 352, 356, 360 or a sequence with at least 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 7 or family 7-like a single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 8 or family 8-like sequence.

A family 8 or family 8-like sequence includes the parent sequence and sequences of clones that are derived from the parent (8.1, SEQ ID NO. 36) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 8.1 through a

process of optimization, CDR sequences and full length sequences of 8.1 in are numbered according to Table 8 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
8.1	SEQ ID NO. 361 GYYWS	SEQ ID NO. 362 EINHSGSTNY NPSLKS	SEQ ID NO. 363 GPIPATAIPDAF D	SEQ ID NO. 364 QVQLQQWGAGLLKPS ETLSLTCAVYGGSFSG YYWSWIRQPPGKGLE WIGEINHSGSTNYNPS LKSRVTISVDTSKNQF SLKLSSVTAADTAVYY CARGPIPATAIPDAFDI WGQGTMVTVSS

Table 8. This shows SEQ ID NOs of family 8 CDR sequences and of family 8 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 8. Family 8-like sequences are variants that have certain percentage sequence identity with Family 8 sequences as set out herein.

In one aspect, the invention relates to a family 8 or family 8-like immunoconjugate comprising a human V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 363 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 363.

In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 363.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 363 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 8 or family 8-like sequence comprises a immunoconjugate comprising at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 361 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 362 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 363 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 361 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 362 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 363 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for sdAb 8.1 as in Figure 8.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 364 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 8 or family 8-like a single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 9 or family 9-like sequence.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 9 or family 9-like sequence. In one embodiment, two V_H domains comprising a family 9 or a family-9 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 9 or a family-9 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or

confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.

In one embodiment, one V_H domain comprising a family 9 or a family-9 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence.

A family 9 or family 9 sequence includes the parent sequence and sequences that are derived from the parent (9.1; SEQ ID NO. 368) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 9.1 through a process of optimization, CDR sequences and full-length sequences of 9.1 in are numbered according to Table 9 as shown below.

Name	CDR1	CDR2	CDR3	VH Full length sequence
9.1	SEQ ID NO. 365 GHYWS	SEQ ID NO. 366 DINHSGSTNYN PSLKS	SEQ ID NO. 367 DYGDSRSLFDY	SEQ ID NO. 368 QVQLQQWGAGLLKPSETL SLTCAVYGGSFSGHYWSW IRQPPGKGLEWIGDINHSG STNYNPSLKSRVTISVDTS KNQFSLKLSSVTAADTAVY YCVRDYGDSRSLFDYWGQ GTLVTVSS

Table 9. This shows SEQ ID NOs of family 9 CDR sequences and of family 9 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 9. Family 9-like sequences are variants that have certain percentage sequence identity with Family 9 sequences as set out herein.

In one aspect, the invention relates to a family 9 or family 9-like immunoconjugate comprising a human V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 367 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 367.

In one embodiment, the immunoconjugate comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain and wherein said human V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 367 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 367. In one embodiment,

homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 367.

In one embodiment, the single V_H domain antibody comprises a CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 363 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 9 or family 9-like sequence comprises a immunoconjugate comprising at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 365 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 366 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 367 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 365 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 366 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 367 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for clone 9.1 as in Figure 9.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 368 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid

substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 9 or family 9-like a single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 10 or family 10-like sequence.

In one embodiment, two V_H domains comprising a family 10 or a family-10 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 10 or a family-10 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.

In one embodiment, one V_H domain comprising a family 10 or a family-10 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence. A family 10 or family 10 sequence includes the parent sequence and sequences that are derived from the parent (10.1) or a part thereof, for example a CDR3 sequence, and V_H sequences of or parts thereof that are derived from the parent 10.1 through a process of optimization, CDR sequences and full length sequences of 10.1 in are numbered according to Table 10 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
10.1	SEQ ID NO. 369 SYGMY	SEQ ID NO. 370 FMSYDGSNKY YVDSVKG	SEQ ID NO. 371 GDYDFWSGY PDYD	SEQ ID NO. 372 QVQLVESGGGLVQPGG SLRLSCAASGFTFSSYG MHWVRQAPGKGLEWV AFMSYDGSNKYYVDSV KGRFTISRDNSKNTLYL QMNSLRAEDTAVYYCA KGDYDFWSGYPDYDM DVWGQGTTVTVSS

Table 10. This shows SEQ ID NOs of family 10 CDR sequences and of family 10 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are

shown in Figure 10. Family 10-like sequences are variants that have certain percentage sequence identity with Family 10 sequences as set out herein.

In one aspect, the invention relates to a family 10 or family 10-like immunoconjugate comprising a human V_H domain comprising a CDR3 sequence comprising SEQ ID NO. 371 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 371.

In one embodiment, the family 10 or family 10-like immunoconjugate comprises at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain and wherein said human V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 371 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 371. In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 371.

In one embodiment, said V_H domain comprises at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 369 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 370 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 371 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 369 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 370 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 371 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for clone 10.1 as in Figure 10.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 372 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 10 or family 10-like a single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a V_H domain comprising a family 11 or family 11-like sequence. In one embodiment, two V_H domains comprising a family 11 or a family-11 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 11 or a family-11 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.

In one embodiment, one V_H domain comprising a family 11 or a family-11 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence.

A family 11 or family 11 sequence includes the parent sequence and sequences that are derived from the parent (11.1, SEQ ID NO. 376) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 11.1 through a process of optimization, CDR sequences and full-length sequences of 11.1 in are numbered according to Table 11 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence

11.1	SEQ ID NO. 373 SYGMY	SEQ ID NO. 374 VISYDGSNKNY ADSVKG	SEQ ID NO. 375 GGNALYSSG WPDD	SEQ ID NO. 376 EVQLVESGGGLVKPGGS LRLSCAASGFNLISYGMY WVRQAPGKGLEWVAVIS YDGSNKNYADSVKGRFTI SRDNSKNTLFLQMNSLRV EDTAVYYCAKGGNALYS SGWPDDGFDIRGQGTMV TVSS

Table 11. This shows SEQ ID NOs of family 11 CDR sequences and of family 11 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 11. Family 11-like sequences are variants that have certain percentage sequence identity with Family 11 sequences as set out herein.

In one embodiment, the V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 375 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 375. In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 375.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 375 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 11 or family 11-like sequence comprises an immunoconjugate comprising or consisting of at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 373 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 374 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 375 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 373 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 374 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 375 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for sdAb 11.1 as in Figure 11.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 376 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 11 or family 11-like immunoconjugates have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples.

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 12 or family 12-like sequence. In one embodiment, two V_H domains comprising a family 12 or a family-12 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 12 or a family-12 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from family 1, 5, 6, 12 or 13 or family 1, 5, 6, 12 or 13-like sequence.

In one embodiment, one V_H domain comprising a family 12 or a family-12 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or 14 or a family 2, 3, 4, 7, 9, 10, 11 or 14-like sequence.

A family 12 or family 12-like sequence includes the parent sequence and sequences that are derived from the parent (12.1, SEQ ID NO. 380) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 12.1 through a process of optimization, CDR sequences and full-length sequences of 12.1 in are numbered according to Table 12 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
12.1	SEQ ID NO. 377 NFGMH	SEQ ID NO. 378 VISYDGNSKYYAD TVKG	SEQ ID NO. 379 GLWPPMDV	SEQ ID NO. 380 QVQLVESGGGVVQPG RSLRLSCAASGFTFSN FGMHWARQAPGKGLE WVAVISYDGNSKYYAD TVKGRFTISRDNSKNT LYLEMNSLRADDTAVY YCAKGLWPPMDVRGQ GTTVTVSS

Table 12. This shows SEQ ID NOs of family 12 CDR sequences and of family 12 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 12. Family 12-like sequences are variants that have certain percentage sequence identity with Family 12 sequences as set out herein.

In one embodiment, the V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 379 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 379. In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 379.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ

ID NO. 379 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 12 or family 12-like sequence comprises an immunoconjugate comprising at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 377 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 378 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 379 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 377 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 378 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 379 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for clone 12.1 as in Figure 12.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 380 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 12 or family 12-like immunoconjugates have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 13 or family 13-like sequence. In one embodiment, two V_H domains comprising a family 13 or a family-13 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 13 or a family-13 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from family 1, 5, 6, 12 or 13 or family 1, 5, 6, 12 or 13-like sequence.

In one embodiment, one V_H domain comprising a family 13 or a family-13 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or 14 or a family 2, 3, 4, 7, 9, 10, 11 or 14-like sequence.

A family 13 or family-like 13 sequence includes the parent sequence and sequences that are derived from the parent (13.1, SEQ ID NO. 384) or a part thereof, for example a CDR3 sequence, and V_H sequences of clones or parts thereof that are derived from the parent 13.1 through a process of optimization, CDR sequences and full-length sequences of 13.1 in are numbered according to Table 13 as shown below.

Name	CDR1	CDR2	CDR3	V_H Full length sequence
13.1	SEQ ID NO. 381 DYWMT	SEQ ID NO. 382 NIKQDGSEKYY VDSVKG	SEQ ID NO. 383 DRGGAVALY HNGMDM	SEQ ID NO. 384 EVQLVESGGGSVQPGG SLRLSCAASGFTFSDYW MTWVRQVPGKGLEWV ANIKQDGSEKYYVDSVK GRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCAR DRGGAVALYHNGMDM GGQGTTVTVSS

Table 13. This shows SEQ ID NOs of family 13 CDR sequences and of family 13 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 13. Family 13-like are variants sequences that have certain percentage sequence identity with Family 13 sequences as set out herein.

In one embodiment, the V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 383 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 383. In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 383.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 383 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 13 or family 13-like sequence comprises an immunoconjugate comprising at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 381 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 382 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 383 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 381 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 382 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 383 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for clone 13.1 as in Figure 13.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 384 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 13 or family 13-like a single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 14 or family 14-like sequence. In one embodiment, two V_H domains comprising a family 14 or a family-14 like sequence may be combined for a bivalent immunoconjugate. In another embodiment, one V_H domain comprising a family 14 or a family-14 like sequence may be combined with a second V_H domain as described herein that binds to the same a epitope, part, domain, subunit or confirmation of PSMA for a bivalent immunoconjugate. The second V_H domain may be selected from a family 2, 3, 4, 7, 9, 10, 11 or a family 2, 3, 4, 7, 9, 10, 11 -like sequence.

In one embodiment, one V_H domain comprising a family 14 or a family-14 like sequence may be combined with a second V_H domain as described herein that binds to a different epitope, part, domain, subunit or confirmation of PSMA for a biparatopic immunoconjugate. The second V_H domain may be selected from a family 1, 5, 6, 12 or 13 or a family 1, 5, 6, 12 or 13-like sequence.

A family 14 or family 14 sequence includes the parent sequence and sequences of clones that are derived from the parent (14.1) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 14.1 through a process of optimization, CDR sequences and full-length sequences of 14.1 in are numbered according to Table 14 as shown below.

Name	CDR1	CDR2	CDR3	VH Full length sequence

14.1	SEQ ID NO. 385 SYDIN	SEQ ID NO. 386 WMNPNSGNTG YAQKFQG	SEQ ID NO. 387 GNGPGITGTT DY	SEQ ID NO. 388 KCSWWSLGEVKKPGAS VKVSCKASGYTFTSYDI NWVRQATGQGLEWMG WMNPNSGNTGYAQKF QGRVTMTRNTSISTAYM ELSSLRSEDTAVYYCAR GNGPGITGTTDYWGQG TLVTVSS

Table 14. This shows SEQ ID NOs of family 14 CDR sequences and of family 14 full-length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 14. Family 14-like sequences are variants that have certain percentage sequence identity with Family 14 sequences as set out herein.

In one embodiment, the V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 387 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 387. In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 387.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 385 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 14 or family 14-like sequence comprises an immunoconjugate comprising at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 385 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 386 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 387 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 385 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 386 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 387 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for clone 14.1 as in Figure 14.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 388 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 14 or family 14-like a single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples

In one aspect, the invention relates to an immunoconjugate capable of binding human PSMA comprising a human V_H domain comprising a family 15 or family 15-like sequence. In one embodiment, the immunoconjugate comprises at least two one single V_H domain antibodies capable of binding PSMA, preferably human PSMA, of family 15 or family 15 sequence. Family 15 single V_H domain antibodies include the parent sequence and sequences that are derived from the parent (15.1) or a part thereof, for example a CDR3 sequence, and V_H sequences or parts thereof that are derived from the parent 15.1 through a process of optimization, CDR sequences and full-length sequences of 15.1 in are numbered according to Table 15 as shown below.

Name	CDR1	CDR2	CDR3	VH Full length sequence
15.1	SEQ ID NO. 389 DYGMS	SEQ ID NO. 390 GINWNGDRTGY ADSVKG	SEQ ID NO. 391 ENVIVPAATY	SEQ ID NO. 392 EVQLVESGGGVV RPGGSLRLSCAA SGFTFDDYGMSW VRQAPGKGLEWV SGINWNGDRTGY ADSVKGRFTISRD NAKNSLYLQMNSL RAEDTALYYCGR ENVIVPAATYWGQ GTLVTVSS

Table 15. This shows SEQ ID NOs of family 15 CDR sequences and of family 15 full length V_H sequences that are within the scope of the invention. Corresponding sequences are shown in Figure 15. Family 15-like sequences are variants that have certain percentage sequence identity with Family 15 sequences as set out herein.

In one embodiment, the V_H domain comprises at least one antigen binding site comprising a CDR3 sequence comprising SEQ ID NO. 391 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology to SEQ ID NO. 391. In one embodiment, homology is at least 90%. In one embodiment, the V_H domain comprises a CDR3 of SEQ ID NO. 391.

In one embodiment, the single V_H domain antibody comprises at least one antigen binding site comprising hypervariable region CDR3 said CDR3 having the amino acid sequence SEQ ID NO. 391 or a sequence having at least 70%, at least 80%, at least 90%, or at least 95% homology thereto. In one embodiment, the family 15 or family 15-like sequence comprises an immunoconjugate comprising at least one immunoglobulin single domain antibody capable of binding PSMA wherein said domain is a human V_H domain comprising at least one antigen binding site comprising hypervariable regions CDR1, CDR2 and CDR3, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 389 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 390 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto, and said CDR3

comprises or consists of the amino acid sequence SEQ ID NO. 391 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

In one embodiment, said CDR1 comprises or consists of the amino acid sequence SEQ ID NO. 389 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR2 comprises or consists of the amino acid sequence SEQ ID NO. 390 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, said CDR3 comprises or consists of the amino acid sequence SEQ ID NO. 391 or a sequence with at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto. In one embodiment, the CDR sequences of the V_H domain are as shown for clone 15.1 as in Figure 15.

In one embodiment, the V_H domain comprises or consists of SEQ ID NO. 392 or a sequence with at least 40%, 50%, 60%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% homology thereto.

In another embodiment, the V_H domain is selected from one of the sequences above, but comprises one or more amino acid substitutions, for example 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions. In one embodiment, the one or more amino acid substitution is in one or more of the framework areas. In another embodiment, the one or more amino acid substitution is in one or more of the CDRs. In one embodiment, the amino acid substitutions are in the framework and CDR sequences.

The family 15 or family 15-like a single V_H domain antibodies have KD, Koff, KA, Kd, EC_{50} and IC_{50} values as further described herein and as shown in the examples

In one aspect, the single V_H domain antibody comprises a CDR3 sequence selected from a family 1 or family 1-like, family 2 or family 2-like, family 3 or family 3-like, family 4 or family 4-like, family 5 or family 5-like, family 6 or family 6-like, family 7 or family 7-like, family 8 or family 8-like, family 9 or family 9-like, family 10 or family 10-like, family 11 or family 11-like, family 12 or family 12-like, family 13 or family 13-like, family 14 or family 14-like or a family 15 or family 15-like CDR3 sequence combined with a CDR1 and CDR2 sequence from another family listed herein.

For example, the single V_H domain antibody comprises a family 1 or family 1-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 2 to 15.

In another aspect, the single V_H domain antibody comprises a family 2 or family 2-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1, 3 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 3 or family 3-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1, 2, 4 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 4 or family 4-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in Table 1 any of Tables 1 to 3, 5 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 5 or family 5-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 4, 6 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 6 or family 6-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 5, 7 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 7 or family 7-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 6, 8 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 8 or family 8-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 7, 9 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 9 or family 9-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 8, 10 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 10 family 10-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 4, 11 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 11 or family 11-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 10, 12 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 12 or family 12-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 11, 13 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 13 or family 13-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 12, 14 to 15. Various combinations are possible as would be appreciated by a skilled person.

In another aspect, the single V_H domain antibody comprises a family 15 or family 15-like CDR3 sequence combined with a CDR1 and a CDR2 sequence from one or two other families as shown in any of Tables 1 to 14. Various combinations are possible as would be appreciated by a skilled person.

The invention also relates to an immunoconjugate comprising a first V_H domain selected from one of the V_H domains described above and listed in any of Tables 1 to 15 linked to a second V_H domain selected from a described above and listed in any of Tables 1 to 15 wherein said first V_H domain competes for binding to PSMA with the second V_H domain in a competitive assay.

The invention also relates to an immunoconjugate comprising a first V_H domain selected from one of the V_H domains described above and listed in any of Tables 1 to 15 linked to a second V_H domain selected from a described above and listed in any of Tables 1 to 15 wherein said first V_H domain does not compete for binding to PSMA with the second V_H domain in a competitive assay.

In preferred embodiment, the immunoconjugate is biparatopic or multiparatopic and comprises a first single V_H domain antibody selected from single domain antibodies 1.1 to 1.20 as shown in Table 1, and a second single V_H domain antibody selected from 2.1 to 2.25 as shown in Table 2. For example, the first single V_H domain antibody is selected from single domain antibodies 1.1, 1.8-1.20 as shown in Table 1, and a second single V_H domain antibody is selected from 2.1, 2.2, 2.11-2.19, 2.22-2.25 as shown in Table 2. In another embodiment, the immunoconjugate comprises a first single domain antibody selected from single domain antibodies 2.1 to 2.25 as shown in Table 2 and a second V_H single domain antibody selected from 1.1 to 1.20 as shown in Table 1. For example, the first single V_H domain antibody is selected from single domain antibodies 2.1, 2.2, 2.11-2.19, 2.22-2.25 as shown in Table 2, and the second single V_H domain antibody is selected from 1.1, 1.8-1.20 as shown in Table 1. Single domain antibodies are linked with a $(G_4S)n$ linker, preferably a $(G_4S)n$. In further preferred embodiments, the immunoconjugate is selected from an immunoconjugate that comprises the following components: single V_H domain antibody-linker- single V_H domain antibody as follows:
1.1-6GS-2.1, 1.8-6GS-2.1, 1.1-6GS-2.17, 1.1-6GS-2.15, 1.1-6GS-2.22, 1.16-6GS-2.1, 1.16-6GS-2.17, 1.16-6GS-2.15, 1.16-6GS-2.22, 1.11-6GS-2.1, 1.11-6GS-2.17, 1.11-6GS-2.15, 1.11-6GS-2.22, 1.18-6GS-2.1, 1.18-6GS-2.17, 1.18-6GS-2.15, 1.18-6GS-2.22, 1.17-6GS-2.1, 1.17-6GS-2.17, 1.17-6GS-2.15 or 1.17-6GS-2.22.

In another embodiment, single V_H domain antibodies as described above can be replaced with
Binding molecules, e.g. antibodies, antibody fragments or antibody mimetics, that bind to the same epitope on human PSMA as a single domain antibodies (i.e., antibodies that have the ability to cross-compete for binding to PSMA with any of the single domain antibodies described herein). The single domain antibodies described herein can thus be used as a reference antibody. In preferred embodiments, the reference antibody for cross-competition studies is single domain antibody 1.1, 2.1, 3.1, 4.1, 5.1, 6.1, 7.1, 8.1, 9.1, 10.1, 11.1, 12.1, 13.1, 14.1 or 15.1. Such cross-competing antibodies can be identified based on their ability to cross-compete with any of single domain antibodies described herein in standard PSMA binding assays. For example, BIAcore® analysis, ELISA assays or flow cytometry may be

used to demonstrate cross-competition with the single domain antibodies of the current invention. In one embodiment, the invention provides a binding agent capable of binding human PSMA wherein any one of the single domain antibodies described above displaces the binding agent in a competitive assay.

Suitable linkers to connect the binding moieties of the immunoconjugate include peptide linker, for example linkers that include GS residues such as $(Gly_4Ser)_n$, where n=from 1 to 10, *e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. As used herein, GS designates Gly_4Ser. $(Gly_4Ser)_n$ is also expressed as nGS herein.

In another embodiment, the immunoconjugate may comprise a further moiety that is specific for a different antigen to provide a bispecific immunoconjugate. As used herein, the term "bispecific immunoconjugate" thus refers to a polypeptide that comprises a immunoconjugate as described herein which has a binding site that has binding specificity for PSMA, and a second polypeptide domain which has a binding site that has binding specificity for a second target, *i.e.*, the bispecific immunoconjugate has specificity for two targets. The first target and the second target are not the same, *i.e.* are different targets, *e.g.*, proteins; both may be present on a cell surface. Accordingly, a bispecific immunoconjugate as described herein can selectively and specifically bind to a cell that expresses (or displays on its cell surface) the first target and the second target. In another embodiment, the immunoconjugate comprises more than two antigen-binding moieties.

In another embodiment, more than two moieties are joined together providing a multispecific immunoconjugate. A multispecific polypeptide agent as described herein can in addition to binding PSMA bind one or more additional targets, *i.e.*, a multispecific polypeptide can bind at least two, at least three, at least four, at least five, at least six, or more targets, wherein the multispecific polypeptide agent has at least two, at least, at least three, at least four, at least five, at least six, or more target binding sites respectively.

As used herein, the term "target" refers to a biological molecule (e.g., antigen, peptide, polypeptide, protein, lipid, carbohydrate) to which a polypeptide domain which has a binding site can selectively bind. The target can be, for example, an intracellular target (such as an intracellular protein target) or a cell-surface target (such as a membrane protein, *e.g.*, a receptor protein). Preferably, a target is a cell-surface target, such as a cell-surface protein. Preferably, the first cell-surface target and second cell-surface target are both present on a cell. In one embodiment, the target is an immunooncology target.

Multispecific molecules of the present invention can be constructed using methods known art.

If desired, bispecific or multispecific immunoconjugates can be linked to an antibody Fc region or fragment thereof, comprising one or both of C_H2 and C_H3 domains, and optionally a hinge region. For example, vectors encoding bispecific or multispecific immunoconjugates linked as a single nucleotide sequence to an Fc region or fragment thereof can be used to prepare such polypeptides.

In one embodiment, the further moiety may serve to prolong the half-life of the immunoconjugate. The second moiety may comprise a protein, for example and antibody, or part thereof that binds a serum albumin, *e.g.*, human serum albumin (HSA) or mouse serum albumin (MSA). The further moiety may comprise a V_H domain that binds serum albumin, e.g., human serum albumin (HSA) or mouse serum albumin (MSA).

The further moiety may comprise a serum albumin, *e.g.* a human serum albumin (HSA) or a variant thereof such as HSA C34S. Further provided is an immunoconjugate as described herein comprising a V_H domain and an Fc domain, *e.g.*, wherein the V_H domain is fused to an Fc domain. Further provided is an immunoconjugate that comprises a second variable domain that specifically binds a second antigen, where the second antigen is an antigen other than human PSMA. The second antigen may be a cluster of differentiation (CD) molecule or a Major Histocompatibility Complex (MHC) Class II molecule.

In one embodiment, the immunoconjugate of the invention is labelled with a detectable or functional label. A label can be any molecule that produces or can be induced to produce a signal, including but not limited to fluorescers, radiolabels, enzymes, chemiluminescers, a nuclear magnetic resonance active label or photosensitizers. Thus, the binding may be detected and/or measured by detecting fluorescence or luminescence, radioactivity, enzyme activity or light absorbance.

In still other embodiments, the immunoconjugate of the invention is coupled to at least one therapeutic moiety, such as a drug, an enzyme or a toxin. In one embodiment, the therapeutic moiety is a toxin, for example a cytotoxic radionuclide, chemical toxin or protein toxin. For example, the PSMA immunoconjugate of the invention, can be coupled to a radioactive isotope such as an α-, β-, or γ-emitter, or a β- and γ-emitter.

Toxin-conjugated forms of the PSMA immunoconjugates of the present invention preferably mediate specific cell killing of PSMA-expressing cells at picomolar concentrations.

In another aspect, the PSMA immunoconjugates of the invention are modified to increase half-life, for example by a chemical modification, especially by PEGylation, or by incorporation in a liposome or using a serum albumin protein.

In one embodiment, the immunoconjugate of the invention is covalently modified. The term “covalently modified/covalent modification" includes modifications of an immunoconjugate according to the present invention, *e.g.*, of a specified sequence herein; with an organic proteinaceous or non-proteinaceous derivatizing agent, fusions to heterologous polypeptide sequences, and post-translational modifications. Covalent modified polypeptides, *e.g.*, of a specified sequence, still have the functional properties described herein, for example the ability to bind the human PSMA or, Covalent modifications are generally introduced by reacting targeted amino acid residues with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. Certain post-translational modifications are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deaminated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl, tyrosine or threonyl residues, methylation of the [alpha]-amino groups of lysine, arginine, and histidine side chains. Covalent modifications, *e.g.*, include fusion proteins comprising a immunoconjugate according to the present invention, *e.g.*, of a specified sequence and their amino acid sequence variants, such as immunoadhesins, and N-terminal fusions to heterologous signal sequences.

The immunoconjugates of the invention have certain functional properties as further described below. These and other pharmacological activities of the immunoconjugates of the invention may be demonstrated in standard test methods for example as described in the art.

The immunoconjugates of the invention can be internalised into a cell along with the prostate-specific membrane antigen. Immunoconjugates of the invention bind specifically to epitopes on the extracellular domain of human PSMA. In one embodiment, immunoconjugates of the invention specifically bind PSMA in its dimeric form. Immunoconjugates of the invention can be conjugated to a toxic moiety and used to ablate or kill PSMA-expressing prostatic or cancerous cells.

A single V_H domain antibody used in the immunoconjugates of the invention can bind live cells, such as a tumor cell or a prostate cell, such as human PSMA expressing CHO cells, LNCaP cells as shown in the examples and accompanying Tables. In a further aspect, the present invention provides single domain antibodies that bind to PSMA with an EC50 value of between 100 nM and 100 pM, such as at an average EC50 value of 100nM or less, even more preferably at an average EC50 value of 90 nM or less, such as less than 80, 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM or even less, such as less than 500, 400, 300, 200, 100 pM, or even less, such as less than 4 pM, preferably as measured in a FMAT binding assay. In particular, EC50 values are shown in Table 19. In one embodiment, immunoconjugates of the invention are capable of binding specifically to human PSMA and to cynomolgus monkey PSMA.

Potency is normally expressed as an IC_{50} value, in nM unless otherwise stated. In functional assays, IC_{50} is the concentration of a binding member that reduces a biological response by 50% of its maximum. IC_{50} may be calculated by plotting % of maximal biological response as a function of the log of the binding member concentration, and using a software program to fit a sigmoidal function to the data to generate IC_{50} values. Methods for measuring IC_{50} are well known in the art. For example, to determine the IC_{50}, a HIS ZAP Cell Killing assay may be employed to determine IC_{50}. EC_{50} designates the half maximal effective concentration.

In another aspect, the invention relates to an immunoconjugate comprising or consisting of at least one immunoglobulin single domain antibody directed against PSMA, preferably human PSMA, wherein said domain is a human V_H domain and has an IC_{50} of about 0.2 to about 1000 nM or more, for example 0.2 to 900, 0.2 to 800, 0.2 to 700, 0.2 to 600, 0.2 to 500, 0.2 to 400, 0.2 to 300, 0.2 to 200, 0.2 to 100, 0.2 to 50, 0.2 to 40, 0.2 to 30, 0.2 to 20, 0.2 to 10, 0.2 to 9, 0.2 to 8, 0.2 to 7, 0.2 to 6, 0.2 to 5, 0.2 to 4, 0.2 to 3, 0.2 to 2 or 0.2 to 1 when tested as described in the examples.

Additionally, binding kinetics and affinity (expressed as the equilibrium dissociation constant, KD) of a single V_H domain antibody for binding PSMA may be determined, *e.g.*, using surface plasmon resonance such as BIAcore® or Octet, or KD may be estimated from pA2 analysis. In particular, the molecules of the invention are very potent (i.e., EC50 values as measured, e.g., in the experimental part in the pM range).

In a further aspect, the present invention provides a single domain antibody as described herein, wherein said sdAb binds to said PSMA with an average KD value of between 100 nM and 10 pM, such as at an average KD value of 90 nM or less, even more preferably at an

average KD value of 80 nM or less, such as less than 70, 60, 50, 40, 30, 20, 10, 5 nM or even less, such as less than 4, 3, 2, or 1 nM, such as less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20 pM, or even less such, as less than 10 pM. Preferably, the KD is determined as shown in the examples.

In one embodiment, a single V_H domain antibody used in the molecules of the invention has a binding affinity to PSMA with an affinity constant of at least about 10^7 M^{-1}, preferably about 10^9 M^{-1}, and more preferably, about 10^{10} M^{-1} to 10^{11} M^{-1} or higher. In one embodiment, a single V_H domain antibody used in the molecules of the invention has a Kon of 1.00E+04 to 1.00E+6 (1/Ms). In one embodiment, a single V_H domain antibody used in the molecules of the invention has a Koff of 1.00E-03 to 1.00E-05 (1/s).

Single V_H domain antibodies used in the molecules of the invention have shown excellent stability, including heat and serum stability (see examples). Furthermore, immunoconjugates of the invention show rapid tumor targeting as shown in the examples. Furthermore, single V_H domain antibodies used in the molecules of the invention also show high specificity for human PSMA and low uptake in non-target tissues (see examples).

In one embodiment, immunoconjugates of the invention show fast blood clearance. In one embodiment, immunoconjugates of the invention show low renal retention.

In one embodiment, an immunoconjugate of the invention may have one or more property select from the following non-limiting list:

a) high-affinity binding to human and/or cynomolgus prostate-specific membrane antigen in its native form occurring on the surface of tumor cells,
b) internalization by a tumor cell,
c) low uptake in non-target tissues,
d) rapid tumor targeting,
e) binding strongly to LNCaP cells, but not or only minimally to cells which lack expression of prostate-specific membrane antigen and/or
f) binding to a unique epitope on PSMA.

The nucleic acid of the single domain antibodies described herein are set out below. These can be combined using linkers described herein to generate a nucleic acid construct for expression.

Sequences comprising or consisting of SEQ ID NOs. 393 to 410 as shown below which encode V_H domains of family 1 comprising or consisting of SEQ ID NO. 4 to 80.

SEQ ID NO. 393 (encodes V_H domain 1.1)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCATGAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATGATGGTACCACA
GACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAGTAT
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 394 (encodes V_H domain 1.2)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGATAATAATAATAGCACA
GAGTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAGCA
CGCTGTATCTGCAAATGAACAGCCTGAGCGCCGAGGACACGGCCGTATATTACTGTGT
GAAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACTGTCTCTTCA

SEQ ID NO. 395 (encodes V_H domain 1.3)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCA
GGCTCCAGGGAAGGGACTGGAGTGGGTCTCAAGTATTGGTGATAATAATAATAGCACA
GACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAGTA
CGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGT
GAAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACTGTCTCCTCA

SEQ ID NO. 396 (encodes V_H domain 1.4)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGATGGAACCACATACTAC
GCAGACTCCGTGAAGGGCCGTTTCACCATCTCCAGAGACAATTCCAAGAGCACGCTGT
ATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGAT
GGTGTCCACTGGGGCCAGGGAACCCTGGTCACTGTCTCCTCA

SEQ ID NO. 397 (encodes V_H domain 1.5)

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCACTTATGCCATGAGCTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAAAATGATCGAACCACA
TACTACGTAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAGCAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCG
AAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACTGTCTCTTCA

SEQ ID NO. 398 (encodes V_H domain 1.6)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGATAATAATAGAACCACA
TACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAGCA
CGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGC
GAAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 399 (encodes V_H domain 1.7)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGATGGAACCACATACTAC
GCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAGCACGCTGT
ATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGAT
GGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 400 (encodes V_H domain 1.8)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCATGAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATGATGGTACCACA
GACTACGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 401 (encodes V_H domain 1.9)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGATACCACA
GACTACGCAGACAACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 402 (encodes V_H domain 1.10)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGCTACCACA
GACTACGCAGACTTCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 403 (encodes V_H domain 1.11)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGCTACCACA
GACTACGCAGACGCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATA
CGCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTG
AAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCASEQ ID NO.
404 (encodes V_H domain 1.12)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGCTACCACA
GACTACGCAGACGCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATA
CGCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTG
AAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
SEQ ID NO. 405 (encodes V_H domain 1.13)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACCATACCACA
GACTACGCAGCCGACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATA
CGCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTG
AAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
SEQ ID NO. 406 (encodes V_H domain 1.14)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGCTACCACA
GACTACGCAGACGTCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
SEQ ID NO. 407 (encodes V_H domain 1.15)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACCATACCACA
GACTACGCAGCCTTCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
SEQ ID NO. 408 (encodes V_H domain 1.16)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA

GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACCATACCACA
GACTACGCAGACACCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 409 (encodes V_H domain 1.17)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGATACCACA
GACTACGCAGACGCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATA
CGCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTG
AAAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 410 (encodes V_H domain 1.18)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGCTACCACA
GACTACGCAGCCTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 411 (encodes V_H domain 1.19)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACGATACCACA
GACTACGCAGCCTACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO. 412 (encodes V_H domain 1.20)
GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGTTTTAGCAGCTATGCCCTCAGTTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAAGTATTGGTGAGAATAACCATACCACA
GACTACGCAGCCACCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTCGAGGACACGGCCGTCTATTACTGTGTGA
AAGATGGTGTCCACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NOs. 413 to 437 as shown below encode V_H domains of family 2 comprising or consisting of SEQ ID NO. 84 to 180.

SEQ ID NO. 413 (encodes V_H domain 2.1)
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA

GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATGGAAGTAATAAAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 414 (encodes V_H domain 2.2)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATGGAAGTAATAAAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 415 (encodes V_H domain 2.3)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGCTTCAGTGGCTATGGCATGCACTGGGTCCGCC
AGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCACATATATCATATGATGGAAGTAATAG
ATACTATGCAGAATCCGTGAAGGGCCGATTCACCATCTCCAGAGAGAATTCCAAGAACA
CGCTGTCTCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGC
GAAAGATCCGGCCTGGGGATTACGTTTGGGGGAGTTATCGTCCTATGATTTTGACATTT
GGGGCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 416 (encodes V_H domain 2.4)

CAGGTCACCTTGAAGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTCTCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCG
AGAGATCCGGCCTGGGGATTACGTTTGGGGGAGTTATCGTCCTATGATTTTGAAATCTG
GGGCCAAGGGACAATGGTCACCGTCTCCTCA

SEQ ID NO. 417 (encodes V_H domain 2.5)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
ACTGTCTCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGATCCGGCCTGGGGATTACGTTTGGGGGAGTTATCGTCCTATGATTTTGAAATTTGG
GGCCAAGGGACAATGGTCACCGTCTCTTCA

SEQ ID NO. 418 (encodes V_H domain 2.6)

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTATATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGATCCGGCCTGGGGATTACGTTTGGGGGAACTATCGTCCTATAAATTTGAAATCTGG
GGCCAAGGGACAATGGTCACCGTCTCTTCA

SEQ ID NO. 419 (encodes V_H domain 2.7)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCACTTATATCATATGATGGAAGTAATAAAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGCAATCGTCCTATGCTTTTGATATCTGGG
GCCAAGGGACAATGGTCACCGTCTCCTCA

SEQ ID NO. 420 (encodes V_H domain 2.8)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGTCAGTTATATCATATGATGGAAGTAATAAAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAACTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGCAATCGTCCTATGCTTTTGAAATCTGGG
GCCAAGGTACAATGGTCACCGTCTCCTCA

SEQ ID NO. 421 (encodes V_H domain 2.9)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGTTGAGGACACGGCTGTGTATTACTGTGCGA
AAGATCCGGCCTGGGGATTACGTTTGGGGGAGCAATCGTCCTATGCTTTTGAAATCCGG
GGCCAGGGGACAACGGTCACCGTCTCTTCA

SEQ ID NO. 422 (encodes V_H domain 2.10)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATATATATCATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAAGAC

GCTGTCTCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCG
AAAGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCATATGATTTTGATATCTG
GGGCCAAGGGACAATGGTCACCGTCTCCTCA

SEQ ID NO. 423 (encodes V_H domain 2.11)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCCTCCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGACGAGAGTAATAAAT
ACTATGCACCCAGCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACCGTCTCCTCA

SEQ ID NO. 424 (encodes V_H domain 2.12)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATAAGAGTAATAAAT
ACTATGCAGACAAGGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 425 (encodes V_H domain 2.13)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCCTCCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATGCGAGTAATAAAT
ACTATGCAGACAACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 426 (encodes V_H domain 2.14)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCGTGCACTGGGTCCGCC
AGGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATGCGAGTAATAAA
TACTATGCAGACAACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGG
GGCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 427 (encodes V_H domain 2.15)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCCTCCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATAAGAGTAATAAAT
ACTATGCAGACAAGGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 428 (encodes V_H domain 2.16)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCGCGCACTGGGTCCGCC
AGGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATAAGAGTAATAAA
TACTATGCAGACAAGGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGG
GGCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 429 (encodes V_H domain 2.17)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATGCGAGTAATAAAT
ACTATGCAGACAACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 430 (encodes V_H domain 2.18)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCCAGCACTGGGTCCGCC
AGGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATGCGAGTAATAAA
TACTATGCAGACAACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGG
GGCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 431 (encodes V_H domain 2.19)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCTTCCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGATGCGAGTAATAAAT
ACTATGCAGACAACGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA

AGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 432 (encodes V_H domain 2.20)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAGCTTCAGTGGCTATGGCATGCACTGGGTCCGCC
AGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAATTATATCATATGATGGAAGTAATAG
ATACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACA
CGCTGTCTCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGC
GAAAGATCCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGAAATTT
GGGGCCAAGGGACAATGGTCACCGTCTCCTCA

SEQ ID NO. 433 (encodes V_H domain 2.21)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTCTCTACAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGATCCGGCCTGGGGATTACGTTTGGGGAAATTATCGTCCTATGATTTTGAAATCTGG
GGCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 434 (encodes V_H domain 2.22)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCACGCACTGGGTCCGCC
AGGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGACGGGAGTAATAA
ATACTATGCAGCCCCGGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACA
CGCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGC
GAAAGACGCGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCT
GGGGCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 435 (encodes V_H domain 2.23)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCACGCACTGGGTCCGCC
AGGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGACGAGAGTAATAAA
TACTATGCATCCAGCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGACCGGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGG
GGCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 436 (encodes V_H domain 2.24)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCATGCACTGGGTCCGCCA

GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGACGAGAGTAATAAAT
ACTATGCAAGGCTGGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGACACGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGGG
GCCAAGGGACAATGGTCACTGTCTCTTCA

SEQ ID NO. 437 (encodes V_H domain 2.25)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTGGCTATGGCCTCCACTGGGTCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCATATATATCATATGACCTGAGTAATAAAT
ACTATGCAAGGGGGGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGACGTGGCCTGGGGATTACGTTTGGGGGAGTCATCGTCCTATGATTTTGATATCTGG
GGCCAAGGGACAATGGTCACTGTCTCCTCA

In one aspect, the invention also relates to nucleic acid sequences comprising or consisting of SEQ ID NOs. 438 to 461 as shown below which encode V_H domains of family 3 comprising or consisting of SEQ ID NO. 184 to 276.

SEQ ID NO. 438 (encodes V_H domain 3.1)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATGACATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGATGAGGACACGGCTCTATATTACTGTGCGA
GAGATCGTATAGTGGGAGGTAGGGTCCCTGATGCTTTTGATATCTGGGGCCAAGGGAC
AATGGTCACCGTCTCTTCA

SEQ ID NO. 439 (encodes V_H domain 3.2)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATATCATATGATGGAAGTAATAAAT
ATTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAAAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAA
AGATCGTATAGTGGGAGCCAGGGTCCCTGATGCTTTTGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 440 (encodes V_H domain 3.3)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCCTCATTAGCTATGGCATGAACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATATCATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG

CTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATAGTGGGAGCTAGGGTCCCTGATGCTTTTGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 441 (encodes V_H domain 3.4)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGCGGTCCAGCCTGGGAGGTCCCTGAG
ACTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCC
AGGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAG
ATATTATGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACA
CGCTTTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCG
AAAGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGA
CAATGGTCACCGTCTCCTCA

SEQ ID NO. 442 (encodes V_H domain 3.5)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 443 (encodes V_H domain 3.6)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 444 (encodes V_H domain 3.7)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTCATCTGCAAATGGACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACTGTCTCTTCA

SEQ ID NO. 445 (encodes V_H domain 3.8)

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAGGGAACC
CTGGTCACTGTCTCCTCA

SEQ ID NO. 446 (encodes V_H domain 3.9)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATATCATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCTTCA

SEQ ID NO. 447 (encodes V_H domain 3.10)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTCATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACTGTCTCCTCA

SEQ ID NO. 448 (encodes V_H domain 3.11)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTCATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACTGTCTCCTCA

SEQ ID NO. 449 (encodes V_H domain 3.12)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA

AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 450 (encodes V_H domain 3.13)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACTGTCTCCTCA

SEQ ID NO. 451 (encodes V_H domain 3.14)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTCATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACTGTCTCCTCA

SEQ ID NO. 452 (encodes V_H domain 3.15)

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTCATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 453 (encodes V_H domain 3.16)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTCATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 454 (encodes V_H domain 3.17)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA

GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 455 (encodes V_H domain 3.18)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGACTGGGTGGCATTTATAACATATGATGGAAGTAATAGAT
ACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTTTATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCTGTATATTACTGTGCGAA
AGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACA
ATGGTCACCGTCTCCTCA

SEQ ID NO. 456 (encodes V_H domain 3.19)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATGACATATGATGGAAGTAATAGA
TACTATGCAGACGCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
GAGATCGTATAGTGGGAGGTAGGGTCCCTGATGCTTTTGATATCTGGGGCCAAGGGAC
AATGGTCACCGTCTCTTCA

SEQ ID NO. 457 (encodes V_H domain 3.20)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTCAGACATATGATGGCAGTAATAGA
TACTATGCAGACGCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
GAGATCGTATAGTGGGAGGTAGGGTCCCTGATGCTTTTGATATCTGGGGCCAAGGGAC
AATGGTCACCGTCTCTTCA

SEQ ID NO. 458 (encodes V_H domain 3.21)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTCAGACATATGATGGCAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
GAGATCGTATAGTGGGAGGTAGGGTCCCTGATGCTTTTGATATCTGGGGCCAAGGGAC
AATGGTCACCGTCTCTTCA

SEQ ID NO. 459 (encodes V_H domain 3.22)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTCAGACATATGATGCCAGTAATAGA
TACTATGCAGACGCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
GAGATCGTATAGTGGGAGGTAGGGTCCCTGATGCTTTTGATATCTGGGGCCAAGGGAC
AATGGTCACCGTCTCTTCA

SEQ ID NO. 460 (encodes V_H domain 3.23)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATAACATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTTTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTATATTACTGTGCGA
AAGATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGAC
AATGGTCACTGTCTCCTCA

SEQ ID NO. 461 (encodes V_H domain 3.24)

AGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGAC
TCTCCTGTGCAGCCTCTGGATTCCCCTTAATTAGCTATGGCATGAATTGGGTCCGCCAG
GCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATAACATATGATGGAAGTAATAGATA
CTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGC
TTTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTATATTACTGTGCGAAA
GATCGTATTGTGGGAGCTAGGGTCCCTGATGCTTATGATATCTGGGGCCAAGGGACAAT
GGTCACTGTCTCCTCA

SEQ ID NOs. 4462, 463, 464 and 465 as shown below encode V_H domains of family 4 comprising or consisting of SEQ ID NO. 280 to 292.

SEQ ID NO. 462 (encodes V_H domain 4.1)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCTTGAGA
CTCTCCTGTGTAGCCTCTGGATTCCCCTTCATTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCGGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTGTATTATTGTGCGA
AAGAGAGGATTTTTGGAGTGCTTACCCCTGATGATTTTGATATCTGGGGCCAAGGGACA
ACGGTCACCGTCTCCTCA

SEQ ID NO. 463 (encodes V_H domain 4.2)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTCATTAGCTATGGCATGCACTGGGTCCGCCA

GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGAGAGGATTTTTGGAGTGCTTACCCCTGATGATTTTGATATCTGGGGCCAAGGGACA
ACGGTCACTGTCTCCTCA

SEQ ID NO. 464 (encodes V_H domain 4.3)

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCCCCTTCATTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAGCTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTGTATTATTGTGCGA
AAGAGAGGATTTTTGGCGTGCTTACCCCTGATGATTTTGAAATCTGGGGCCAAGGGACA
ACGGTCACCGTCTCCTCA

SEQ ID NO. 465 (encodes V_H domain 4.4)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCACTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAGA
TACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGACCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGAGAGGATTTTTGGAGCGCTTACCCCTGATGATTTTGATATCTGGGGCCAAGGGACA
ACGGTCACCGTCTCTTCA

SEQ ID NOs. 466 or 467 as shown below encode V_H domains of family 5 comprising or consisting of SEQ ID NO. 296 and 300.

SEQ ID NO. 466 (encodes V_H domain 5.1)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAATAACTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAATTATATCATATGATGGAAATACTAAAT
ATTATACAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAATAGCCTGAGAGTTGAGGACACGGCTGTGTATTACTGTGCGAA
AGGTTTATGGCCTTCGGACGTCTGGGGCCAAGGGACCACGGTCACTGTCTCTTCA

SEQ ID NO. 467 (encodes V_H domain 5.2)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAATAACTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAATTATATCATATGATGGAAATAGTAAAT
ATTATACAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGCAAATGAATAGCCTGAGAGTTGAGGACACGGCTGTGTATTACTGTGCGAA
AGGTTTATGGCCTTCGGACGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA

SEQ ID NOs. 468 to 474 as shown below encode V_H domains of family 6 comprising or consisting of SEQ ID NO. 304 to 328.

SEQ ID NO. 468 (encodes V_H domain 6.1)

CAGGTGCAGCTACAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCCC
TCACCTGCACTGTCTCTGGTGGCTCCATCAGCAATAGTGGTTATTACTGGAGCTGGGTC
CGCCAGCACCCAGGGAAGGACCTGGAGTGGATTGGGTTCATCTATTACAATGGGAGCA
TCCACTACAACCCGTCCCTCAAGAGTCGAGTTATCATATCAGTAGACACGTCTAAGAAC
CAGTTCTCCCTGAAAATGAACTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTGC
GAGAGACGGGGATGACTACGGTGACTACTTGAGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

SEQ ID NO. 469 (encodes V_H domain 6.2)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCC
CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAATAGTGGTTATTACTGGAGCTGGAT
CCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTTCATCTATTACAATGGGAGC
ATCCACTACAACCCGTCCCTCAAGAGTCGAGTTATCATATCAGTAGACACGTCTAAGAA
CCAGTTCTCCCTGAAAATGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTG
CGAGAGACGGGGATGACTACGGTGACTACTTGAGGGGCCAGGGAACCCTGGTCACCG
TCTCCTCA

SEQ ID NO. 470 (encodes V_H domain 6.3)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCC
CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAATAGTGGTTATTACTGGAGCTGGGT
CCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTTCATCTATTACAATGGGAGC
ATCCACTACAACCCGTCCCTCAAGAGTCGAGTTATCATATCAGTAGACACGTCTAAGAA
CCAGTTCTCCCTGAAACTGAACTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTG
CGAGAGACGGGGATGACTACGGTGACTACTTGAGGGGCCAGGGAACCCTGGTCACCG
TCTCCTCA

SEQ ID NO. 471 (encodes V_H domain 6.4)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCC
CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAATAGTGGTTATTACTGGAGCTGGAT
CCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTTCATCTATTACAATGGGAGC
ATCCACTACAACCCGTCCCTCAAGAGTCGAGTTATCATATCAGTAGACACGTCTAAGAA
CCAGTTCTCCCTGAAACTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGT
GCGAGAGACGGGGATGACTACGGTGACTACTTGAGGGGCCAGGGAACCCTGGTCACC
GTCTCCTCA

SEQ ID NO. 472 (encodes V_H domain 6.5)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCC
CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAATAGTGGTTATTACTGGAGCTGGGT

CCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTTCATCTATTACAATGGGAGC
ATCCACTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAA
CCAGTTCTCCCTGAAAATGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTG
CGAGAGACGGGGATGACTACGGTGACTACTTGAGGGGCCAGGGAACCCTGGTCACCG
TCTCCTCA

SEQ ID NO. 473 (encodes V_H domain 6.6)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCC
CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAATAGTGGTTATTACTGGAGCTGGGT
CCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTTCATCTATTACAATGGGAGC
ATCCACTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAA
CCAGTTCTCCCTGAAACTGAACTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGTG
CGAGAGACGGGGATGACTACGGTGACTACTTGAGGGGCCAGGGAACCCTGGTCACCG
TCTCCTCA

SEQ ID NO. 474 (encodes V_H domain 6.7)

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCACAGACCCTGTCC
CTCACCTGCACTGTCTCTGGTGGCTCCATCAGCAATAGTGGTTATTACTGGAGCTGGGT
CCGCCAGCACCCAGGGAAGGGCCTGGAGTGGATTGGGTTCATCTATTACAATGGGAGC
ATCCACTACAACCCGTCCCTCAAGAGTCGAGTTACCATATCAGTAGACACGTCTAAGAA
CCAGTTCTCCCTGAAACTGAGCTCTGTGACTGCCGCGGACACGGCCGTGTATTACTGT
GCGAGAGACGGGGATGACTACGGTGACTACTTGAGGGGCCAGGGAACCCTGGTCACC
GTCTCCTCA

SEQ ID NOs. 475 to 482 as shown below w encode V_H domains of family 7 comprising or consisting of SEQ ID NO. 332 to 360.

SEQ ID NO. 475 (encodes V_H domain 7.1)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACGATGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGC
GAGAGATTCCCTTATAGTGGGAGAGAGGGGCTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCA

SEQ ID NO. 476 (encodes V_H domain 7.2)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACGATGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGC

GAGAGATAACCTTATAGTGGGAGAGAGGGGCTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCA

SEQ ID NO. 477 (encodes V_H domain 7.3)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACGGGGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGC
GAGAGATTCCCTTATAGTGGGAGAGAGGGGCTACT

SEQ ID NO. 478 (encodes V_H domain 7.4)

GGGGCCAGGGAACCCTGGTCACCGTCTCCTCA
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACCAGGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGC
GAGAGATTCCCTTATAGTGGGAGAGAGGGGCTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCA

SEQ ID NO. 479 (encodes V_H domain 7.5)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACCCCGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGC
GAGAGATTCCCTTATAGTGGGAGAGAGGGGCTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCA

SEQ ID NO. 480 (encodes V_H domain 7.6)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACGAGGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGC
GAGAGATTCCCTTATAGTGGGAGAGAGGGGCTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCA

SEQ ID NO. 481 (encodes V_H domain 7.7)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA

GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACATCGGAAGTGAGAAA
TACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTC
ACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGCG
AGAGATTCCCTTATAGTGGGAGAGAGGGGCTACTGGGGCCAGGGAACCCTGGTCACC
GTCTCCTCA

SEQ ID NO. 482 (encodes V_H domain 7.8)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTAGCTATTGGATGTACTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAATCACGATGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGCGC
GAGAGATACCCTTATAGTGGGAGAGAGGGGCTACTGGGGCCAGGGAACCCTGGTCAC
CGTCTCCTCA

SEQ ID NO. 483 as shown below encodes a V_H domain of family 8 comprising or consisting of SEQ ID NO. 364.

SEQ ID NO. 483

CAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCC
CTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTTACTACTGGAGCTGGATCCGCCA
GCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGAAATCAATCATAGTGGAAGCACCAAC
TACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAACCAGTT
CTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGCGAGA
GGCCCCATACCAGCCACTGCTATACCCGATGCTTTTGATATCTGGGGCCAAGGGACAAT
GGTCACTGTCTCCTCA

SEQ ID NO. 484 as shown below encodes a V_H domain of family 9 comprising or consisting of SEQ ID NO. 368.

SEQ ID NO. 484

GAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGGAGACCCTGTCC
CTCACCTGCGCTGTCTATGGTGGGTCCTTCAGTGGTCACTACTGGAGCTGGATCCGCC
AGCCCCCAGGGAAGGGGCTGGAGTGGATTGGGGACATAAATCATAGTGGAAGCACCAA
CTACAACCCGTCCCTCAAGAGTCGAGTCACCATATCAGTAGACACGTCCAAGAATCAGT
TCTCCCTGAAGCTGAGCTCTGTGACCGCCGCGGACACGGCTGTGTATTACTGTGTGAG
AGACTACGGTGACTCCCGTAGCCTTTTTGACTACTGGGGCCAGGGAACCCTGGTCACC
GTCTCTTCA

SEQ ID NO. 485 as shown below encodes a V_H domain of family 10 comprising or consisting of SEQ ID NO. 372.

SEQ ID NO. 485

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGGCATGCACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATGTCATATGATGGCAGTAATAAA
TACTATGTAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGA
AAGGCGATTACGATTTTTGGAGTGGTTACCCCGACTACGATATGGACGTCTGGGGCCAA
GGGACCACGGTCACCGTCTCCTCA

SEQ ID NO. 486 as shown below encode a V_H domain of family 11 comprising or consisting of SEQ ID NO. 376.

SEQ ID NO. 486

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCAAGCCTGGAGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCAACTTGATTAGCTATGGCATGTACTGGGTCCGCCA
GGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAA
AACTATGCAGACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAATAC
GCTGTTTCTGCAAATGAACAGCCTGAGAGTTGAGGACACGGCTGTGTATTACTGTGCGA
AAGGGGGGAATGCCTTGTATAGCAGTGGCTGGCCCGATGATGGTTTTGATATCAGGGG
CCAAGGGACAATGGTCACTGTCTCCTCA

SEQ ID NO. 487 as shown below encode a V_H domain of family 12 comprising or consisting of SEQ ID NO. 380.

SEQ ID NO. 487

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAACTTTGGCATGCACTGGGCCCGCCA
GGCTCCAGGCAAGGGACTGGAGTGGGTGGCAGTAATATCATATGATGGAAATAGTAAAT
ACTATGCAGACACCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACG
CTGTATCTGGAAATGAACAGCCTGAGAGCTGATGACACGGCTGTGTATTACTGTGCGAA
AGGCCTATGGCCCCCAATGGACGTCAGGGGCCAAGGGACCACGGTCACCGTCTCCTC
A

SEQ ID NO. 488 as shown below encode a V_H domain of family 13 comprising or consisting of SEQ ID NO. 384.

SEQ ID NO. 488

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTCGGTCCAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTGACTATTGGATGACCTGGGTCCGCCA
GGTTCCAGGGAAGGGGCTGGAGTGGGTGGCCAACATAAAGCAAGATGGAAGTGAGAA
ATACTATGTGGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACT
CACTATATCTGCAAATGAATAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCG
AGAGATCGAGGAGGAGCAGTGGCCCTTTATCACAACGGTATGGACATGGGGGGCCAAG
GGACCACGGTCACTGTCTCTTCA

SEQ ID NO. 4899 as shown below encode a V_H domain of family 14 comprising or consisting of SEQ ID NO. 388.

SEQ ID NO. 489

GAAGTGCAGCTGGTGGAGTCTGGGGGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGGCTTCTGGATACACCTTCACCAGTTATGATATCAACTGGGTGCGACAGG
CCACTGGACAAGGGCTTGAGTGGATGGGATGGATGAACCCTAACAGTGGTAACACAGG
CTATGCACAGAAGTTCCAGGGCAGAGTCACCATGACCAGGAACACCTCCATAAGCACA
GCCTACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGA
GAGGGAACGGGCCCGGTATAACTGGAACTACTGACTACTGGGGCCAGGGAACCCTGG
TCACTGTCTCTTCA

SEQ ID NO. 490 as shown below encodes a V_H domain of family 15 comprising or consisting of SEQ ID NO. 392.

SEQ ID NO. 490

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTCACCTTTGATGATTATGGCATGAGCTGGGTCCGCCA
AGCTCCAGGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGATCGTACC
GGTTATGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTC
CCTGTATCTGCAAATGAACAGTCTGAGAGCCGAGGACACGGCCTTGTATTACTGTGGGA
GAGAGAATGTTATAGTACCAGCTGCTACCTACTGGGGCCAGGGAACCCTGGTCACCGT
CTCCTCA

A nucleic acid according to the present invention may comprise DNA or RNA and may be wholly or partially synthetic or recombinantly produced. Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses a RNA molecule with the specified sequence in which U is substituted for T, unless context requires otherwise.

The nucleic acid may be in the form of a construct, for example a plasmid, vector, transcription or expression cassette.

The invention also relates to an isolated recombinant host cell comprising one or more nucleic acid construct as described above. The host cell may be a bacterial, viral, mammalian or other suitable host cell. In one embodiment, the cell is an E. coli cell. In another embodiment, the cell is a yeast cell. In another embodiment, the cell is a Chinese Hamster Ovary (CHO) cell.

Methods for preparing or generating the polypeptides, nucleic acids, host cells, products and compositions described herein using *in vitro* expression libraries can comprise the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding amino acid sequences; and

b) screening said set, collection or library for amino acid sequences that can bind to / have affinity for PSMA and

c) isolating the amino acid sequence(s) that can bind to / have affinity for PSMA.

In the above methods, the set, collection or library of amino acid sequences may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) amino acid sequences will be clear to the person skilled in the art (see for example Phage Display of Peptides and Proteins: A Laboratory Manual, Academic Press; 1st edition (October 28, 1996) Brian K. Kay, Jill Winter, John McCafferty).

A single V_H domain antibody used in the molecules of the invention described herein can be expressed in a transgenic rodent. The transgenic rodent, for example a mouse, may have a reduced capacity to express endogenous antibody genes. Thus, in one embodiment, the rodent has a reduced capacity to express endogenous light and/or heavy chain antibody genes. The rodent may therefore comprise modifications to disrupt expression of endogenous light and/or heavy chain antibody genes so that no functional light and/or heavy chains are produced.

Human heavy chain only antibodies capable of binding human PSMA can be produced by a method comprising

a) immunising a transgenic rodent with an PSMA antigen wherein said rodent expresses a nucleic acid construct comprising unrearranged human heavy chain V genes and is not capable of making functional endogenous light or heavy chains,

b) isolating human heavy chain only antibodies.

V_H domains can be produced by a method comprising

a) immunising a transgenic mouse with an PSMA antigen wherein said mouse expresses a nucleic acid construct comprising human heavy chain V genes and is not capable of making functional endogenous light or heavy chains,

b) generating a library of sequences comprising V_H domain sequences from said mouse and

c) isolating sequences comprising V_H domain sequences from said libraries.

Further steps may include identifying a single V_H domain antibody or heavy chain only antibody that binds to human PSMA, for example by using functional assays as shown in the examples.

In one embodiment, the rodent is a mouse. The mouse may comprise a non-functional endogenous lambda light chain locus. Thus, the mouse does not make a functional endogenous lambda light chain. In one embodiment, the lambda light chain locus is deleted in part or completely or rendered non-functional through insertion, inversion, a recombination event, gene editing or gene silencing. For example, at least the constant region genes C1, C2 and C3 may be deleted or rendered non-functional through insertion or other modification as described above. In one embodiment, the locus is functionally silenced so that the mouse does not make a functional lambda light chain.

Furthermore, the mouse may comprise a non-functional endogenous kappa light chain locus. Thus, the mouse does not make a functional endogenous kappa light chain. In one embodiment, the kappa light chain locus is deleted in part or completely or rendered non-functional through insertion, inversion, a recombination event, gene editing or gene silencing. In one embodiment, the locus is functionally silenced so that the mouse does not make a functional kappa light chain.

The mouse having functionally-silenced endogenous lambda and kappa L-chain loci may, for example, be made as disclosed in WO 2003/000737, which is hereby incorporated by reference in its entirety.

Furthermore, the mouse may comprise a non-functional endogenous heavy chain locus. Thus, the mouse does not make a functional endogenous heavy chain. In one embodiment, the heavy chain locus is deleted in part or completely or rendered non-functional through insertion, inversion, a recombination event, gene editing or gene silencing. In one embodiment, the locus is functionally silenced so that the mouse does not make a functional heavy chain.

For example, as described in WO 2004/076618 (hereby incorporated by reference in its entirety), all 8 endogenous heavy chain constant region immunoglobulin genes (μ, δ, γ3, γ1, γ2a, γ2b, ε and α) are absent in the mouse, or partially absent to the extent that they are non-functional, or genes δ, γ3, γ1, γ2a, γ2b and ε are absent and the flanking genes μ and α are partially absent to the extent that they are rendered non-functional, or genes μ, δ, γ3, γ1, γ2a,

γ2b and ε are absent and α is partially absent to the extent that it is rendered non-functional, or δ, γ3, γ1, γ2a, γ2b, ε and α are absent and μ is partially absent to the extent that it is rendered non-functional. By deletion in part is meant that the endogenous locus gene sequence has been deleted or disrupted, for example by an insertion, to the extent that no functional endogenous gene product is encoded by the locus, *i.e.*, that no functional product is expressed from the locus. In another embodiment, the locus is functionally silenced.

In one embodiment, the mouse comprises a non-functional endogenous heavy chain locus, a non-functional endogenous lambda light chain locus and a non-functional endogenous kappa light chain locus. The mouse therefore does not produce any functional endogenous light or heavy chains. Thus, the mouse is a triple knockout (TKO) mouse.

The transgenic mouse may comprise a vector, for example a Yeast Artificial Chromosome (YAC) for expressing a heterologous heavy chain locus. YACs are vectors that can be employed for the cloning of very large DNA inserts in yeast. As well as comprising all three cis-acting structural elements essential for behaving like natural yeast chromosomes (an autonomously replicating sequence (ARS), a centromere (CEN) and two telomeres (TEL)), their capacity to accept large DNA inserts enables them to reach the minimum size (150 kb) required for chromosome-like stability and for fidelity of transmission in yeast cells. The construction and use of YACs is well known in the art (*e.g.*, Bruschi, C.V. and Gjuracic, K. Yeast Artificial Chromosomes, Encyclopedia of Life Sciences, 2002 Macmillan Publishers Ltd, Nature Publishing Group / www.els.net).

For example, the YAC may comprise a plethora of human V_H, D and J genes in combination with mouse immunoglobulin constant region genes lacking C_H1 domains, mouse enhancer and regulatory regions.

Alternative methods known in the art may be used for deletion or inactivation of endogenous mouse or rat immunoglobulin genes and introduction of human V_H, D and J genes in combination with mouse immunoglobulin constant region genes lacking C_H1 domains, mouse enhancer and regulatory regions.

Transgenic mice can be created according to standard techniques as illustrated in the examples. The two most characterised routes for creating transgenic mice are via pronuclear microinjection of genetic material into freshly fertilised oocytes or via the introduction of stably transfected embryonic stem cells into morula or blastocyst stage embryos. Regardless of how the genetic material is introduced, the manipulated embryos are

transferred to pseudo-pregnant female recipients where pregnancy continues and candidate transgenic pups are born.

The main differences between these broad methods are that ES clones can be screened extensively before their use to create a transgenic animal. In contrast, pronuclear microinjection relies on the genetic material integrating to the host genome after its introduction and, generally speaking, the successful incorporation of the transgene cannot be confirmed until after pups are born.

There are many methods known in the art to both assist with and determine whether successful integration of transgenes occurs. Transgenic animals can be generated by multiple means including random integration of the construct into the genome, site-specific integration, or homologous recombination. There are various tools and techniques that can be used to both drive and select for transgene integration and subsequent modification including the use of drug resistance markers (positive selection), recombinases, recombination-mediated cassette exchange, negative selection techniques, and nucleases to improve the efficiency of recombination. Most of these methods are commonly used in the modification of ES cells. However, some of the techniques may have utility for enhancing transgenesis mediated via pronuclear injection.

Further refinements can be used to give more efficient generation of the transgenic line within the desired background. As described above, in preferred embodiments, the endogenous mouse immunoglobulin expression is silenced to permit sole use of the introduced transgene for the expression of the heavy-chain only repertoire that can be exploited for drug discovery. Genetically-manipulated mice, for example TKO mice that are silenced for all endogenous immunoglobulin loci (mouse heavy chain, mouse kappa chain and mouse lambda chain) can be used as described above. The transfer of any introduced transgene to this TKO background can be achieved via breeding, (either conventional or with the inclusion of an IVF step to give efficient scaling of the process). However, it is also possible to include the TKO background during the transgenesis procedure. For example, for microinjection, the oocytes may be derived from TKO donors. Similarly, ES cells from TKO embryos can be derived for use in transgenesis. Triple knock-out mice into which transgenes have been introduced are referred to herein as TKO/Tg. In one embodiment, the mouse is as described in WO2016/062990.

In another aspect of the present invention, there is provided a pharmaceutical composition comprising an immunoconjugate according to the present invention and optionally a

pharmaceutically accepTable carrier. The immunoconjugate of the present invention or compositions can be administered by any convenient route. The compounds may be administered by any route, including oral and parenteral administration. Parenteral administration includes, for example, intravenous, intramuscular, intraarterial, intraperitoneal, intranasal, rectal, intravesical, intradermal, topical or subcutaneous administration. Compositions can take the form of one or more dosage units.

The composition of the invention can be in the form of a liquid, *e.g.*, a solution, emulsion or suspension. The liquid can be useful for delivery by injection, infusion (*e.g.*, IV infusion) or sub-cutaneously. The liquid compositions of the invention, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides, polyethylene glycols, glycerin, or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; and agents for the adjustment of tonicity such as sodium chloride or dextrose. A composition can be enclosed in an ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material.

In specific embodiments, it can be desirable to administer an immunoconjugate of the present invention or compositions locally to the area in need of treatment, or by intravenous injection or infusion.

The amount of the immunoconjugate of the present invention that is effective/active in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

The compositions of the invention comprise an effective amount of an immunoconjugate of the present invention such that a suitable dosage will be obtained. The correct dosage of the compounds will vary according to the particular formulation, the mode of application, and its particular site, host and the disease being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

Typically, this amount is at least about 0.01% of an immunoconjugate of the present invention by weight of the composition.

Preferred compositions of the present invention are prepared so that a parenteral dosage unit contains from about 0.01 % to about 2% by weight of the immunoconjugate of the present invention.

For intravenous administration, the composition can comprise from about typically about 0.1 mg/kg to about 250 mg/kg of the animal's body weight, preferably, between about 0.1 mg/kg and about 20 mg/kg of the animal's body weight, and more preferably about 1 mg/kg to about 10 mg/kg of the animal's body weight.

The present compositions can take the form of suitable carriers, such aerosols, sprays, suspensions, or any other form suitable for use. Other examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

The pharmaceutical compositions can be prepared using methodology well known in the pharmaceutical art. For example, a composition intended to be administered by injection can be prepared by combining an immunoconjugate of the present invention with water so as to form a solution. A surfactant can be added to facilitate the formation of a homogeneous solution or suspension.

The invention furthermore relates to a method for the prevention and/or treatment of cancer, in particular prostate cancer, comprising administering an immunoconjugate of the invention to a patient, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoconjugate and/or of a pharmaceutical composition of the invention. In particular, the invention relates to a method for the prevention and/or treatment of cancer, in particular prostate cancer, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an immunoconjugate or a pharmaceutical composition of the invention.

The invention also relates to an immunoconjugate of the invention for use in the treatment of disease. The invention also relates to an immunoconjugate of the invention for use in the treatment of cancer, in particular prostate cancer or a prostatic disorder. "Prostate cancer" refers to all stages and all forms of cancer arising from the tissue of the prostate gland. The invention also relates to the treatment of a disease characterized by aberrant expression of PSMA.

In another aspect, the invention relates to the use of an immunoconjugate of the invention in the treatment of disease. In another aspect, the invention relates to the use of an immunoconjugate of the invention in the manufacture of a medicament for the treatment of cancer, in particular prostate cancer or a prostatic disorder.

The immunoconjugates of the invention are also useful for the treatment, prevention, or amelioration of cancer, in particular prostate cancer or a prostatic disorder. A prostatic disorder refers to any disease that afflicts the prostate gland in the male reproductive system. The prostate gland is dependent on the hormonal secretions of the testes. Expression of PSMA has been detected in other cancers, more specifically in the neovasculature associated with these cancers. A wide range of carcinomas, including conventional (clear cell) renal cell, transitional cell of the bladder, testicular-embryonal, neuroendocrine, colon, and breast, and the different types of malignancies were found consistently and strongly to express PSMA in their neovasculature.

The immunoconjugate of the invention may be administered as the sole active ingredient or in combination with one or more other therapeutic and/or cytotoxic moiety. In one embodiment, the immunoconjugate may be conjugated to a toxic moiety.

In therapies of prostatic disorders, *e.g.*, prostate cancer, the anti-PSMA immunoconjugate can be used in combination with existing therapies. In one embodiment, the single domain antibody is used in combination with an existing therapy or therapeutic agent, for example an anti-cancer therapy. Thus, in another aspect, the invention also relates to a combination therapy comprising administration of a single domain antibody or pharmaceutical composition of the invention and an anti-cancer therapy. The anti-cancer therapy may include a therapeutic agent or radiation therapy and includes gene therapy, viral therapy, RNA therapy bone marrow transplantation, nanotherapy, targeted anti-cancer therapies or oncolytic drugs. Examples of other therapeutic agents include other checkpoint inhibitors, antineoplastic agents, immunogenic agents, attenuated cancerous cells, tumor antigens, antigen presenting cells such as dendritic cells pulsed with tumor-derived antigen or nucleic acids, immune stimulating cytokines (e.g., IL-2, IFNa2, GM-CSF), targeted small molecules and biological molecules (such as components of signal transduction pathways, e.g. modulators of tyrosine kinases and inhibitors of receptor tyrosine kinases, and agents that bind to tumor- specific antigens, including EGFR antagonists), an anti-inflammatory agent, a cytotoxic agent, a radiotoxic agent, or an immunosuppressive agent and cells transfected with a gene encoding an immune stimulating cytokine (e.g., GM-CSF), chemotherapy. In one embodiment, the single domain antibody is used in combination with surgery. The

immunoconjugate of the invention may be administered at the same time or at a different time as the other therapy, *e.g.*, simultaneously, separately or sequentially.

In another aspect, the invention provides a kit for detecting prostate cancer for diagnosis, treatment, prognosis or monitoring comprising an immunoconjugate of the invention. The kit may also comprise instructions for use. The kits may include a labeled immunoconjugate of the invention as described above and one or more compounds for detecting the label. The invention in another aspect provides an immunoconjugate of the invention packaged in lyophilized form, or packaged in an aqueous medium.

The invention also relates to detection methods using the immunoconjugate of the invention. Given their ability to bind to human PSMA, the human-PSMA-immunoconjugates, disclosed herein can be used to detect PSMA (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. In particular, the invention also relates to *in vitro* or *in vivo* methods for diagnosing or monitoring progression of a cancer, in particular prostate cancer. *In vitro* methods comprise detecting the presence of a PSMA protein in a test sample and comparing this with control sample from a normal subject or with a standard value or standard value range for a normal subject. The sample may be selected from blood, plasma, serum, semen, urine or a tissue biopsy.

The method may include: (a) contacting the sample (and optionally, a reference, *e.g.*, a positive and/ or negative control sample) with an immunoconjugate of the invention and (b) detecting either the immunoconjugate bound to PSMA or unbound immunoconjugate in the sample, to thereby detect PSMA in the biological sample. The immunoconjugate can be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

The invention also relates to an assay for assessing the immunoconjugate of the invention by labelling said immunoconjugate. Such an assay is described in the examples.

In vivo methods may comprise detecting the presence of PSMA *in vivo*, for example by imaging in a subject. In this method, an immunoconjugate of the invention is labeled to detect binding.

As an alternative to labeling the immunoconjugate of the invention, human PSMA can be assayed in biological fluids by a competition immunoassay utilizing PSMA standards labeled with a detectable substance and an unlabeled immunoconjugate. In this assay, the biological sample, the labeled PSMA standards and the immunoconjugate are combined and the amount of labeled PSMA standard bound to the unlabeled immunoconjugate is determined. The amount of human PSMA in the biological sample is inversely proportional to the amount of labeled PSMA standard bound to the immunoconjugate. Similarly, human PSMA can also be assayed in biological fluids by a competition immunoassay utilizing PSMA standards labeled with a detectable substance and an unlabeled immunoconjugate.

Immunoconjugates disclosed herein can be used to inhibit PSMA activity, *e.g.*, in a cell culture containing PSMA, in human subjects or in other mammalian subjects having PSMA with which an immunoconjugate disclosed herein cross-reacts. In one embodiment, a method for inhibiting or increasing PSMA activity is provided comprising contacting PSMA with an immunoconjugate disclosed herein such that PSMA activity is inhibited or increased. For example, in a cell culture containing, or suspected of containing PSMA, an immunoconjugate disclosed herein can be added to the culture medium to inhibit PSMA activity in the culture.

Therefore, in one embodiment, the invention also relates to a method of ablating or killing a cell that expresses PSMA, *e.g.*, a cancerous or non-cancerous prostatic cell. Methods of the invention include contacting the cell, an immunoconjugate of the invention, in an amount sufficient to ablate or kill, the cell. The methods can be used on cells in culture, *e.g.*, *in vitro* or *ex vivo*.

Unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. While the foregoing disclosure provides a general description of the subject matter encompassed within the scope of the present invention, including methods, as well as the best mode thereof, of making and using this invention, the following examples are provided to further enable those skilled in the art to practice this invention and to provide a complete written description thereof. However, those skilled in the art will appreciate that the specifics of these examples should not be read as limiting on the invention, the scope of which should be apprehended from the claims and equivalents thereof appended to this disclosure. Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

All documents mentioned in this specification are incorporated herein by reference in their entirety, including references to gene accession numbers.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

The invention is further described in the non-limiting examples.

EXAMPLES

EXAMPLE 1. Construction of Tg/TKO mice

Mice carrying a heavy-chain antibody transgenic locus in germline configuration within a background that is silenced for endogenous heavy and light chain antibody expression (triple knock-out, or TKO) were created as previously described (WO2004/076618 and WO2003/000737, Ren *et al.*, Genomics, 84, 686, 2004; Zou *et al.*, J. Immunol., 170, 1354, 2003). Briefly, transgenic mice were derived following pronuclear microinjection of freshly fertilised oocytes with a yeast artificial chromosome (YAC) comprising a plethora of human V_H, D and J genes in combination with mouse immunoglobulin constant region genes lacking CH1 domains, mouse enhancer and regulatory regions. Yeast artificial chromosomes (YACs) are vectors that can be employed for the cloning of very large DNA inserts in yeast. As well as comprising all three cis-acting structural elements essential for behaving like natural yeast chromosomes (an autonomously replicating sequence (ARS), a centromere (CEN) and two telomeres (TEL)), their capacity to accept large DNA inserts enables them to reach the minimum size (150 kb) required for chromosome-like stability and for fidelity of transmission in yeast cells. The construction and use of YACs is well known in the art (*e.g.*, Bruschi, C.V. and Gjuracic, K. Yeast Artificial Chromosomes, Encyclopedia of Life Sciences, 2002, Macmillan Publishers Ltd., Nature Publishing Group / www.els.net).

The transgenic founder mice were back crossed with animals that lacked endogenous immunoglobulin expression to create the Tg/TKO lines used in the immunisation studies described.

EXAMPLE 2. Antigen for immunisation

The immunisations used recombinant purified protein or Human Cell Line LNCap. Recombinant human PMSA was purchased from R&D, (cat. no. 4234-ZN), while the LNCap cells were from Sigma Aldrich (cat. no. 89110211-1VL).

EXAMPLE 3. Immunisation Protocol

Briefly, Tg/TKO mice aged 8 – 12 weeks of age each received a total of 50 µg of recombinant purified human PSMA protein, emulsified in Complete Freund's Adjuvant and delivered subcutaneously, or 10 million LNCap cells in PBS delivered intraperitoneally, followed by boosts of 1 – 10µg of the recombinant protein, emulsified in Incomplete Freund's Adjuvant, also administered subcutaneously, given at various intervals following the initial priming. A final dose of the recombinant purified human PSMA protein antigen was administered intraperitoneally, in phosphate buffered saline, in the absence of adjuvant.

Alternative immunisation routes and procedures can also be employed. For example, different adjuvants or immune potentiating procedures may be used instead of Freund's adjuvant. DNA immunisations are often delivered intramuscularly or via a Genegun. Transfected cells or membrane preparations from such cells are often, although not exclusively, administered intraperitoneally.

EXAMPLE 4. Serum ELISA

During and following immunisation, serum was collected from mice and checked for the presence of heavy-chain antibody responses to the immunogen by ELISA. Nunc Maxisorp plates (Nunc cat. no. 443404) were coated overnight at 4°C with 50µl/well of a 1µg recombinant antigen/ml of PBS solution. Following decanting of the antigen solution, plates were washed using PBS (prepared from PBS Tablets, Oxoid cat. no. BR0014G) supplemented with 0.05% (v/v) Tween® 20 (Sigma P1379), followed by washes with PBS without added Tween 20. To block non-specific protein interactions, a solution of 3% (w/v) skimmed milk powder (Marvel®) in PBS was added to the wells and the plate was incubated for at least one hour at room temperature. Dilutions of serum in 3% Marvel™/PBS were prepared in polypropylene tubes or plates and incubated for at least one hour at room temperature prior to transfer to the blocked ELISA plate where a further incubation of at least one hour took place. Unbound protein was then washed away using repetitive washes with PBS/Tween 20 followed by PBS. A solution of biotin-conjugated, goat anti-mouse IgG, Fcgamma subclass 1 specific antibody (Jackson cat. no.115-065-205), prepared in PBS/3% Marvel was then added to each well and a further incubation at room temperature for at least one hour took place. Unbound detection antibody was removed by repeated washing using PBS/Tween 20 and PBS. Neutravidin-HRP solution (Pierce cat. no. 31030) in 3%

Marvel/PBS was then added to the ELISA plates and allowed to bind for at least 30 minutes. Following further washing, the ELISA was developed using TMB substrate (Sigma cat. no. T0440) and the reaction was stopped after 10 minutes by the addition of 0.5M H_2SO_4 solution (Sigma cat. no. 320501). Absorbances were determined by reading at an optical density of 450nm. Alternative assays, such as ELISPOT assays, may also be used to check for immunisation induced heavy-chain antibody responses.

EXAMPLE 5. Generation of Libraries from Immunised Mice

a) processing tissues, RNA extraction and cDNA manufacture

Spleen, inguinal and brachial lymph nodes were collected into RNAlate® from each immunised animal. For each animal, 1/2 of the spleen and 4 lymph nodes were processed separately. Initially, the tissues were homogenised; following transfer of tissues to Lysing matrix D bead tubes (MP Bio. Cat. no. 116983001), 600µl of RLT buffer containing β-mercaptoethanol (from Qiagen RNeas® kit cat. no. 74104) was added before homogenisation in a MP Bio Fastprep96 homogeniser (cat# 116010500) at 1600rpm for 60 seconds. The tubes containing the homogenised tissues were transferred to ice and debris was pelleted by centrifugation at 1200rpm for 5 minutes. A 400µl sample of the supernatant was removed and used for RT-PCR.

Initially, RNA was extracted using Qiagen RNeasy® kit (cat. no. 74104) following the manufacturer's protocol. Each RNA sample was then used to make cDNA using Superscript III RT-PCR high-fidelity kit (Invitrogen cat. no. 12574-035). For each spleen and lymphnodes RNA sample, 5 RT-PCR reactions were performed, each with V_H_J/F (long) primer in combination with a primer for V_H1, V_H2, V_H3, V_H4 or V_H6 family. Details of the primers are below.

Table 16. Primers for V10

V1a/pelB(long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTBCAGCTGGTGCAGTCTGGGGCTGAGG SEQ ID No. 491
V2/pelB(long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGATCACCTTGAAGGAGTCTGG SEQ ID No. 492
V3/pelB(long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**SAGGTGCAGCTGGTGGAGTCTGGGGGAGG SEQ ID No. 493
V4-4/pelB(long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTGCAGCTGCAGGAGTCGGG SEQ ID No. 494
V6/pelB(l	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTACA

ong)	GCTGCAGCAGTCAGG SEQ ID No. 495
VH_J/F(long)	**CCGTGGTGATGGTGGTGATGGCTACCGCCACCCTCGAG**TGARGAGACRGTGACC SEQ ID No. 496

Residues in **bold** have homology with pUCG3

Table 17 Primers for V23

VH1-2 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGG SEQ ID No. 497
VH1-3 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTCCAGCTCGTGCAGTCTGGGGCTGAGG SEQ ID No. 498
VH1-18 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTTCAGCTGGTGCAGTCTGGAGCTGAGG SEQ ID No. 499
VH1-24 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTCCAGCTGGTACAGTCTGGGGCTGAGG SEQ ID No. 500
VH2 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGRTCACCTTGAAGGAGTCTGG SEQ ID No. 501
VH3-7 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**GAGGTGCAGCTGGTGGAGTCTGGGGGAGG SEQ ID No. 502
VH3-9 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**GAAGTGCAGCTGGTGGAGTCTGGGGGAGG SEQ ID No. 503
VH3-11 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTGCAGCTGGTGGAGTCTGGGGGAGG SEQ ID No. 504
VH3-23 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**GAGGTGCAGCTGTTGGAGTCTGGGGGAGG SEQ ID No. 505
VH3-23 (long)	GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCCGAGGTGCAGCTGTTGGAGTCTGGGGGAGG SEQ ID No. 506
VH4-4 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTGCAGCTGCAGGAGTCGGG SEQ ID No. 507
VH4-34 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTGCAGCTACAGCAGTGGGGC SEQ ID No. 508
VH6-1 (long)	**GCCGCTGGATTGTTATTACTCGCGGCCCAGCCGGCCATGGCC**CAGGTACAGCTGCAGCAGTCAGG SEQ ID No. 509
VH_J/F(long)	**CCGTGGTGATGGTGGTGATGGCTACCGCCACCCTCGAG**TGARGAGACRGTGACC SEQ ID No. 510

Residues in **bold** have homology with pUCG3

The code for the choice of nucleotide for degenerate primer is shown below.

R A, G

Y	C, T
M	A, C
K	G, T
S	C, G
W	A, T
B	C, G, T
V	A, C, G
D	A, G, T
N	A, C, G, T

Mastermixes were prepared for the RT-PCR reactions, based on the following tube reaction components:

12.5µl 2x reaction mix
0.5µl forward primer (10µM)
0.5µl reverse primer (10µM)
0.5µl enzyme mix
500ng - 1µg RNA
Up to 25µl with water

The RT-PCR reactions were carried out in a thermal cycler using the following conditions:

55^{0}C 20min
94^{0}C 2min
35 cycles of 94^{0}C 15sec
58^{0}C 30sec
68^{0}C 30sec
68^{0}C 5min
Hold at 4^{0}C

Products in the range of 370bp were confirmed by gel electrophoresis.

For each mouse, the V_H products amplified for a given family from the 1/2 spleen and each of the 4 lymph nodes were then pooled for purification using Thermo/Fermentas GeneJet PCR purification kit (cat. no. K0702) which was used according to the manufacturer's instructions, with the products eluted in 50µl of water.

a) **Cloning into phagemid vector**

The phagemid vector, pUCG3, was employed in these studies. A PCR-based method was used to construct the V_H phagemid libraries from the amplified V_H sequences. The following procedure was used:

A linearised version of pUCG3 was created using PCR; with the following primers:

pUCG3-pHENAPmut4 GGCCATGGCCGGCTGGGCCGCGAG SEQ ID No. 511

pUCG3-pHENAPmut5mycHis

TCATCGAGGGTGGCGAGCGAACAAAAACTCATCTCAGAAGAATCTGAATCATCACACAT CACACGGGAGCTAGACTGTTGAAAGTTGTTTAGCAAAACC SEQ ID No. 512

Phusion High fidelity PCR master mix with GC buffer (cat. no. F532L, NEB) was used for the PCR reactions which comprised the following reagents:

Phusion GC 2x mix	25µl
pUCG3	5-10ng
Primers (10 µM)	1.25µl of each
DMSO	1.5µl
Nuclease-free H_2O	to final volume of 50µl

The cycling conditions used were:

98°C 30 seconds

10 cycles of

- 98°C 10 seconds
- 58°C 20 seconds
- 68°C 2 minutes, 30 seconds

20 cycles of

- 98°C 10 seconds
- 58°C 20 seconds
- 68°C 3 minutes

68°C 5 minutes

4°C hold

The PCR product (3152bp) was gel purified using Fermentas GeneJet Gel purification kit (cat. no. K0691), according to the manufacturer's instructions, with final elution in 40µl of elution buffer.

The purified V_H RT-PCR products were employed as megaprimers with the linearised pUCG3 to give phagemid products for transformation and library creation, based on the following reactions;

Phusion GC 2x mix	25µl
Linearised pUCG3	800ng
V_H PCR product	200ng
DMSO	1.5µl
Nuclease-free H_2O	to 50µl final volume

PCR was performed as follows:

98°C 30sec

98°C 10sec
58°C 20sec } 10 cycles
72°C 2min
72°C 5min
Hold at 10°C

The products of PCR were analysed on a 1% (w/v) agarose gel.

The various family V_H/phagemid products were purified using Fermentas PCR purification kit (cat. no. K0702) according to the manufacturer's instructions with the final elution being in 25µl H_2O; this eluate was used for transformations of TG1 *E. coli* (Lucigen, cat. no. 60502-2) by electroporation using BioRad 2 x 0.2cm cuvettes (BioRad cat. no. 165-2086) in a Bio-Rad GenePulser Xcell and pre-warmed recovery medium (Lucigen, proprietary mix). 22µl of the purified products were added to 160µl of cells for the electroporation, with 2 electroporations being performed for each V_H/phagemid product at 2000v. Electroporated cells were pooled and recovered in 50ml Falcon tubes incubated for 1 hour at 37°C with shaking at 150rpm. A 10-fold dilution series of an aliquot of the transformations was performed and plated in petri dishes containing 2xTY agar supplemented with 2% (w/v) glucose and 100µg/ml ampicillin. Resulting colonies on these dishes were used to estimate the library size. The remainder of the transformation was plated on large format Bioassay dishes containing 2xTY agar supplemented with 2% (w/v) glucose and 100µg/ml ampicillin. All agar plates were incubated overnight at 30°C. 10 ml of 2xTY broth was added to the large format bioassay dishes and colonies were scraped and OD_{600} measured (OD of 1.0 = 5 x 10^8 cells/ml). Aliquots were stored at -80°C in cryovials after addition of 50% (v/v) glycerol solution or used directly in a phage selection process.

EXAMPLE 6. Selection strategies for isolation of PSMA binders

Preparation of library phage stocks and phage display selections were performed according to published methods (Antibody Engineering, edited by Benny Lo, chapter 8, p161-176, 2004). In most cases, phage display combined with a panning approach was used to isolate binding V_H domains. However, a variety of different selection methods are well described in the art, including soluble selection and selections performed under stress (*e.g.* heat). Selections to promote internalising anti-PSMA V_H were also conducted with monovalent and multivalent phage (patent US2009170792 (A1) — 2009-07-02). Briefly, blocked phage in ice-cold cell media were added to 4ml ice-cold cell media containing 2.5 x 10^6 LnCAP cells. Phage and cells were incubated on ice for 2 hours, mixing occasionally to prevent cell clumping. Unbound or weakly bound phage were removed by washing five times in ice-cold PBS. The phage were then allowed to internalise by incubating the cells in media at 37°C

before removing phage bound to the outside of the cells with a 5 minutes wash step in a low pH cell-stripping buffer at 4°C. The cells were then lysed to harvest internalised phage using trimethylamine. Both the stripped and internalised fractions were neutralised with Tris buffer before being used to infect *E.coli*. The phage outputs were analysed as described for panning selections on recombinant proteins.

EXAMPLE 7. Assays for target binding

V_H from the different selections were screened in one or more of the following assays to identify specific V_H capable of binding PMSA.

a) Binding ELISA

Following selections of the libraries, specific V_H antibodies were identified by phage ELISA following published methods (Antibody Engineering, edited by Benny Lo, chapter 8, p161-176, 2004). Phage ELISAs were performed against target protein and an unrelated antigen as control. In some cases, purified or crude extracts of V_H domains were assayed by ELISA instead of using a phage ELISA. In these cases, bacterial periplasmic extracts or purified V_H were used.

Small-scale bacterial periplasmic extracts were prepared from 1ml cultures, grown in deep well plates. Starter cultures were used to inoculate 96-well deep well plates (Fisher, cat. no. MPA-600-030X) containing 2XTY broth (Melford cat. no. M2130), supplemented with 0.1% (w/v) glucose + 100µg/ml ampicillin at 37°C with 250rpm shaking. When OD_{600} had reached 0.6-1, V_H production was induced by adding 100µl of 2XTY, supplemented with IPTG (final concentration 1mM) and ampicillin and the cultures were grown overnight at 30°C with shaking at 250rpm. *E. coli* were pelleted by centrifugation at 3200rpm for 10 mins and supernatants discarded. Cell pellets were resuspended in 30-100µl of ice cold extraction buffer (20% (w/v) sucrose, 1mM EDTA & 50mM Tris-HCl pH 8.0) by gently pipetting. Cells were incubated on ice for 30 minutes and then centrifuged at 4500rpm for 15 mins at 4°C. Supernatants were transferred to polypropylene plates and used, following incubation in Marvel/PBS blocking solution, in the ELISA.

The purified V_H were obtained by using the V_H C-terminal 6xHIS tag for Ni-NTA affinity chromatographic purification of the periplasmic extracts. A starter culture of each V_H was grown overnight in 5ml 2XTY broth (Melford cat. no. M2103) supplemented with 2% (w/v) glucose + 100µg/ml ampicillin at 30°C with 250rpm shaking. 50µl of this overnight culture was then used to inoculate 50ml 2XTY supplemented with 2% (w/v) glucose + 100µg/ml ampicillin and incubated at 37°C with 250rpm shaking for approximately 6-8 hours (until

OD_{600} = 0.6-1.0). Cultures were then centrifuged at 3200rpm for 10 mins and the cell pellets resuspended in 50ml fresh 2XTY broth containing 100µg/ml ampicillin + 1mM IPTG. Shake flasks were then incubated overnight at 30°C and 250rpm. Cultures were again centrifuged at 3200rpm for 10 mins and supernatants discarded. Cell pellets were resuspended in 1ml ice cold extraction buffer (20% (w/v) sucrose, 1mM EDTA & 50mM Tris-HCl pH 8.0) by gently pipetting and then a further 1.5ml of 1:5 diluted ice cold extraction buffer added. Cells were incubated on ice for 30 minutes and then centrifuged at 4500rpm for 15 mins at 4°C. Supernatants were transferred to 50ml Falcon tubes containing imidazole (Sigma cat. no. I2399 - final concentration 10mM) and 0.5ml of nickel agarose beads (Qiagen, Ni-NTA 50% soln, cat. no. 30210) pre- equilibrated with PBS buffer. V_H binding to the nickel agarose beads was allowed to proceed for 2 hours at 4°C with gentle shaking. The nickel agarose beads were then transferred to a polyprep column (BioRad cat. no. 731-1550) and the supernatant discarded by gravity flow. The columns were then washed 3 times with 5ml of PBS+0.05% Tween® followed by 3 washes with 5ml of PBS containing imidazole at a concentration of 20mM. V_H were then eluted from the columns by the addition of 250µl of PBS containing imidazole at a concentration of 250mM. Imidazole was then removed from the purified V_H preparations by buffer exchange with NAP-5 columns (GE Healthcare, 17-0853-01) and then eluting with 1ml of PBS. Yields of purified V_H were estimated spectrophotometrically and purity was assessed using SDS PAGE.

Alternatively anti-PSMA V_H were purified from the supernatants of W3110 *E coli* with pJExpress vector. For this procedure up to 400ml cultures were grown at 37°C with 250rpm shaking in TB media before being induced overnight with 1mM IPTG overnight. The resulting supernatants were harvested and V_H purified on AKTA Pure using a Ni-Sepharose excel column (HiScale 16, GE Healthcare). Yields of purified V_H were estimated spectrophotometrically and purity was assessed using SDS PAGE.

The binding ELISA for crude or purified V_H was similar to the serum ELISA and phage ELISA, previously described, mostly differing in the final detection steps. Briefly, antigen was immobilised on Maxisorb plates (Nunc cat. no. 443404) by adding 50µl volumes at 0.1 – 2µg/ml in PBS and incubating at 4°C overnight. Following coating, the antigen solution was aspirated and the plates were washed using PBS (prepared from PBS Tablets, Oxoid cat. no. BR0014G) supplemented with 0.05% Tween® 20 (Sigma cat. no. P1379), followed by washes with PBS without added Tween® 20. To block non-specific protein interactions, a solution of 3% skimmed milk powder (Marvel®) in PBS was added to the wells and the plate was incubated for at least one hour at room temperature. Dilutions of periplasmic extract or purified V_H in 3% Marvel®/PBS were prepared in polypropylene tubes or plates and

incubated for at least one hour at room temperature prior to transfer to the blocked ELISA plate where a further incubation of at least one hour took place. Unbound protein was then washed away using repetitive washes with PBS/Tween followed by PBS. A solution of HRP-conjugated anti-Myc Ab (Santa Cruz cat. no. SC-40), prepared at 1:50 dilution in PBS/3% Marvel was then added to each well and a further incubation at room temperature for at least one hour took place. Unbound detection antibody was removed by repeated washing using PBS/Tween® and PBS. The ELISA was then developed using TMB substrate (Sigma cat. no. T0440) and the reaction was stopped after 10 minutes by the addition of 0.5M H_2SO_4 solution (Sigma cat. no. 320501). Absorbances were determined by reading at 450nm.

b) FMAT Direct cell Binding Assay

Periplasmic extracts from *E.coli* were screened for production of PSMA-binding-His-tagged V_H using Fluorescence Microvolume Assay Technology (FMAT), a fluorescence-based platform that detects fluorescence localized to beads or cells settled at the bottom of microwells (Dietz *et al., Cytometry 23*:177-186 (1996), Miraglia *et al., J. Biomol. Screening 4*:193-204 (1999). CHO TREX human and cynomolgus cell lines were generated in-house using full-length human and cynomolgus PSMA using standard procedures. LnCAP cells were purchased from Sigma Aldrich.

Peripreps were tested by single point screening for the presence of V_H that bound specifically to CHO human PSMA, CHO cyno PSMA and LnCAP cells with no binding to CHO parental cells in an FMAT Direct Binding Assay. Cells were resuspended at 0.1 x 10^6 cells/ml in FMAT assay buffer (pH 7.4) containing PBS, 0.1% Bovine Serum Albumin, 0.01% Sodium Azide and 120nM DRAQ5 (Thermo Scientific cat. no. 62251) added to the cell suspension. Peripreps (10µl) were transferred into 384 well black clear-bottomed assay plates (Costar cat. no. 3655) and 10µl of 6nM mouse Anti-His (Millipore cat. no. 05-949)/12nM Goat Anti-Mouse Alexa Fluor-488 (Jackson Immunolabs cat. no. 115-545-071) mix added. The DRAQ5 stained cells (20µl per well) were then added and the assay plates incubated for 2 hours at room temperature. Plates were read in the FL2 (502nm-537nm) and FL5 (677-800nm) channels on the TTP Mirrorball plate reader following excitation at 488nm and 640nm. Data was gated on FL5 perimeter and peak intensity and the FL2 median mean fluorescence intensity of the gated data used for determination of V_H binding.

For titrations, V_H purified via the terminal His tag were serially diluted in FMAT assay buffer then binding was measured as described above (Figure 16). Improved monovalent variants show similar properties to the parent V_H (16b and 16c). Biparatopic immunoconjugates were also tested (Figure 16d and e) and showed good binding to human PSMA CHO cells. The

linker length used was $(G_4S)_6$. The Table below shows the different CDR3 sequences of the V_H families identified together with their binding characteristics.

Table 18 (prepared form phagemid and periprep)

CDR3	VH family	rhPSMA	Cyno PSMA CHO	Human PSMA CHO	CHO parent	LnCAP
DPAWGLRLGESSSYDFDI SEQ ID No.513	VH3-30	Y	Y	Y	N	Y
DRIVG**G**RVPDA**F**DI SEQ ID No.514	VH3-30	Y	Y	Y	N	Y
ERIFGVLTPDDFDI SEQ ID No.515	VH3-30	Y	Y	Y	N	Y
GLWPSDV 516	VH3-30	Y	Y	Y	N	Y
GLWPPMDV SEQ ID No.517	VH3-30	Y	Y	Y	N	Y
GDYDFWSGYPDYDMDV SEQ ID No.518	VH3-30	Y	Y	Y	N	Y
GGNALYSSGWPDD SEQ ID No. 519	VH3-30	Y	Y	Y	N	Y
DGVH SEQ ID No.520	VH3-23	Y	Y (weak)	Y	N	Y
ENVIVPAATY SEQ ID No.521	VH3-20	Y	Y	Y	N	Y
DSLIVGERGY SEQ ID No. 522	VH3-07	Y	Y (weak)	Y (weak)	N	Y (Weak)
DRGGAVALYHNGMDM SEQ ID No. 523	VH3-07	Y	N	Y	N	Y
DYGDSRSLFDY SEQ ID No. 524	VH4-34	N	Y	Y	N	Y
GPIPATAIPDAFDI SEQ ID No. 525	VH4-34	N	Y	Y	N	Y
DGDDYGDY SEQ ID No. 526	VH4-41	Y	Y (Very Weak)	Y	N	Y
GNGPGITGTTDY SEQ ID No. 527	VH1-08	Y/N	Y	Y	N	Y

Table 19 EC50 values for anti-PSMA V_H binding to PSMA expressing cell lines (prepared from purified V_H)

EC50	huPSMA CHO	cynoPSMA CHO	DU145 PSMA	LNCap
2.1	1.097E-10	3.667E-10	2.304E-10	6.07E-11
2.18	1.044E-10	3.370E-10	2.496E-10	3.54E-11
2.17	1.004E-10	3.082E-10	2.181E-10	1.13E-11
2.15	9.212E-11	3.335E-10	1.663E-10	8.41E-11

2.14	1.103E-10	4.269E-10	2.023E-10	3.32E-11
2.22	1.232E-10	6.129E-10	2.293E-10	1.53E-10
1.8	1.029E-10	3.099E-10	9.455E-11	1.473E-10
1.10	7.182E-11	1.518E-10	6.699E-11	1.328E-10
1.11	8.634E-11	2.168E-10	7.604E-11	1.189E-10
1.12	5.023E-11	1.097E-10	4.15E-11	1.992E-10
1.13	5.127E-11	1.154E-10	4.564E-11	3.862E-11
1.14	5.884E-11	1.45E-10	5.201E-11	8.329E-11
1.16	6.805E-11	1.458E-10	5.938E-11	7.539E-11
1.17	3.338E-11	9.127E-11	3.099E-11	5.853E-11
1.18	5.858E-11	1.237E-10	4.949E-11	4.239E-11

Table 20 EC_{50} values for anti-PSMA V_H binding to human PSMA-CHO. The linker length used was $(G_4S)_6$. (prepared from purified V_H)

No	Construct	EC50
1	1.1-2.1	3.616E-10
2	1.1-2.17	2.639E-10
3	1.1-2.15	1.948E-10
4	1.1-2.22	1.784E-10
5	1.16-2.1	3.057E-10
6	1.16-2.17	3.327E-10
7	1.16-2.15	1.967E-10
8	1.16-2.22	2.250E-10
9	1.11-2.1	2.871E-10
10	1.11-2.17	2.805E-10
11	1.11-2.15	2.100E-10
12	1.11-2.22	2.187E-10
13	1.18-2.1	2.938E-10
14	1.18-2.17	2.778E-10
15	1.18-2.15	1.921E-10
16	1.18-2.22	1.958E-10
17	1.17-2.1	3.252E-10
18	1.17-2.15	2.986E-10
19	1.17-2.17	1.921E-10
20	1.17-2.22	1.989E-10

Each individual V_H clone as identified above was sequenced from the phagemid and grouped based on V_H germline and CDR3 amino acid similarity into separate families. Representative clones were further characterised. Variants, including germlined variants, were generated by standard methods of parent clones e.eg 1.1 and 2.1. Figure 1 shows the sequences of clones 1.1 to 1.20 isolated as described herein above and grouped into a single family.

Clones 1.8-1.20 are variants of 1.1. Figure 2 shows the sequences of clones 2.1 to 2.25 isolated as described herein above and grouped into a single family. Clones 2.2, 2.11-2.19, 2.22-2.25 are variants of 2.1. Figure 3 shows the sequences of clones 3.1 to 3.24 isolated as described herein above and grouped into a single family. Clones 3.20-3.25 are variants of 3.1.

EXAMPLE 8 – Characterisation of V_H

a) Specificity of anti-PMSA

The specificity of individual V_H for target antigen was confirmed by ELISA, following the methods described in Example 7(a). V_H were tested for binding to PMSA and shown not to cross react with irrelevant proteins.

b) Measurement of Binding Kinetics and epitope binding using Octet

Binding kinetics of purified anti-PSMA V_H antibodies were measured on a ForteBio Octet RED 384 instrument. Recombinant PMSA was diluted to 20µg/ml in sodium acetate buffer, pH 5 (ForteBio, cat. no. 18-1069) and coupled to ARG2G biosensors (ForteBio cat. no. 18-5092) using amine-coupling chemistry (NHS-EDC amine-coupling, ForteBio cat. nos. 18-1067 and 18-1033), followed by quenching in ethanolamine (ForteBio cat. no. 18-1071). Binding kinetics of anti-PSMA V_H antibodies were then determined by preparing each V_H antibody in dilution series (typically 1:2 dilution series starting with 15µg/ml, V_H at the highest concentration), and then measuring binding of the different V_H concentrations to the PSMA-coupled biosensors. V_H binding kinetics were then determined from the (blank subtracted) sensorgram trace using 1:1 binding models and ForteBio Octet DataAnalysis software. Binding affinities from 1-150nM and in the subnanomolar range were detected and examples of the binding parameters are shown in Table 21 below.

Table 21

	KD (nM)	Kdis (1/s)
2.1	1.64	4.56E-04
1.1	2.44	1.54E-03
3.1	3.78	4.52E-04

Further family members in particular variants of parent molecules were also tested as below using 1:2 dilution series starting with 0.375µg/ml. Binding affinities in the nanomolar and picomolar range were detected as shown in Tables 22 and 23.

Table 22 Family 1

Clone	KD (nM)	Kdis (1/s)
1.8	1.95	1.04E-03
1.10	0.67	4.18E-04
1.11	0.80	4.95E-04
1.12	0.55	4.28E-04
1.14	0.46	3.35E-04
1.16	0.44	3.65E-04
1.17	0.61	5.51E-04
1.18	0.59	5.72E-04

Table 23 Family 2

Clone	KD (nM)	Kdis (1/s)
2.1	0.32	2.28E-04
2.13	0.99	7.43E-04
2.17	0.76	7.26E-04
2.15	4.72	3.44E-03
2.12	1.56	1.57E-03
2.22	2.62	2.44E-03

Single domain antibodies purified from periplasmic extracts using Ni-NTA chromatography (via the C-terminal His-tag) as in example 7a were also tested. Results are shown in the Table below. Binding affinities in the nanomolar range were detected.

Table 24

clone number	KD (nM)	Kdiss (1/s)
4.1	45	1.4 x 10-2
5.1	30	9.1 x 10-3
12.1	3.9	1.37E-03
10.1	95	1.85 x 10-3
11.1	26	0.00149
7.1	41	4.783 x 10-4
13.1	4.2	6 x 10-4
6.1	16	3.65 x 10-3
14.1	17	1.1 x 10-3

c) Measurement of Internalization of Cynomolgus PSMA-Binding V_H using Fluorescence Microvolume Assay Technolog

Internalization of purified V_H was measured using the pH-sensitive fluorescent dye pHrodo® green. Anti-His antibody (Millipore cat. no. 05-949) was labelled with pHrod® Green STP

ester (Molecular Probes cat. no. P35369) according to the manufacturer's instructions. All samples and reagents were prepared in internalization buffer (pH 7.4) containing PBS and 0.1% Bovine Serum Albumin. CHO cells expressing cynomolgus PSMA were resuspended at 0.1 x 10^6 cells/ml and 120 nM DRAQ5 added to the cell suspension. V_H (10µl) were transferred into 384-well black clear-bottomed assay plates (Costar cat. no. 3655) and 10µl of 40nM pHrodo® green labelled Anti-His antibody added followed by 20 µl DRAQ5 stained cells. Plates were incubated at 37°C for 2hr then equilibrated to room temperature. Fluorescence emission in the FL2 (502nm-537nm) and FL5 (677-800nm) channels were measured on TTP Mirrorball plate reader following excitation at 488nm and 640nm. Data was gated on FL5 perimeter and peak intensity and the FL2 median mean fluorescence intensity of the gated data used for determination of V_H internalization (Figure 17).

Internalization of variants of single domain antibodies 1.1 and 1.2 was measured using the pH sensitive fluorescent dye pHrodo® green as described above except serially diluted V_H were pre-incubated with pHrodo® green labelled Anti His antibody for 30 minutes at room temperature prior to addition of DRAQ5 stained CHO human PSMA clone 1A10 cells (20µl). Plates were incubated for 2 hour at room temperature then fluorescent emission measured. Activity of the V_H in the assay is shown in Table 25 below.

Table 25

Name	pH® RodoGreen Internalization Assay human PSMA Average EC_{50} (M)
1.8	5.0E-10
1.10	6.4E-10
1.11	3.7E-10
1.12	5.7E-10
1.14	4.4E-10
1.16	4.8E-10
1.17	2.9E-10
1.18	3.1E-10
2.1	8.0E-10
2.13	5.8E-10
2.17	8.0E-10
2.15	7.2E-10
2.12	5.3E-10
2.22	6.7E-10

d) Measurement of Internalization of PSMA Binding V_H using the His-ZAP Assay

Internalization of PSMA binding V_H His tagged was assessed using an anti-His antibody conjugated to saporin toxin (His-ZAP Advanced targeting Systems IT52). The His-ZAP reagent binds to the V_H and is internalized through the V_H interaction with PSMA on the cell

surface. Saporin toxin is released from the complex in the endosome and inactivates ribosomes eventually resulting in cell death.

CHO cells expressing human or cynomolgus PSMA (400 cells per well in a 30 µl volume) were seeded into 384-well black clear-bottomed tissue culture-treated assay plates (Costar cat. no. 3712) in Hams F12 (Sigma cat. no. N6658) media containing 10% foetal bovine serum, 2mM L-glutamine, 10µg/ml blasticidin, 300µg/ml Zeocin, penicillin/streptomycin, 1µg/ml tetracycline and incubated overnight in a CO_2 incubator at 37°C. Purified V_H were serially diluted in media then an equal volume of 40nM His-ZAP added. Following incubation for 30 minutes at 37°C the V_H/His-ZAP samples (10µl) were transferred to the cell assay plates and incubated for 48 hours in a CO_2 incubator at 37°C. His-ZAP control wells (cells with His-ZAP reagent) and background controls (media only) were set up on each plate for data normalization. Cell viability was determined following the 48 hour incubation using the Cell Titer-Glo Cell Viability assay (Promega cat. no. G7571) according to the manufacturer's instructions. Relative luminescent signal (RLU) was measured using the BMG PHERAstar plate reader. The data was normalized by subtraction of the RLU signal obtained in the absence of cells and expression as a percentage of the background-corrected signal of the His-ZAP control wells Examples are given in Figure 18.

For LnCAP assays, cells (2000 per well in a 100 µl volume) were seeded into 96-well TC-treated plates (Costar 3340) in RPMI 1640 media containing 10% foetal bovine serum, 2 mM L-glutamine and penicillin/streptomycin. Purified V_H were serially diluted in media, then an equal volume of 60 nM His-ZAP (Advanced targeting Systems IT52) was added. Following incubation for 30 minutes at 37 °C the V_H/His-ZAP samples (100 µl) were transferred to the cell assay plates and incubated for 96 hours in CO_2 incubator at 37 °C. Cell viability was measured using the Cell Titer-Glo Cell Viability assay and data analysed as described above Examples are given in Figure 19.

The ability of variants of single domain antibodies 1.1 and 2.1 to internalize with a bound saporin conjugated anti His antibody, resulting in toxin mediated cell death, was determined. Assays were performed as described above except CHO human PSMA clone 1A10 cells were used for human PSMA assays and plates were incubated for 72 hours in a CO_2 incubator at 37°C prior to measurement of cell viability. Activity of the single domain antibodies tested in the assay is shown in Table 26 below.

Table 26

	human PSMA Average EC_{50} (M)	cyno PSMA EC_{50} (M)
1.8	2.6E-11	1.4E-09
1.10	2.1E-11	1.3E-09
1.11	1.4E-11	4.1E-10
1.12	1.8E-11	9.7E-10
1.14	1.7E-11	7.9E-10
1.16	1.7E-11	4.2E-10
1.17	1.5E-11	5.6E-10
1.18	2.3E-11	4.8E-10
2.1	1.4E-11	5.2E-11
2.13	2.7E-11	8.0E-11
2.17	3.5E-11	7.0E-11
2.15	6.9E-11	1.6E-10
2.12	1.6E-11	9.1E-11
2.22	6.9E-11	1.8E-10

Biparatopic molecules were tested and showed the following EC50 values.

Table 27

	human PSMA Average EC_{50} (M)	cyno PSMA Average EC_{50} (M)
1.1-6GS-2.1	1.4E-11	1.2E-11
1.1-6GS-2.17	1.2E-11	1.1E-11
1.1-6GS-2.15	1.3E-11	8.6E-12
1.1-6GS-2.22	8.8E-12	7.8E-12
1.16-6GS-2.1	1.4E-11	1.3E-11
1.16-6GS-2.17	1.5E-11	1.1E-11
1.16-6GS-2.1	1.8E-11	1.1E-11
1.16-6GS-2.22	2.0E-11	1.1E-11
1.11-6GS-2.1	1.7E-11	1.2E-11
1.11-6GS-2.17	7.9E-12	8.1E-12
1.11-6GS-2.15	1.1E-11	9.0E-12
1.11-6GS-2.22	1.0E-11	9.5E-12
1.18- 6GS-2.1	5.7E-12	5.8E-12
1.18-6GS-2.17	1.2E-11	5.6E-12
1.18-6GS-2.15	1.4E-11	1.0E-11
1.18-6GS-2.22	1.5E-11	1.4E-11
1.17-6GS-2.1	1.3E-11	1.5E-11
1.17-6GS-2.17	1.4E-11	1.0E-11
1.17-6GS-2.15	1.5E-11	1.2E-11
1.17-6GS-2.22	1.8E-11	1.2E-11

EXAMPLE 9 – Stability of V_H

V_H from the different CDR3 families were tested for developability characteristics.

a) Heat Stability: HPLC Size Exclusion Chromatography

Purified V_H were subjected to size exclusion chromatography. Briefly, purified V_H were stored l in PBS buffer for 0-14 days at either 4oC or 40oC, and then analysed at various time points using a Waters H-Class Bio UPLC containing a PDA detector (detection at 280nm) with separation on a Waters ACQUITY BEH 125Å SEC column. Samples were injected in 10µl volumes and were run in a mobile phase containing 200 mM NaCl, 100 mM sodium phosphate, pH 7.4 + 5% propan-1-ol at a flow rate of 0.4ml/min. Data were collected for 6 minutes and the percentage of monomer remaining after storage as compared to that present at the start (T=0) was calculated. Parent molecues showed high stability and variants were also tested.

Concentration of samples varied: Monovalent 1.1 variants: 5.0 mg/ml

Monovalent 2.1 variants: 3.5 mg/ml

Results are shown in the Tables below.

Table 27

	%Area T0 Monomer 4°C				% Area T0 Monomer 40°C				
	0	4	7	14	0	1	4	7	14
1.8	100.00	100.47	99.06	102.71	100.00	98.65	97.70	90.78	88.64
1.10	100.00	100.75	99.74	101.47	100.00	97.73	94.35	82.99	85.89
1.11	100.00	101.34	100.41	103.26	100.00	98.34	97.92	90.95	100.75
1.12	100.00	100.97	103.69	110.61	100.00	97.62	97.03	87.86	100.99
1.14	100.00	101.44	101.09	109.51	100.00	97.55	95.03	83.69	88.01
1.16	100.00	101.44	100.84	107.00	100.00	97.24	93.57	82.10	88.46
1.17	100.00	101.06	100.29	108.35	100.00	98.44	100.56	93.92	108.68
1.18	100.00	100.36	101.41	106.39	100.00	98.38	98.70	88.09	95.31

Table 28

	%Area T0 Monomer 4°C				% Area T0 Monomer 40°C				
	0	4	7	14	0	1	4	7	14
2.1	100.00	100.85	98.69	101.05	100.00	99.75	100.07	100.59	100.55
2.13	100.00	103.11	100.91	99.78	100.00	99.80	99.92	100.30	100.34
2.17	100.00	101.89	99.62	99.64	100.00	100.10	101.00	101.17	101.50
2.15	100.00	102.20	99.85	99.20	100.00	99.46	100.23	100.28	101.03
2.12	100.00	100.06	99.56	99.66	100.00	99.51	99.92	100.84	101.71
2.22	100.00	100.76	99.91	100.48	100.00	99.12	99.88	100.23	102.02

Table 29

	%Area T0 Monomer 4oC	% Area T0 Monomer 40oC

	0	4	7	14	0	1	4	7	14
1.1-6GS-2.1	100.00	109.56	68.16	68.02	100.00	105.79	94.77	64.98	64.58
1.1-6GS-2.17	100.00	116.15	75.23	75.20	100.00	111.31	101.98	68.73	63.67
1.1-6GS-2.15	100.00	111.01	72.93	73.21	100.00	106.51	95.09	70.50	58.28
1.1-6GS-2.22	100.00	116.88	80.88	80.66	100.00	110.00	105.22	74.44	75.37
1.16-6GS-2.1	100.00	135.96	110.01	110.26	100.00	101.92	116.20	106.53	106.74
1.16-6GS-2.17	100.00	125.26	106.57	106.10	100.00	117.55	110.34	100.59	96.83
1.16-6GS-2.15	100.00	136.57	117.42	118.25	100.00	121.10	117.79	107.33	106.12
1.16-6GS-2.22	100.00	122.20	105.46	104.15	100.00	100.32	104.92	97.31	93.30
1.11-6GS-2.1	100.00	76.33	98.05	97.37	100.00	96.51	95.53	N/A	98.38
1.11-6GS-2.17	100.00	45.23	98.17	97.40	100.00	96.51	94.72	N/A	90.52
1.11-6GS-2.15	100.00	101.17	98.87	98.68	100.00	97.23	94.78	N/A	87.98
1.11-6GS-2.22His	100.00	102.38	100.84	99.07	100.00	98.45	100.47	N/A	82.62
1.18-6GS-2.1	100.00	102.42	98.53	97.83	100.00	92.81	90.11	85.86	85.82
1.18-6GS-2.17	100.00	101.18	97.79	97.30	100.00	92.66	87.07	84.42	79.49
1.18-6GS-2.15	100.00	100.57	N/A	N/A	100.00	88.40	93.87	N/A	N/A
1.18-6GS-2.22	100.00	102.69	97.73	97.54	100.00	94.07	91.87	86.53	100.98
1.17-6GS-2.1	100.00	101.15	98.97	97.85	100.00	97.08	96.59	95.85	97.40
1.17-6GS-2.17	100.00	98.88	98.94	99.34	100.00	96.16	95.61	98.86	92.37
1.17-6GS-2.15	100.00	97.67	N/A	N/A	100.00	99.14	99.77	N/A	N/A
1.17-6GS-2.22	100.00	100.20	97.98	98.47	100.00	101.00	102.12	102.52	100.19

Long term stability of monovalent single domain antibodies up to 35 days was also tested and showed a good profile.

b) Heat stability: Mirror ball

Purified V_H samples were incubated for 0-8 days at 40°C and then tested for binding to CHO cells expressing cynomolgus PSMA using the FMAT Direct Binding Assay as detailed in Examples 7(b). Molecules tested showed good stability.

c) Assessment of V_H Serum stability using a Homogenous Time Resolved Fluorescence (HTRF) Assay.

Purified V_H were mixed with cynomolgus monkey serum and incubated for 0-7 days at 37°C. Samples were then assessed for binding to PSMA using an HTRF assay. Briefly, PSMA (R&D Systems cat. no. 4234-ZN) was biotinylated using the Pierce EZ-Link Micro-Sulfo-NHS-LC- Biotinylation kit. (Thermo Scientific cat. no. 21935). For HTRF binding assays all samples and reagents were prepared in HTRF assay buffer containing PBS, 0.1% (w/v) BSA and 0.4M Potassium Fluoride. V_H (C-terminally His-Myc tagged) were incubated with 3nM biotinylated PSMA, 1.5nM Streptavidin cryptate (Cisbio cat. no. 610SAKLA) and 10nM Anti-

Myc-Alexa Fluor-647 (AbD Serotec cat. no. MCA2200AF647) in a total assay volume of 10µl in black 384-shallow-well plates (Costar cat. no. 3676) for a minimum of 3 hours at room temperature. Time-resolved fluorescent emission at 620nm and 665nm was measured following excitation at 337nm on the BMG PHERAstar plate reader.

In another experiment, purified VH were mixed with human serum for 0-7 days at 37°C and then assessed for binding to huPSMA CHO 1A10 cells as described in examples' 7(b) FMAT Direct cell Binding Assay. Data obtained is shown in and EC50 values are shown in Tables 29 and 30 below.

Table 29

VH	EC50
2.1 Day 0	2.49E-10
2.1 Day 1	2.54E-10
2.1 Day 4	2.60E-10
2.1 Day 7	3.01E-10
2.17 Day 0	2.30E-10
2.17 Day 1	2.10E-10
2.17 Day 4	2.28E-10
2.17 Day 7	2.38E-10
2.15 Day 0	2.66E-10
2.15 Day 1	4.97E-10
2.15 Day 4	3.93E-10
2.15 Day 7	3.76E-10
2.22 Day 0	3.05E-10
2.22 Day 1	2.91E-10
2.22 Day 4	3.40E-10
2.22 Day 7	3.28E-10

Table 30

VH	EC50
1.8 Day 0	4.09E-10
1.8 Day 1	4.86E-10
1.8 Day 4	4.96E-10
1.8 Day 7	5.42E-10
1.11 Day 0	2.34E-10
1.11 Day 1	2.08E-10
1.11 Day 4	2.27E-10
1.11 Day 7	2.78E-10
1.16 Day 0	1.65E-10
1.16 Day 1	2.43E-10
1.16 Day 4	2.42E-10
1.16 Day 7	2.36E-10
1.17 Day 0	2.73E-10
1.17 Day 1	2.53E-10
1.17 Day 4	2.59E-10
1.17 Day 7	2.74E-10

1.18 Day 0	3.04E-10
1.18 Day 1	3.11E-10
1.18 Day 4	3.19E-10
1.18 Day 7	3.13E-10

To assess the serum stability of the different biapartopic combinations the purified biparatopic V_H were mixed with human serum for 0-7 days at 37°C and then assessed for binding to huPSMA CHO cells as described in examples 7(b) FMAT Direct cell Binding Assay. Table 31 shows the EC50 values for the cell binding.

Table 31 EC50 values for biparatopic anti-PSMA V_H binding to PSMA expressing cell line.

Biparatopic immunoconjugate	Days	EC50
1.1-6GS-2.1	0	8.02E-11
	1	1.03E-10
	4	7.86E-11
	7	7.88E-11
1.1-6GS-2.17	0	7.7E-11
	1	9.16E-11
	4	8.49E-11
	7	7.42E-11
1.11-6GS-2.1	0	8.92E-11
	1	6.05E-11
	4	7.36E-11
	7	8.65E-11
1.11-6GS-2.17	0	6.39E-11
	1	7.25E-11
	4	8.44E-11
	7	1.01E-10
1.16-6GS-2.1	0	9.02E-11
	1	8.60E-11
	4	1.00E-10
	7	1.07E-10
1.16-6GS-2.17	0	7.41E-11
	1	9.44E-11
	4	6.28E-11
	7	6.75E-11
1.17-6GS-2.1	0	5.69E-11
	1	4.77E-11
	4	4.58E-11
	7	5.44E-11
1.17-6GS-2.17	0	6.74E-11
	1	3.32E-11
	4	4.73E-11

	7	5.7E-11

d) Assessment of V_H thermal stability

Differential scanning calorimetry (DSC) was conducted using a MicroCal VP-Capillary DSC (Malvern). 300 µl of protein at 0.25 mg/ml in PBS was run using a scan rate of 60°C per minute between 10 and 90°C. Data was analysed using the MicroCal software. Results are shown in Table 32 for monovalent single domain antibodies and in Table 33 for biparatopic immunoconjugates.

Table 32

	T_m (°C)	T_{onset} (°C)	$T_{½}$ (°C)
2.1	73.91	70.12	2.5
2.17	72.55	59.96	7.04
2.15	63.62	46.37	11.75
2.22	71.18	56.74	8.05
1.1	63.92	54.86	4.02
1.11	61.51	52.62	3.25
1.16	60.02	48.77	5.19
1.17	62.15	53.59	3.69
1.18	60.34	51.44	3.69

Table 32

	T_m (°C)	T_{onset} (°C)	$T_{½}$ (°C)
1.1-6GS-2.1	67.63	57.06	6.37
1.1-6GS-2.17	65.60	58.39	3.52
1.1-6GS-2.15	61.28	50.36	3.69
1.1-6GS-2.22	64.39	57.01	3.53
1.16-6GS-2.1	64.18	54.28	9.07
1.16-6GS-2.17	62.98	53.08	5.37
1.16-6GS-2.15	58.97	48.07	4.03
1.16-6GS-2.22	61.54	51.97	4.86
1.11-6GS-2.1	65.75	54.00	7.56
1.11-6GS-2.17	64.36	55.80	4.03
1.11-6GS-2.15	60.05	50.81	3.52

1.11-6GS-2.22	63.07	54.00	4.02
1.18-6GS-2.1	63.89	53.15	9.57
1.18-6GS-2.17	62.98	52.92	5.70
1.18-6GS-2.15	60.75	48.67	7.38
1.18-6GS-2.22	61.75	51.68	5.04
1.17-6GS-2.1	66.58	54.49	7.22
1.17-6GS-2.17	64.84	56.45	4.20
1.17-6GS-2.15	60.69	51.29	3.86
1.17-6GS-2.22	63.23	53.83	4.53

EXAMPLE 10 Imaging studies in mice

V_H were injected in mice (V_H1.1, V_H2.1 and V_H2.1 with half-life extension). The mice contain PSMA positive (+) and PSMA negative (–) tumours. Studies were carried out as follows:

- ~100 MBq of Tc-99m injected activity per mouse
- SPECT/CT at 5min, 30min, 60min, 3hrs, 6hrs & 24hrs.
- images produced for different time points
- Post imaging *ex vivo* biodistribution and autoradiography
- Negative control V_H(αHEL4)

The half-life extended V_H comprises an anti-mouse serum albumin (anti-MSA) V_H with the following sequence:

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVATISDSGSSAD YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARGRYNWNPRALGIWGQGTMVTV SS (SEQ ID NO: 528)

The experiments showed high levels of specific tumor targeting, faster penetration and greater accumulation of the injected dose to PSMA+ tumor, in particular compared to a control monoclonal IgG anti-PSMA antibody. This can be further improved by extending the half life of the V_H. Furthermore, the data shows quick clearance of the naked Humabody® V_H.

EXAMPLE 11 Epitope mapping

In tandem epitope mapping of PSMA binding V_H against each other was carried out using Octet RED 384. V_H binding was then determined from the (reference sensor subtracted) sensorgram trace using 1:1 binding models and ForteBio Octet DataAnalysis software. See also example 8b. The epitope binning results are shown in Table 33. Some clones showed partial blocking.

Table 33

Group 1	Group 2
3.6	1.4
2.1	12.1
11.1	5.1
4.1	13.1
7.1	6.1
14.1	
10.1	
9.1	

In a further experiment, epitope competition between single domain antibodies 1.1 and 2.1 was further characterised. PSMA was coupled onto AR2G biosensors using the amine coupling second generation kit (ForteBio) and then used for epitope binning experiments conducted using the Octet RED384. In these experiments each V_H was diluted to a concentration of 4ug/ml. Biosensors were loaded with no V_H or either 2.1 or 1.1 until binding to PSMA reached saturation level. These sensors were then briefly dipped into PBS/Tween before undergoing a second association step. The second association step involved dipping biosensors into wells containing the same V_H only or both 2.1 and 1.1. The presence of the first VH in the later combination ensured that it continued to saturate its PSMA binding sites. The binding profiles were then studied using the ForteBio Analysis software. These data obtained demonstrate that single domain antibodies 2.1 and 1.2 bind distinct epitopes on PSMA.

EXAMPLE 12 Imaging studies

The following constructs were tested in these studies:

VH 2.1

VH 2.1-HIS, 1.2mg/ml

Sequence:

EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDGSNKY YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQ GTMVTVSSLEGGGSEQKLISEEDLNHHHHHH (SEQ ID NO. 532)

VH 1.1

VH 1.1-HIS

Sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGKGLEWVSSIGENDGTTD

YADSVKGRFTISRDNSKSMLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSSLEGGGS EQKLISEEDLNHHHHHH (SEQ ID NO. 533)

Hel4

HEL-4-HIS

Sequence:

EVQLLESGGGLVQPGGSLRLSCAASGFRISDEDMGWVRQAPGKGLEWVSSIYGPSGSTYY ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCASALEPLSEPLGFWGQGTLVTVSSA AAHHHHHH (SEQ ID NO. 534)

VH 2.1- VH 2.1

VH 2.1-6GS- VH 2.1

Sequence:

EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDGSNKY YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQ GTMVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVESGGGVVQPGRSL RLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDGSNKYYADSVKGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSSAAAHHHHHH (SEQ ID NO. 535)

VH 2.1- VH 1.1

VH 2.1-6GS- VH 1.1

Sequence:

EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDGSNKY YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQ GTMVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLLESGGGLVQPGGSLR LSCAASGFSFSSYAMSWVRQAPGKGLEWVSSIGENDGTTDYADSVKGRFTISRDNSKSML YLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSSAAAHHHHHH (SEQ ID NO. 536)

All V_H domains used in this study were expressed in E.coli. The proteins were purified from filtered supernatant using nickel affinity chromatography and size exclusion chromatography (SEC). After buffer exchange into storage buffer, the some proteins were concentrated using spin concentrators. The protein purity was analysed using SDS-PAGE and analytical SEC. Binding to PSMA was checked using recombinant protein and/or cells expressing PSMA. Stability was checked by heating the protein to 40°C for an extended period of time (ranging from overnight to 4 weeks) and measuring the degree of protein degradation. Aliquots of the proteins were stored at -80°C until use.

Confocal fluorescence microscopy method to test the occurrence of co-localization between the V_H of interest (in a monovalent, bivalent and biparatopic format) and the markers of

endocytosis LAMP-1 (staining lysosome) and EEA-1 (staining early endosome) in a PSMA expressing cell line. An IgG benchmark antibody that binds to PSMA was used as a positive control. The results showed internalisation of V_H constructs.

EXAMPLE 13 POTENCY OF MMAE TOXIN CONJUGATED TO IMMUNOCONJUGATES IN VITRO

The ability of MMAE-toxin-conjugated V_H to internalize into PSMA-expressing cells resulting in cell killing was determined using an *in vitro* cytotoxicity assay. Human cells (DU-145, ATCC HTB-81) stably expressing human PSMA or matched PSMA negative cells were seeded into 384-well black clear-bottomed tissue culture treated assay plates at 3000 cells per well in RPMI 1640 medium containing 10% foetal bovine serum, 2mM L-Glutamine, 1X penicillin/streptomycin, and incubated overnight in a CO_2 incubator at 37°C. Cells were then incubated with serially-diluted MMAE-toxin-conjugated V_H for 48 or 72 hours. Untreated control wells (cells in the absence of toxin-conjugated V_H) and background control wells (media only) were set up on each plate for data normalization. Cell killing was determined following the incubation using the Cell Titer-Glo Cell Viability assay (Promega G7571) according to the manufacturer's instructions. Relative luminescent signal (RLU) was measured using the BMG PHERAstar plate reader. The data was normalized by subtraction of the RLU signal obtained in the background control wells then expressed as a % of the untreated control wells (% survival). Figure 20 illustrates dose response curves obtained using a human-PSMA-expressing human cell line and the matched parent (i.e. non-transfected) PSMA negative cell line in a representative experiment (48 hour incubation). IC_{50} values and maximum % cell killing obtained for the MMAE-conjugated constructs are summarized in Table 34. Crescendo's Humabody® V_H were conjugated to MMAE using HiPEG™ technology (WO 2009/047500; Cong *et al.*, (2012) Bioconjugate Chem. 2012, 23, 248−263); the positive ADC control was generated using ThioBridge™ technology (WO 2016063006; WO 2005/007197; Balan *et al.*, (2007) Bioconjugate Chem., 18, 61-76). The anti-PSMA-MMAE-conjugated V_H specifically killed PSMA positive cells with minimal cell killing observed for the PSMA negative control cell line. The biparatopics that consist of two V_H targeting different epitopes of the PSMA were more potent than the monovalent or bivalent PSMA V_H constructs. The DU145 assay was performed with a 48h and with a 72h HDC incubation. This had an impact on the IC_{50} values measured and the % maximum kill, but was not expected to affect the ranking of the different HDC formats. For screening, a 48h incubation was preferred for higher throughput. Using the 48h incubation none of the constructs tested reached 100 % cell kill (even at the highest concentrations tested). The maximum response levelled off at approx. 70-85 % (see Table 34). Table 35 shows the IC_{50}

values and Figure 21 illustrates the higher maximum % cell killing observed using a 72 hour incubation time (n=1 data).

Table 34. Summary of *in vitro* cytotoxicity data obtained with the human-PSMA-expressing human cell line following a 48 hour incubation.

Construct			DAR	Mean IC_{50} ± SD (nM), Mean Max % Cell kill, *(n number)*	IC_{50} Toxin (nM)
HiPEG™ A-His_6 val-cit-PAB-MMAE	Monovalent	2.1-myc-his	1	1.2 ± 0.7nM Max cell kill 74% *(n= 4)*	1.16
HiPEG™ B-His_6 val-cit-PAB-MMAE	Monovalent	1.1-myc-his	0.9	2.7 ± 2.5nM Max cell kill 73% *(n= 4)*	2.43
HiPEG™ C-His_6 val-cit-PAB-MMAE	Monovalent	3.1-myc-his	1	5.2 ± 2.6nM Max cell kill 59% (*n= 4)*	5.21
HiPEG™ D-His_6 val-cit-PAB-MMAE	Monovalent	HEL4-his		>300nM *(n= 3)*	
HiPEG™ A-2-A-His_6 val-cit-PAB-MMAE	Bivalent	2.1-(G4S)6-2.1	1	0.32 ± 0.2nM Max cell kill 57% *(n= 3)*	0.32
HiPEG™ B-2-B-His_6 val-cit-PAB-MMAE	Bivalent	1.1-(G4S)6-1.1	0.7	18 ± 8nM *(n= 3)* Max cell kill *80% (Estimated)*	12.6
HiPEG™ C-2-C-His_6 val-cit-PAB-MMAE	Bivalent	3.1-(G4S)6-3.1	1	4.5 ± 2.4nM Max cell kill 69% *(n= 3)*	4.54
HiPEG™ A-1-B-His_6 val-cit-PAB-MMAE	Biparatopic	2.1-(G4S)2-1.1	1	0.67 ± 0.3nM Max cell kill 75% *(n= 4)*	0.67
HiPEG™ A-2-B-His_6 val-cit-PAB-MMAE	Biparatopic	2.1-(G4S)6-1.1	1	0.37 ± 0.1nM Max cell kill 78% *(n= 3)*	0.37
HiPEG™ B-1-A-His_6 val-cit-PAB-MMAE	Biparatopic	1.1-(G4S)2-	1	0.13 ± 0.1nM Max cell kill 79%	0.13

		2.1		*(n= 3)*	
HiPEG™ B-2-A-His_6 val-cit-PAB-MMAE	Biparatopic	1.1-(G4S)6-2.1	1	0.15 ± 0.1nM Max cell kill 79% *(n= 3)*	0.15
ThioBridge™ anti-PSMA val-cit-PAB-MMAE	Control ADC	control ADC	4	0.03 ± 0.02nM Max cell kill 82% *(n= 3)*	0.13

Table 35. Summary of *in vitro* cytotoxicity data obtained with the human-PSMA-expressing human cell line following a 72 hour incubation.

Construct	**Format**	**VH**	**DAR**	**IC_{50} (nM)**	**IC_{50} (toxin) nM**
HiPEG™ A-His_6 val-cit-PAB-MMAE	monovalent	2.1-myc-his	1	0.55	0.55
HiPEG™ B-His_6 val-cit-PAB-MMAE	monovalent	1.1-myc-his	0.9	4.1	3.69
HiPEG™ A-2-A-His_6 val-cit-PAB-MMAE	bivalent	2.1-(G4S)6-2.1	1	0.19	0.19
HiPEG™ B-2-B-His_6 val-cit-PAB-MMAE	bivalent	1.1-(G4S)6-1.1	0.7	21	14.7
HiPEG™ A-2-B-His_6 val-cit-PAB-MMAE	biparatopic	2.1-(G4S)6-1.1	1	0.29	0.29
HiPEG™ B-1-A-His_6 val-cit-PAB-MMAE	biparatopic	1.1-(G4S)2-2.1	1	0.1	0.1
ThioBridge™ anti-PSMA val-cit-PAB-MMAE	mAb	Control ADC	4	0.042	0.168

The order of potency observed for the monovalent constructs was V_H2.1>V_H1.1>V_H3.1.

The V_H2.1 bivalent construct was observed to be approximately 3-fold more potent than the V_H2.1 monovalent construct, however the max kill% was reduced when using the bivalent construct.

The V_H1.1 bivalent construct was observed to be approximately 6-fold less potent than the V_H1.1 monovalent construct.

The V_H3.1 bivalent construct had comparable activity to the V_H3.1 monovalent construct, with a slightly improved max kill % being observed.

The order of the VH in the biparatopic construct was found to impact on activity, with 2.5 to 4.6-fold differences being observed. The biparatopic construct in the orientation (N-C) V_H2.1-V_H1.1 was observed to be less active than when in the orientation V_H1.1-VH2.1.

The linker length was observed to have a minimal impact on activity (1.2 to 2.2 fold) in the experiments performed. However for some constructs tested the (G4S)6 linker version was slightly more active.

The biparatopic constructs showed improved activity over the monovalent constructs. The most active biparatopic was found to be approximately 7-fold more active than the most active monovalent.

The most active biparatopic was 4.7-fold less active than the control anti-PSMA mAb; however the DAR for the mAb was 4, whereas the DAR for the biparatopic construct was 1. All constructs showed the expected potency, with the biparatopic 1.1-2.1HDC being of comparable potency when compared to the control ADC, when calculating the IC_{50} with respect to the toxin concentration.

Procedure for the Preparation of Humabody™ drug conjugates (HDCs)

A stock solution of conjugation reagent, HiPEG™ val-cit-PAB-MMAE (Figure 22), was prepared in MeCN prior to performing conjugation reactions. A solution of Humabody™ (0.9 mg/mL in PBS; 20 mM EDTA, pH 7.5) was mixed gently with HiPEG™ val-cit-PAB-MMAE reagent (1.5 equiv. per Humabody™; 5% (v/v) final MeCN concentration) and incubated at 22 ^oC for 19 h. After 19 h, the conjugation reaction was mixed with an equal volume of 600 mM sodium phosphate buffer (150 mM NaCl; 20 mM EDTA), pH 7.5 and cooled to 4 ^oC. A stock solution of 1 mg/mL $NaBH_4$ solution was prepared in 0.1 M NaOH. Two aliquots each of $NaBH_4$ solution, (10 equiv. per reagent), were added to the cooled conjugation reaction with a 30 min interval between additions. After a further 30 min interval, the crude mixture was purified by hydrophobic interaction chromatography (HIC) using a TOSOH ToyoPearl Phenyl-650S column. The sample was bound and washed onto the column using 50 mM sodium phosphate (2 M NaCl), pH 7 (buffer A) and eluted using a gradient of 50 mM sodium phosphate (20% v/v isopropanol), pH 7 (buffer B). Fractions containing the mono-loaded product were pooled and concentrated using Vivaspin20 concentrators fitted with 5 kDa MWCO PES membranes. The concentrated fractions were buffer exchanged into DPBS

using PD10 columns and the buffer exchanged material sterile filtered using 0.2 µm PVDF syringe filtration unit.

The HiPEG val-cit-PAB-MMAE moiety is attached via a C terminal His6-tag on a V_H. Two histidines are needed for attachment of each "payload" toxin molecule. Humabody V_H, DAR=1 species were purified for use in cytotoxicity studies, in some instances an exact DAR of 1 was not achieved (see table below). In the examples herein a single MMAE moiety was attached, but multiple payloads are possible (DARs > 1).

Procedure for the Preparation of control ADC with Drug : Antibody Ratio (DAR) of 3.5

Positive control antibody Pro_006 is an anti-PSMA antibody composed of heavy and light chain sequences described within US8470330 and exemplified as antibody 006.

Conjugation 1: A solution of mAb Pro_006 (5.07 mg/mL) in reaction buffer (20 mM sodium phosphate, 150 mM NaCl; 20 mM EDTA, pH 7.5), was warmed to 40 °C for 15 min. TCEP (5 mM, 2 equiv. per mAb) was added to the mAb solution, mixed gently and incubated at 40 °C for 1 h. A stock solution of conjugation reagent, mc-val-cit-PAB-MMAE (Figure 23) was prepared in DMF at 2.8 mM. The reduced mAb was cooled to 22 °C, diluted to 4.2 mg/mL with reaction buffer and mc-val-cit-PAB-MMAE (5.25 equiv. per mAb) was added. The conjugation mixture was incubated at 22 °C for 2 h. The crude conjugation mixture was treated with 50 mM *N*-acetyl-L-cysteine (20 equiv. over reagent) at 22 °C for 30 min. The reaction mixture was diafiltered against DPBS using a Vivaspin20 concentrator fitted with 30 kDa MWCO PES membranes. The diafiltered ADC solution was buffer exchanged into DPBS using a Centripure P50 column. The DAR of the sample was assessed by HIC (average DAR = 3.21).

Conjugation 2: A solution of mAb Pro_006 (5.07 mg/mL) in reaction buffer (20 mM sodium phosphate 150 mM NaCl; 20 mM EDTA), pH 7.5 was warmed to 40 °C for 15 min. TCEP (5 mM, 2.75 equiv. per mAb) was added to the mAb solution, mixed gently and incubated at 40 °C for 1 h. A stock solution of conjugation reagent, mc-val-cit-PAB-MMAE (Figure 23) was prepared in DMF at 4.0 mM. The reduced mAb was cooled to 22 °C, diluted to 4.2 mg/mL with reaction buffer and mc-val-cit-PAB-MMAE (7 equiv. per mAb) was added. The conjugation mixture was incubated at 22 °C for 2 h. The crude conjugation mixture was treated with 50 mM *N*-acetyl-L-cysteine (20 equiv. over reagent) at 22 °C for 30 min. The reaction mixture was diafiltered against DPBS using a Vivaspin20 concentrator fitted with 30 kDa MWCO PES membranes. The diafiltered ADC solution was buffer exchanged into DPBS using a Centripure P50 column. The DAR of the sample was assessed by HIC (average DAR = 4.52).

Production of average DAR 3.5 ADC: ADC 1 (DAR 3.21) and ADC 2 (DAR 4.52) were mixed in a 4:1 mol ratio to prepare an ADC with intermediate DAR. The resulting sample was sterile filtered using 0.2 µm PVDF syringe filtration unit. The DAR of the sample was assessed by HIC (average DAR = 3.45).

***In vitro* potency of half-life extended HDCs**

The *in vitro* potency of half-life extended HDCs was assessed using the DU145 cell killing assay (72h).

This material described in Table 36 was generated to test the effect of adding a half-life extension moiety to the HDCs. Half-life-extended versions (HLE) were generated using the MSA-binding V_H (SEQ ID No.528). *In vitro* potency was assessed using the DU145 cell killing assay (72h). Figure 24 A & B show IC_{50} values observed in the PSMA-DU145 cytotoxicity assay (72h) for: Control ADC mAB-MMAE (■), 2.1 6GS-1.1-MMAE biparatopic (▲), 2.1-6GS-1.1-MMAE-HLE half-life extended biparatopic (▼), HEL4-MMAE Monovalent (●) and HEL4-HLE-MMAE Monovalent half-life extended (I), for (A) DU145 expressing PSMA and (B) DU145 parental cells that have not been modified to express PSMA.

Table 36 IC_{50} values PSMA-DU145 cytotoxicity assay (72h):

Format	**VH**	**Name**	**DAR**	**IC_{50} (nM)**	**IC_{50} (toxin) (nM)**	**Average Max Cell Kill %**
Monovalent	HEL4	HiPEG™ HEL4-His val-cit-PAB-MMAE	1	>100	>100	
Biparatopic	1.1-6GS-2.1	HiPEG™ 1.1-6GS-2.1-His val-cit-PAB-MMAE	1	0.27	0.27	86
Biparatopic-HLE	1.1-6GS-2.1-6GS-half life extension	HiPEG™ 1.1-6GS-2.1-6GS- half life extension -His val-cit-PAB-MMAE	1	0.82	0.82	82
Monovalent -HLE	HEL4-6GS-half life extension	HiPEG™ HEL4-6GS- half life extension -His val-cit-PAB-MMAE	1	>100	>100	

mAb	Control PSMA mAb-MMAE	Pro_006-mc-val-cit-PAB-MMAE	3.5	0.061	0.2135	89

CLAIMS:

1. An immunoconjugate of the formula A-(L-D)n wherein

   A is an antigen-binding moiety comprising a first human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA, optionally comprising a second human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA and optionally comprising a third human single heavy chain variable immunoglobulin (V_H) domain antibody,

   L is a linker, and

   D is an auristatin or a derivative thereof and n is 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

2. An immunoconjugate according to claim 1 wherein D is MMAE, MMAF, or a derivative thereof.
3. An immunoconjugate according to claim 1 or claim 2 wherein D is MMAE or a derivative thereof conjugated to the antigen-binding moiety via a valine-citrulline (vc) linker (vc-MMAE).
4. An immunoconjugate according to claim 1 or claim 2 wherein D is MMAF or a derivative thereof conjugated to the antigen-binding moiety via a maleimidocaproyl linker (mc-MMAF).
5. An immunoconjugate according to any one of claims 1 to 4 wherein L-D is vedotin or mafodotin.
6. An immunoconjugate according to any one of the preceding claims comprising a first human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding human PSMA and a second human single heavy chain variable immunoglobulin V_H domain antibody capable of binding human PSMA.
7. An immunoconjugate according to any one of the preceding claims wherein said first V_H domain and said second V_H domain bind to the same epitope on human PSMA.
8. An immunoconjugate according to any one of claims 1 to 6 wherein said first single V_H domain antibody binds to a first epitope on PSMA and said second single V_H domain antibody binds to a second epitope on PSMA wherein said first and said second epitope are not identical.
9. An immunoconjugate according to a preceding claim wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 3 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.
10. An immunoconjugate according to a claim 9 wherein said first and/or second single V_H domain antibody comprises CDR1 and CDR2 sequences wherein said CDR1

sequence comprises SEQ ID NO. 1 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and said CDR2 sequence comprises SEQ ID NO. 2 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

11. An immunoconjugate according to claim 9 or 10 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 4 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

12. An immunoconjugate according to any of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 83 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

13. An immunoconjugate according to claim 12 wherein said first and/or second single V_H domain antibody comprises CDR1 and CDR2 sequences wherein said CDR1 sequence comprises SEQ ID NO. 81 or a sequence with at least 60%, at least 70%, at least 80%, at least 90% or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 82 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

14. An immunoconjugate according to claim 12 or 13 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 84 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

15. An immunoconjugate according to any of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 183 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

16. An immunoconjugate according to claim 15 wherein said first and/or second single V_H domain antibody comprises CDR1 and 2 sequences wherein said CDR1 sequence comprises SEQ ID NO. 181 or a sequence with at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and said CDR2 sequence comprises SEQ ID NO. 182 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

17. An immunoconjugate according to claims 15 or 16 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 184 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

18. An immunoconjugate according to any of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 279 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

19. An immunoconjugate according to claim 18 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 277 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 278 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.
20. An immunoconjugate according to claims 18 or 19 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 280 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.
21. An immunoconjugate according to any of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 295 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.
22. An immunoconjugate according to claim 21 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 293 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 294 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.
23. An immunoconjugate according to claim 21 or 22 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 296 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.
24. An immunoconjugate according to any of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 303 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.
25. An immunoconjugate according to claim 24 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 301 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 302 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.
26. An immunoconjugate according to claim 24 or 25 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 304 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.
27. An immunoconjugate according to any one of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 331 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

28. An immunoconjugate according to claim 27 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 329 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 330 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

29. An immunoconjugate according to claim 27 or 28 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 332 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

30. An immunoconjugate according to any one of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 363 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

31. An immunoconjugate according to claim 30 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 361 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 362 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

32. An immunoconjugate according to claim 30 or 31 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 364 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

33. An immunoconjugate according to any one of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 367 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

34. An immunoconjugate according to claim 33 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 365 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 366 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

35. An immunoconjugate according to claim 33 or 34 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 368 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

36. An immunoconjugate according to any one of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 371 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

37. An immunoconjugate according to claim 36 wherein said first and/or second single V_H domain antibody comprise a CDR1 sequence having SEQ ID NO. 369 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 370 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

38. An immunoconjugate according to claim 36 or 37 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 372 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

39. An immunoconjugate according to any of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 375 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

40. An immunoconjugate according to claim 39 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 373 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 374 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

41. An immunoconjugate according to claim 39 or 40 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 376 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

42. An immunoconjugate according to any one of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 379 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

43. An immunoconjugate according to claim 42 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 377 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 378 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

44. An immunoconjugate according to claim 42 or 43 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 380 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

45. An immunoconjugate according to any of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 383 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

46. An immunoconjugate according to claim 45 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 381 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 382 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

47. An immunoconjugate according to claim 45 or 46 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 384 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

48. An immunoconjugate according to any one of claims 1 to 8 wherein said first and/or second V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 387 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

49. An immunoconjugate according to claim 48 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 385 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 386 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

50. An immunoconjugate according to claim 48 or 49 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 388 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

51. An immunoconjugate according to any one of claims 1 to 8 wherein said first and/or second single V_H domain antibody comprises a CDR3 sequence comprising SEQ ID NO. 391 or a sequence with at least 70%, at least 80%, at least 90% or at least 95% homology thereto.

52. An immunoconjugate according to claim 51 wherein said first and/or second single V_H domain antibody comprises a CDR1 sequence having SEQ ID NO. 389 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto and a CDR2 sequence having SEQ ID NO. 390 or a sequence with at least 70%, at least 80%, at least 90%, or at least 95% homology thereto.

53. An immunoconjugate according to claims 51 or 52 wherein said first and/or second single V_H domain antibody comprises or consists of SEQ ID NO. 392 or a sequence with at least 70%, 75%, 80%, 85%, 90% or 95% homology thereto.

54. An immunoconjugate according to any of claims 1, 2 or 4 to 48 wherein said first and /or second single V_H domain antibody have the same V_H domain.

55. An immunoconjugate according to claim 8 in which a) the first single V_H domain antibody is selected from a single V_H domain antibody according to any of claims 12-20, 27-29, 33-41, 48-50 and in which b) the second single V_H domain antibody is

selected from a single V_H domain antibody according to any of claims 9-11, 21-26, 42-47.

56. An immunoconjugate according to claim 9 in which a) the first single V_H domain antibody is selected from a single V_H domain antibody according to any of claims 12-14, and in which b) the second single V_H domain antibody is selected from a single V_H domain antibody according to any of claims 9-11.

57. An immunoconjugate according to claim 8 or 9 in which the first single V_H domain antibody is located C or N terminally to the second single V_H domain antibody.

58. An immunoconjugate according to claim 8 in which a) the first single V_H domain antibody can compete with a Humabody® V_H comprising SEQ ID NO: 4 for binding to human PSMA and/or can block the binding of a Humabody® V_H comprising SEQ ID NO: 4 to human PSMA and in which b) the second single V_H domain antibody can compete with a Humabody® V_H comprising SEQ ID NO: 76 for binding to human PSMA and/or can block the binding of a Humabody® V_H comprising SEQ ID NO:76 to human PSMA.

59. An immunoconjugate according to any one of the preceding claims wherein said first and second single V_H domain antibodies are covalently linked by a peptide.

60. An immunoconjugate according to claim 59 wherein the peptide is a length in the range of from 3 to 50 amino acids long.

61. An immunoconjugate according to claim 59 or 60 wherein the linker comprises glycine and serine amino acid residues.

62. An immunoconjugate according to claim 61 wherein the peptide linker consists of the formula $(Gly_4Ser)_n$, where n = from 1 to 10.

63. An immunoconjugate according to claim 61 or claim 62 wherein the peptide linker consists of the formula $(Gly_4Ser)_n$, where n = 2, 3, 4, 5, 6, 7, or 8

64. An immunoconjugate according to any one of the preceding claims wherein the immunoconjugate comprises a half-life extending moiety.

65. An immunoconjugate according to claim 64 wherein said half-life extending moiety is covalently linked to said first or second V_H domain.

66. An immunoconjugate according to claim 55 wherein said half-life extending moiety is selected from the group consisting of an albumin binding moiety, a transferrin binding moiety, a polyethylene glycol molecule, a recombinant polyethylene glycol molecule, human serum albumin, a fragment of human serum albumin, murine serum albumin, a fragment of murine serum albumin and an albumin binding peptide.

67. An immunoconjugate according to any one of the preceding claims wherein said immunoconjugate is capable of being internalised by a cell.

68. An immunoconjugate according to any one of the preceding claims wherein said immunoconjugate is conjugated to a further cytotoxic moiety.

69. An immunoconjugate according to any one of the preceding claims wherein said immunoconjugate is conjugated to a label.

70. An immunoconjugate according to any one of the preceding claims wherein said immunoconjugate is devoid of immunoglobulin light chains.

71. An immunoconjugate according to any one of the preceding claims wherein said first and / or second single V_H domain antibody is obtained from a library obtained from a knock-out mouse in which the endogenous light chain and heavy chain loci are functionally silenced and which expresses a transgene with one or more human heavy chain genes.

72. A pharmaceutical composition comprising an immunoconjugate according to any one of the preceding claim and a pharmaceutical carrier.

73. A method for treating a cancer associated with expression of PSMA comprising administering a therapeutically-effective amount of a immunoconjugate according to any of claims 1 to 71 or a pharmaceutical composition according to claim 72.

74. A method for treating a cancer associated with expression of PSMA, prostate cancer or a prostatic disorder comprising administering a therapeutically-effective amount of a immunoconjugate according to any of claims 1 to 71or a pharmaceutical composition according to claim72.

75. An immunoconjugate according to any of claims 1 to 71 or a pharmaceutical composition according to claim 72 for use as medicament.

76. An immunoconjugate according to any of claims 1 to 71 or a pharmaceutical composition according to claim 72 for use in the treatment of a cancer associated with expression of PSMA, prostate cancer or a prostatic disorder.

77. Use of an immunoconjugate according to claims any of claims 1 to 71 or a pharmaceutical composition according to claim 72 in the manufacture of a medicament for the treatment of a cancer associated with expression of PSMA, prostate cancer or a prostatic disorder.

78. A method for delivering a cytotoxic moiety into a tumor cell comprising exposing said cell to an immunoconjugate according to any one of claims 1 to 71 or a pharmaceutical composition according to claim72.

79. An immunoconjugate according to any one of claims 1 to 71 or a pharmaceutical composition according to claim 72 for use in a method of imaging in a human or animal subject.

80. Use of an immunoconjugate according to any one of claims 1 to 71 or a pharmaceutical composition according to claim 72 for the preparation of a radiolabled compound.

81. A method for imaging a PSMA-expressing tumor or cell, the method comprising contacting the tumor or cell with an effective amount of immunoconjugate according to any one of claims 1 to 71 or a pharmaceutical composition according to claim 72.

82. A method for determining the presence of PSMA in a test sample or detecting a tumor cell by an immunoassay comprising contacting said sample with immunoconjugate according to any one of claims 1 to 71 or a pharmaceutical composition according to claim 72 and at least one detectable label.

83. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an antigen-binding moiety of an immunoconjugate according to any of claims 1 to 71, said antigen binding moiety comprising a first human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA and optionally a second human single heavy chain variable immunoglobulin (V_H) domain antibody capable of binding specifically to human PSMA.

84. A construct comprising a nucleic acid molecule according to claim83.

85. A host cell comprising a nucleic acid according to claim 83 or a construct according to claim84.

86. A kit comprising an immunoconjugate according to any one of claims 1 to 71 or a pharmaceutical composition according to claim72.

87. A method for producing an antigen-binding moiety of an immunoconjugate according to any one of claims 1 to 71 comprising expressing a nucleic acid encoding said antigen-binding moiety in a host cell and isolating the antigen-binding moiety from the host cell culture.

88. A method of determining if a subject has cancer comprising administering to the subject a composition comprising an immunoconjugate according to any one of claims 1 to 71 or a pharmaceutical composition according to claim 72 and obtaining an image of the subject; thereby determining if the subject has cancer.

1.1
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGKGLEWVSSIGENDGTTDYADSVKGRFTISRD
NSKSMLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.2
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIGDNNNSTEYADSVKGRFTISR
DNSKSTLYLQMNSLSAEDTAVYYCVKDGVHWGQGTLVTVSS
1.3
EVQLVESGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGKGLEWVSSIGDNNNSTDYADSVKGRFTISR
DNSKSTLYLQMNSLRAEDTAVYYCVKDGVHWGQGTLVTVSS
1.4
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIGDGTTYYADSVKGRFTISRDNS
KSTLYLQMNSLRAEDTAVYYCAKDGVHWGQGTLVTVSS
1.5
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMSWVRQAPGKGLEWVSSIGENDRTTYYVDSVKGRFTISRD
NSKSTLYLQMNSLRAEDTAVYYCAKDGVHWGQGTLVTVSS
1.6
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIGDNNRTTYYADSVKGRFTISR
DNSKSTLYLQMNSLRAEDTAVYYCAKDGVHWGQGTLVTVSS
1.7
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSSIGDGTTYYADSVKGRFTISRDNS
KSTLYLQMNSLRAEDTAVYYCAKDGVHWGQGTLVTVSS
1.8
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYAMSWVRQAPGKGLEWVSSIGENDGTTDYADSVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.9
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENDGTTDYADSVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.10
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNATTDYADFVKGRFTISRDN
SKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.11
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNDTTDYADNVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.12
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNATTDYADAVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.13
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNHTTDYAADVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.14
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNATTDYADVVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.15
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNHTTDYAAFVKGRFTISRDN
SKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.16
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNHTTDYADTVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.17
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNDTTDYADAVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS

Figure 1

1.18
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNATTDYAASVKGRFTISRDN
SKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.19
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNDTTDYAAVVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS
1.20
EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYALSWVRQAPGKGLEWVSSIGENNHTTDYAATVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCVKDGVHWGQGTLVTVSS

Figure 1 Continued

2.1
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDGSNKYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.2
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDGSNKYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.3
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAHISYDGSNRYYAESVKGRFTISREN
SKNTLSLQMNSLRAEDTAVYYCAKDPAWGLRLGELSSYDFDIWGQGTMVTVSS

2.4
QVTLKESGGGVVQPGRSLKLSCAASGFSFSGYGMHWVRQAPGKGLEWVAVISYDGSNRYYADSVKGRFTISRD
NSKNTLSLQMNSLRAEDTAVYYCARDPAWGLRLGELSSYDFEIWGQGTMVTVSS

2.5
QVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAVISYDGSNRYYADSVKGRFTISRD
NSKNTLSLQMNSLRAEDTAVYYCAKDPAWGLRLGELSSYDFEIWGQGTMVTVSS

2.6
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGELSSYKFEIWGQGTMVTVSS

2.7
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVALISYDGSNKYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGEQSSYAFDIWGQGTMVTVSS

2.8
QVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVSVISYDGSNKYYADSVKGRFTISRD
NSKNTLYLQMNSLRTEDTAVYYCAKDPAWGLRLGEQSSYAFEIWGQGTMVTVSS

2.9
EVQLLESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISRDN
SKNTLYLQMNSLRVEDTAVYYCAKDPAWGLRLGEQSSYAFEIRGQGTTVTVSS

2.10
EVQLVESGGGVVQPGRSLRLSCAASGFTFSGYGMHWVRQAPGKGLEWVAYISYDGSNRYYADSVKGRFTISRD
NSKKTLSLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

Figure 2

2.11
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGLHWVRQAPGKGLEWVAYISYDESNKYYAPSVKGRFTISRDNS
KNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.12
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDKSNKYYADKVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.13
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGLHWVRQAPGKGLEWVAYISYDASNKYYADNVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.14
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGVHWVRQAPGKGLEWVAYISYDASNKYYADNVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.15
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGLHWVRQAPGKGLEWVAYISYDKSNKYYADKVKGRFTISRDNS
KNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.16
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGAHWVRQAPGKGLEWVAYISYDKSNKYYADKVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.17
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDASNKYYADNVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.18
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGQHWVRQAPGKGLEWVAYISYDASNKYYADNVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.19
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGFHWVRQAPGKGLEWVAYISYDASNKYYADNVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFDIWGQGTMVTVSS

2.20
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAIISYDGSNRYYADSVKGRFTISRDN
SKNTLSLQMNSLRAEDTAVYYCAKDPAWGLRLGESSSYDFEIWGQGTMVTVSS

2.21
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NSKNTLSLQMNSLRAEDTAVYYCAKDPAWGLRLGKLSSYDFEIWGQGTMVTVSS

2.22
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGTHWVRQAPGKGLEWVAYISYDGSNKYYAAPVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDAAWGLRLGESSSYDFDIWGQGTMVTVSS

2.23
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGTHWVRQAPGKGLEWVAYISYDESNKYYASSVKGRFTISRDNS
KNTLYLQMNSLRAEDTAVYYCAKDRAWGLRLGESSSYDFDIWGQGTMVTVSS

2.24
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGMHWVRQAPGKGLEWVAYISYDESNKYYARLVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDTAWGLRLGESSSYDFDIWGQGTMVTVSS

2.25
EVQLVESGGGVVQPGRSLRLSCAASGFSFSGYGLHWVRQAPGKGLEWVAYISYDLSNKYYARGVKGRFTISRDNS
KNTLYLQMNSLRAEDTAVYYCAKDVAWGLRLGESSSYDFDIWGQGTMVTVSS

Figure 2 Continued

3.1

EVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMHWVRQAPGKGLEWVAFMTYDGSNRYYADSVKGRFTISRD
NSKNTLYLQMNSLRDEDTALYYCARDRIVGGRVPDAFDIWGQGTMVTVSS

3.2

EVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFISYDGSNKYYADSVKGRFTISKDN
SKNTLYLQMNSLRAEDTAVYYCAKDRIVGARVPDAFDIWGQGTMVTVSS

3.3

EVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLEWVAFISYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDRIVGARVPDAFDIWGQGTMVTVSS

3.4

EVQLVESGGGAVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.5

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.6

EVQLLESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.7

QVQLVESGGGLVQPGGSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLHLQMDSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.8

EVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTLVTVSS

3.9

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLEWVAFISYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.10

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLHLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.11

EVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLHLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.12

EVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

Figure 3

3.13

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.14

EVQLVESGGGVVRPGGSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLHLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.15

EVQLVESGGGLVQPGGSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLHLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.16

EVQLLESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLHLQMNSLRPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.17

EVQLLESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLKPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.18

EVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLDWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLKPEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

3.19

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMHWVRQAPGKGLEWVAFMTYDGSNRYYADAVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCARDRIVGGRVPDAFDIWGQGTMVTVSS

3.20

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMHWVRQAPGKGLEWVAFQTYDGSNRYYADAVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCARDRIVGGRVPDAFDIWGQGTMVTVSS

3.21

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NSKNTLYLQMNSLRAEDTAVYYCARDRIVGGRVPDAFDIWGQGTMVTVSS

3.22

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMHWVRQAPGKGLEWVAFQTYDASNRYYADSVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCARDRIVGGRVPDAFDIWGQGTMVTVSS

3.23

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMHWVRQAPGKGLEWVAFQTYDASNRYYADAVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCARDRIVGGRVPDAFDIWGQGTMVTVSS

3.24

QVQLVESGGGVVQPGRSLRLSCAASGFPLISYGMNWVRQAPGKGLEWVAFITYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRAEDTAVYYCAKDRIVGARVPDAYDIWGQGTMVTVSS

Figure 3 Continued

4.1

QVQLVESGGGVVQPGRSLRLSCVASGFPFSYGMHWVRQAPGKGREWVAVISYDGSNRYYADSVKGRFTISRD
NSKNTLYLQMNSLRPEDTAVYYCAKERIFGVLTPDDFDIWGQGTTVTVSS

4.2

QVQLVESGGGVVQPGRSLRLSCAASGFPFSYGMHWVRQAPGKGLEWVAVISYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKERIFGVLTPDDFDIWGQGTTVTVSS

4.3

EVQLLESGGGVVQPGRSLRLSCAASGFPFSYGMHWVRQAPGKGLEWVAVISYDGANRYYADSVKGRFTISRDN
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4.4

EVQLVESGGGVVQPGRSLRLSCAASGFTFTSYGMHWVRQAPGKGLEWVAVISYDGSNRYYADSVKGRFTISRDN
SKNTLYLQMNSLRPEDTAVYYCAKERIFGALTPDDFDIWGQGTTVTVSS

Figure 4

5.1

QVQLVESGGGVVQPGRSLRLSCAASGFTFNNYGMHWVRQAPGKGLEWVAIISYDGNTKYYTDSVKGRFTISRD
NSKNTLYLQMNSLRVEDTAVYYCAKGLWPSDVWGQGTTVTVSS

5.2

EVQLVESGGGVVQPGRSLRLSCAASGFTFNNYGMHWVRQAPGKGLEWVAIISYDGNSKYYTDSVKGRFTISRDN
SKNTLYLQMNSLRVEDTAVYYCAKGLWPSDVWGQGTTVTVSS

Figure 5

6.1

QVQLQESGPGLVKPSQTLSLTCTVSGGSISNSGYYWSWVRQHPGKDLEWIGFIYYNGSIHYNPSLKSRVIISVDTSK
NQFSLKMNSVTAADTAVYYCARDGDDYGDYLRGQGTLVTVSS

6.2

QVQLQESGPGLVKPSQTLSLTCTVSGGSISNSGYYWSWIRQHPGKGLEWIGFIYYNGSIHYNPSLKSRVIISVDTSK
NQFSLKMSSVTAADTAVYYCARDGDDYGDYLRGQGTLVTVSS

6.3

QVQLQESGPGLVKPSQTLSLTCTVSGGSISNSGYYWSWVRQHPGKGLEWIGFIYYNGSIHYNPSLKSRVIISVDTSK
NQFSLKLNSVTAADTAVYYCARDGDDYGDYLRGQGTLVTVSS

6.4

QVQLQESGPGLVKPSQTLSLTCTVSGGSISNSGYYWSWIRQHPGKGLEWIGFIYYNGSIHYNPSLKSRVIISVDTSK
NQFSLKLSSVTAADTAVYYCARDGDDYGDYLRGQGTLVTVSS

6.5

QVQLQESGPGLVKPSQTLSLTCTVSGGSISNSGYYWSWVRQHPGKGLEWIGFIYYNGSIHYNPSLKSRVTISVDTS
KNQFSLKMSSVTAADTAVYYCARDGDDYGDYLRGQGTLVTVSS

6.6

QVQLQESGPGLVKPSQTLSLTCTVSGGSISNSGYYWSWVRQHPGKGLEWIGFIYYNGSIHYNPSLKSRVTISVDTS
KNQFSLKLNSVTAADTAVYYCARDGDDYGDYLRGQGTLVTVSS

6.7

QVQLQESGPGLVKPSQTLSLTCTVSGGSISNSGYYWSWVRQHPGKGLEWIGFIYYNGSIHYNPSLKSRVTISVDTS
KNQFSLKLSSVTAADTAVYYCARDGDDYGDYLRGQGTLVTVSS

Figure 6

7.1

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHDGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDSLIVGERGYWGQGTLVTVSS

7.2

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHDGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDNLIVGERGYWGQGTLVTVSS

7.3

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHGGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDSLIVGERGYWGQGTLVTVSS

7.4

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHQGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDSLIVGERGYWGQGTLVTVSS

7.5

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHPGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDSLIVGERGYWGQGTLVTVSS

7.6

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHEGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDSLIVGERGYWGQGTLVTVSS

7.7

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHIGSEKYYVDSVKGRFTISRDN
AKNSLYLQMNSLRAEDTAVYYCARDSLIVGERGYWGQGTLVTVSS

7.8

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVANINHDGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDTLIVGERGYWGQGTLVTVSS

Figure 7

8.1

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSRVTISVDTSK
NQFSLKLSSVTAADTAVYYCARGPIPATAIPDAFDIWGQGTMVTVSS

Figure 8

9.1

QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGHYWSWIRQPPGKGLEWIGDINHSGSTNYNPSLKSRVTISVDTSK
NQFSLKLSSVTAADTAVYYCVRDYGDSRSLFDYWGQGTLVTVSS

Figure 9

10.1

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFMSYDGSNKYYVDSVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCAKGDYDFWSGYPDYDMDVWGQGTTVTVSS

Figure 10

11.1

EVQLVESGGGLVKPGGSLRLSCAASGFNLISYGMYWVRQAPGKGLEWVAVISYDGSNKNYADSVKGRFTISRDN
SKNTLFLQMNSLRVEDTAVYYCAKGGNALYSSGWPDDGFDIRGQGTMVTVSS

Figure 11

12.1

QVQLVESGGGVVQPGRSLRLSCAASGFTFSNFGMHWVRQAPGKGLEWVAVISYDGNSKYYADTVKGRFTISRD
NSKNTLYLEMNSLRADDTAVYYCAKGLWPPMDVRGQGTTVTVSS

Figure 12

13.1

EVQLVESGGGSVQPGGSLRLSCAASGFTFSDYWMTWVRQVPGKGLEWVANIKQDGSEKYYVDSVKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARDRGGAVALYHNGMDMGGQGTTVTVSS

Figure 13

14.1

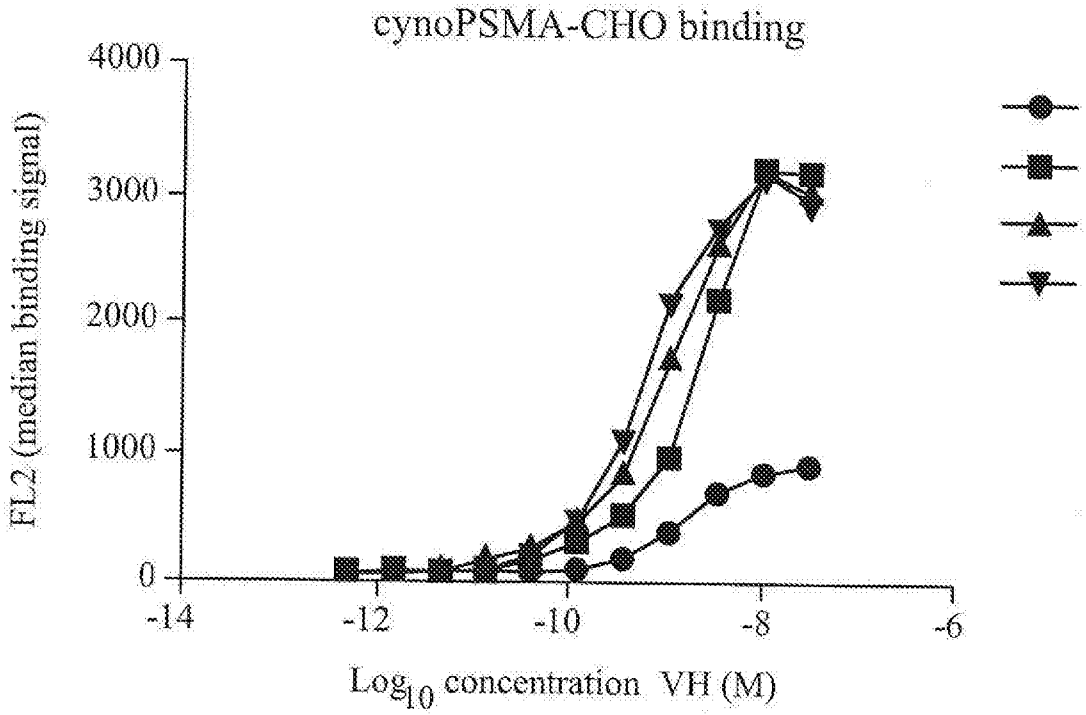
KCSWWSLGEVKKPGASVKVSCKASGYTFTSYDINWVRQATGQGLEWMGWMNPNSGNTGYAQKFQGRVTMT
RNTSISTAYMELSSLRSEDTAVYYCARGNGPGITGTTDYWGQGTLVTVSS

Figure 14

15.1

EVQLVESGGGVVRPGGSLRLSCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGDRTGYADSVKGRFTISR
DNAKNSLYLQMNSLRAEDTALYYCGRENVIVPAATYWGQGTLVTVSS

Figure 15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1000
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-14
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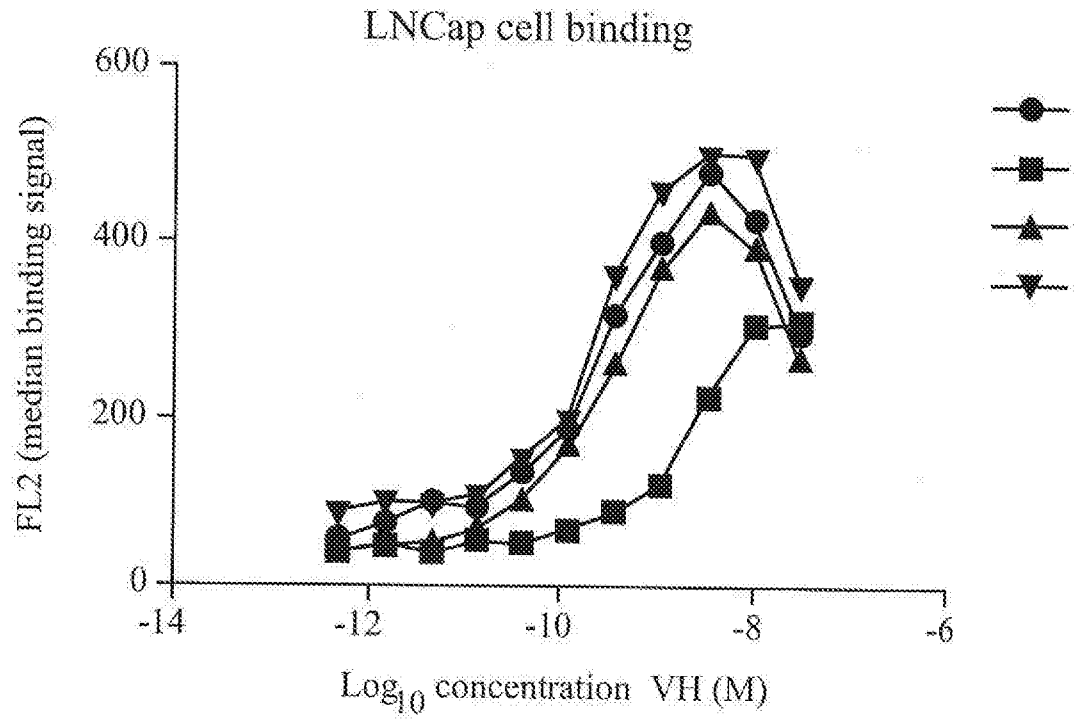
-8
-6
Log10 concentration VH (M)


LNCap cell binding
FL2 (median binding signal)
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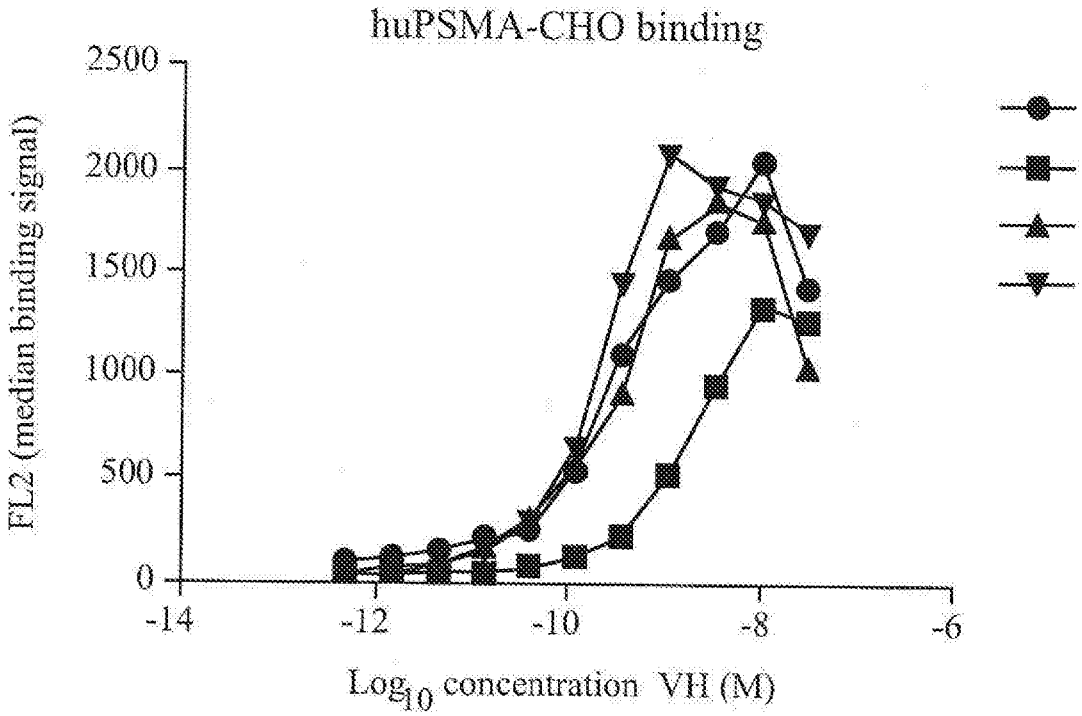
400
200
0
-14
-12
-10
-8
-6
Log10 concentration VH (M)

Figure 16a



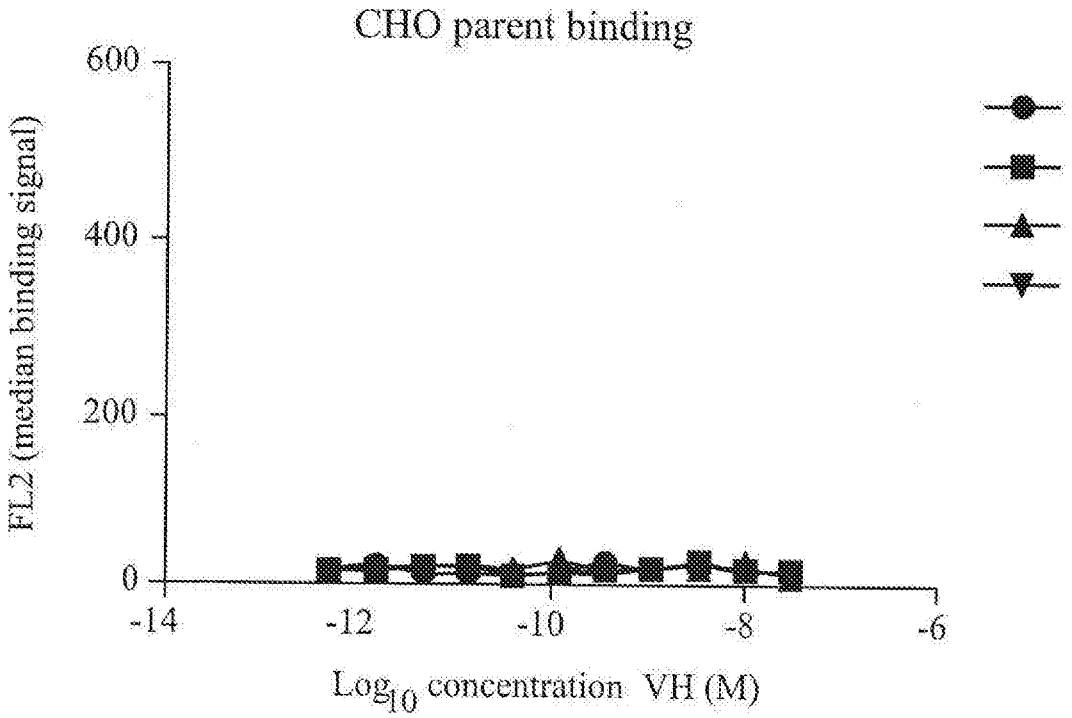
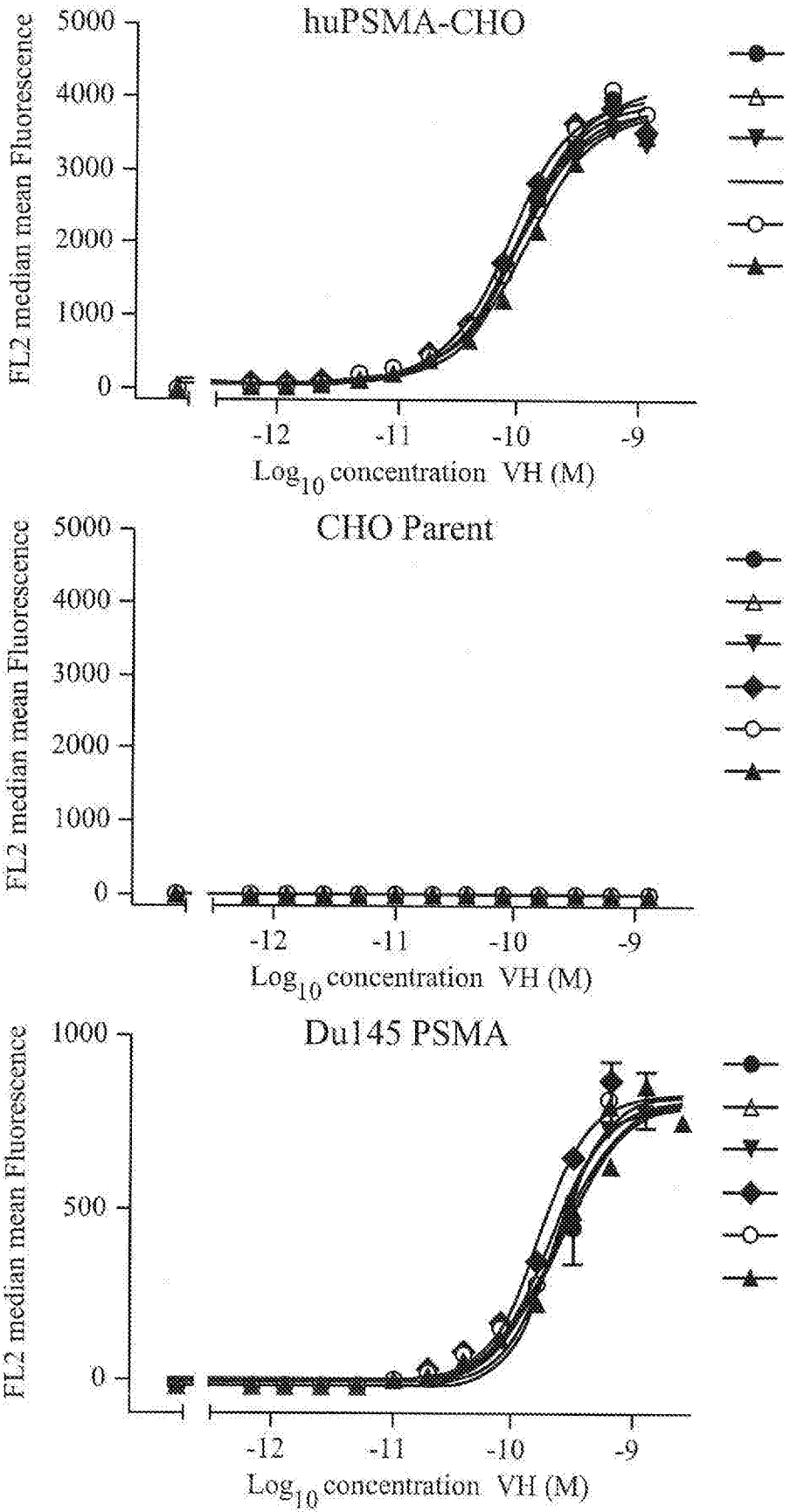


Figure 16a Continued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FL2 median mean Fluorescence
5000
4000
3000
2000
1000
0
-12
-11
-10
-9
Log10 concentration VH (M)
CHO Parent
FL2 median mean Fluorescence
5000
4000
3000
2000
1000
0
-12
-11
-10
-9
Log10 concentration VH (M)
Du145 PSMA
FL2 median mean Fluorescence
1000
500
0
-12
-11
-10
-9
Log10 concentration VH (M)

Figure 16b

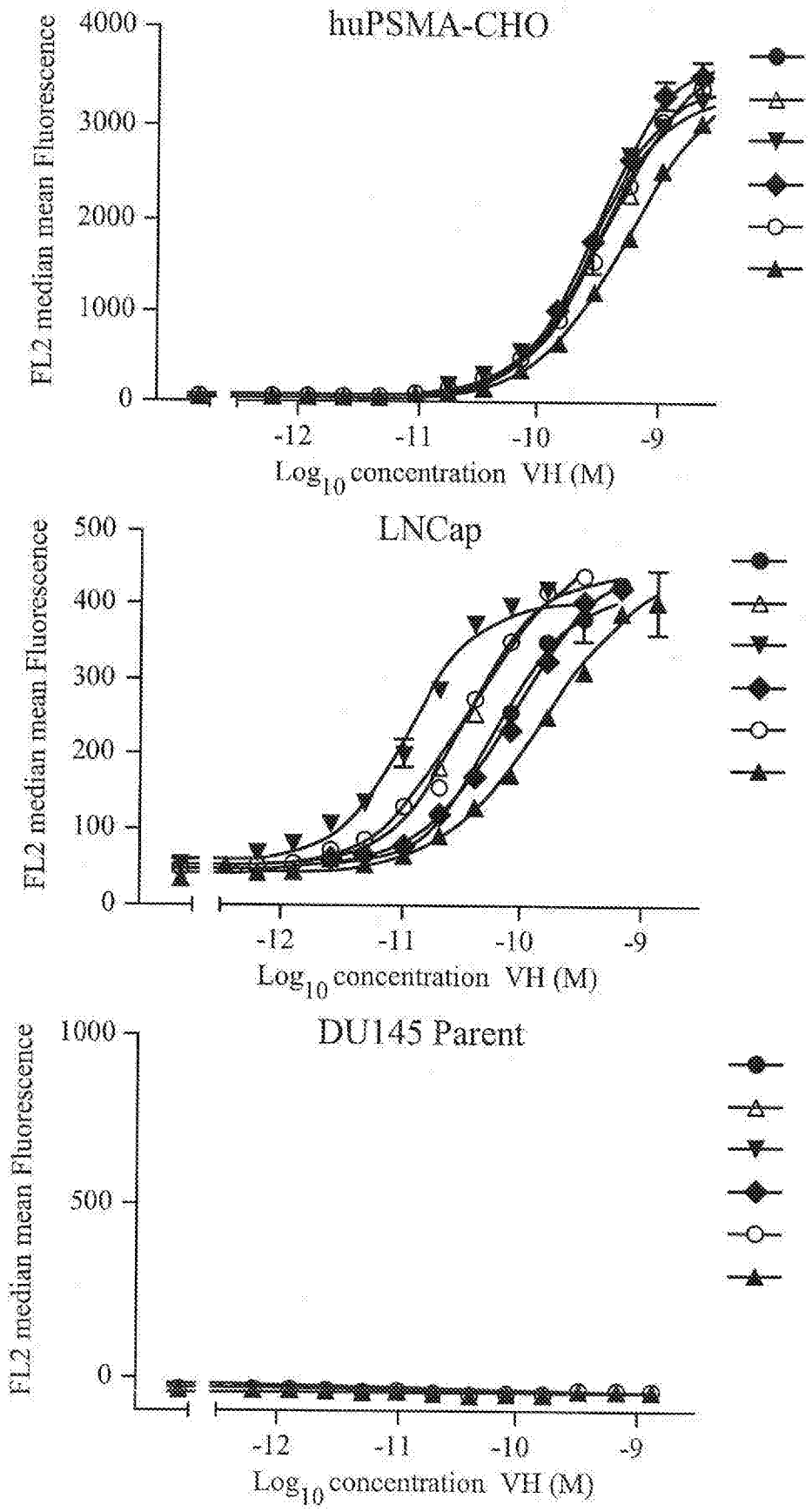

Figure 16b Continued

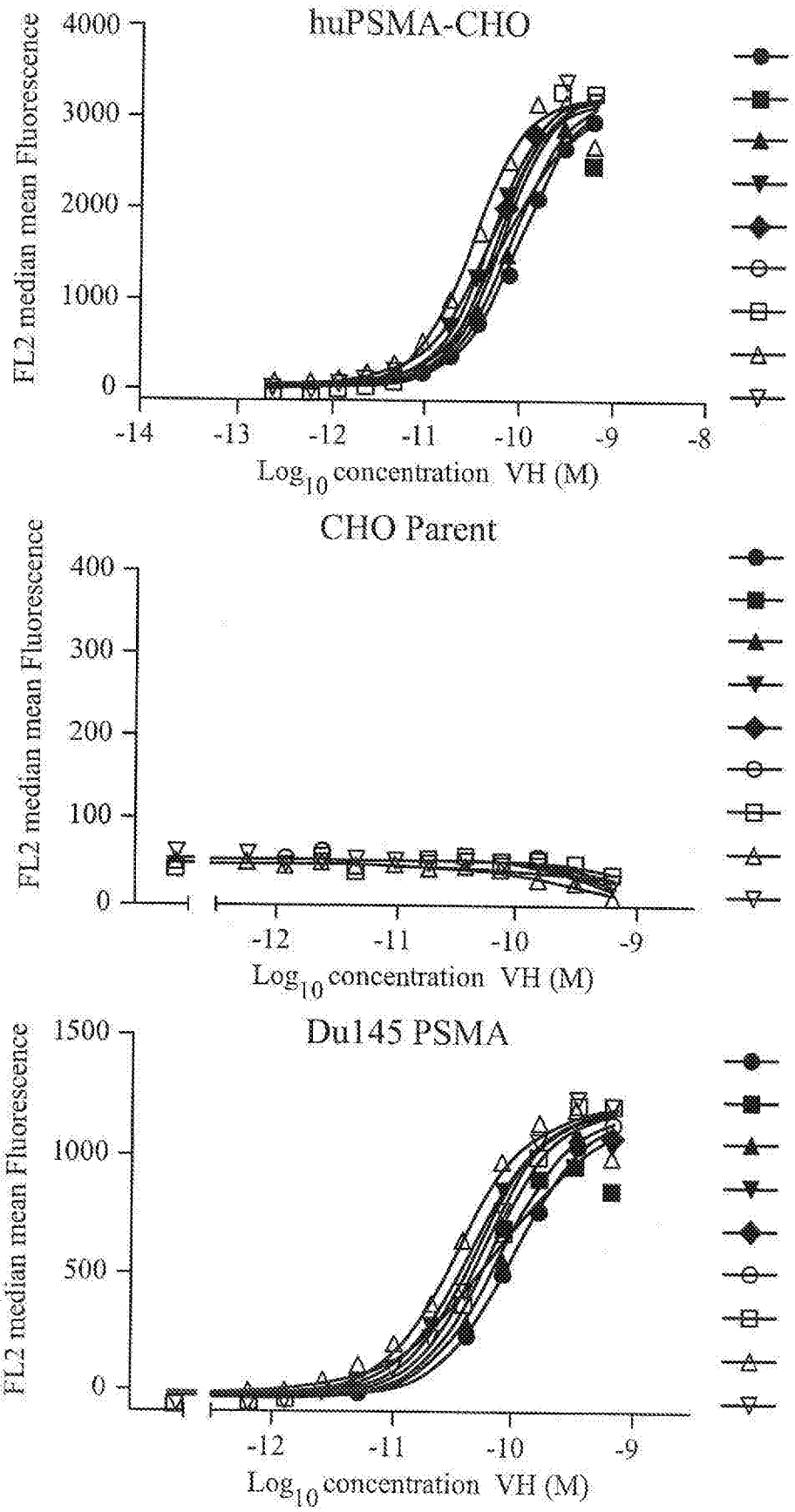
huPSMA-CHO
FL2 median mean Fluorescence
4000
3000
2000
1000
0
-14
-13
-12
-11
-10
-9
-8
Log10 concentration VH (M)
CHO Parent
FL2 median mean Fluorescence
400
300
200
100
0
-12
-11
-10
-9
Log10 concentration VH (M)
Du145 PSMA
FL2 median mean Fluorescence
1500
1000
500
0
-12
-11
-10
-9
Log10 concentration VH (M)

Figure 16c

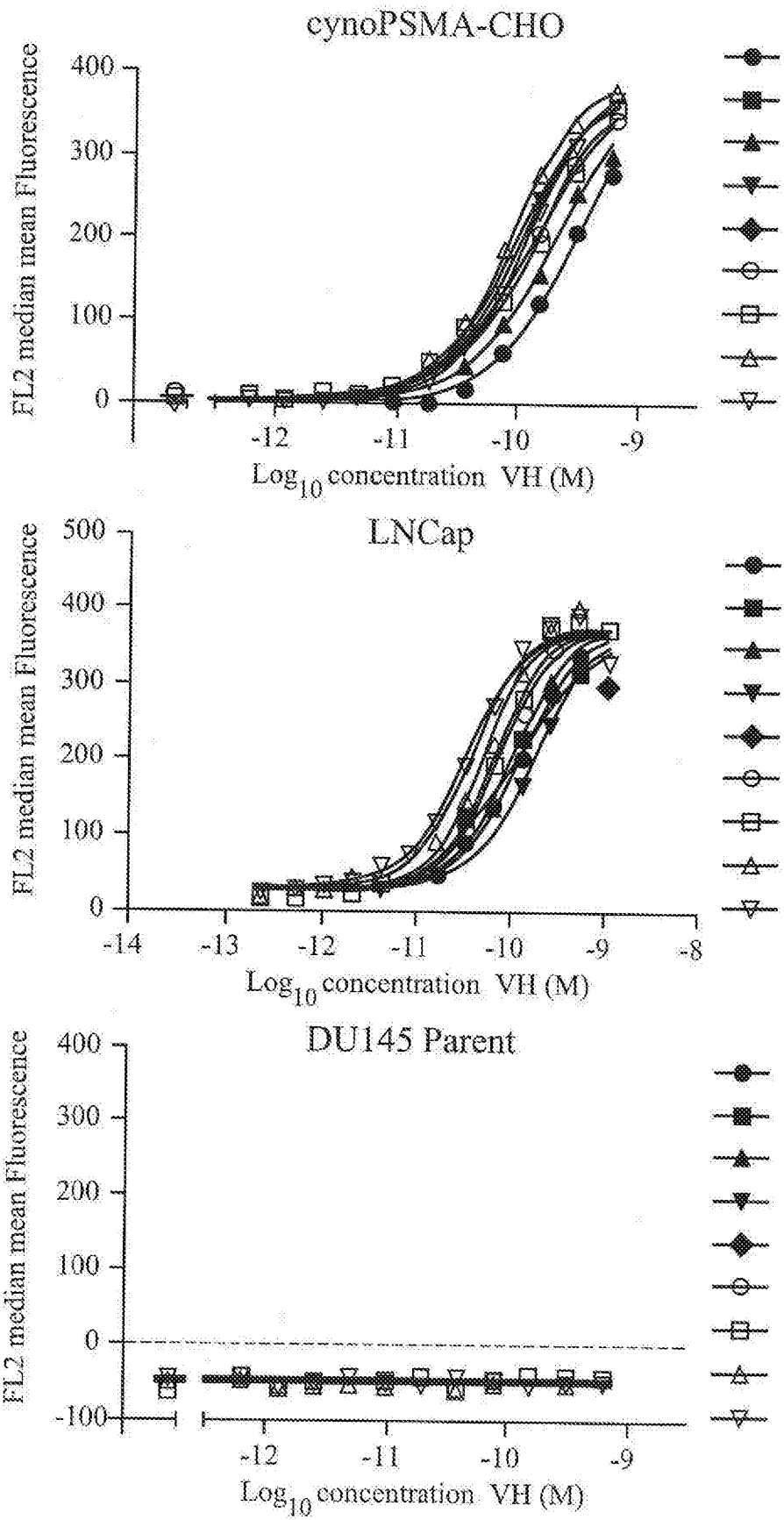

Figure 16c Continued

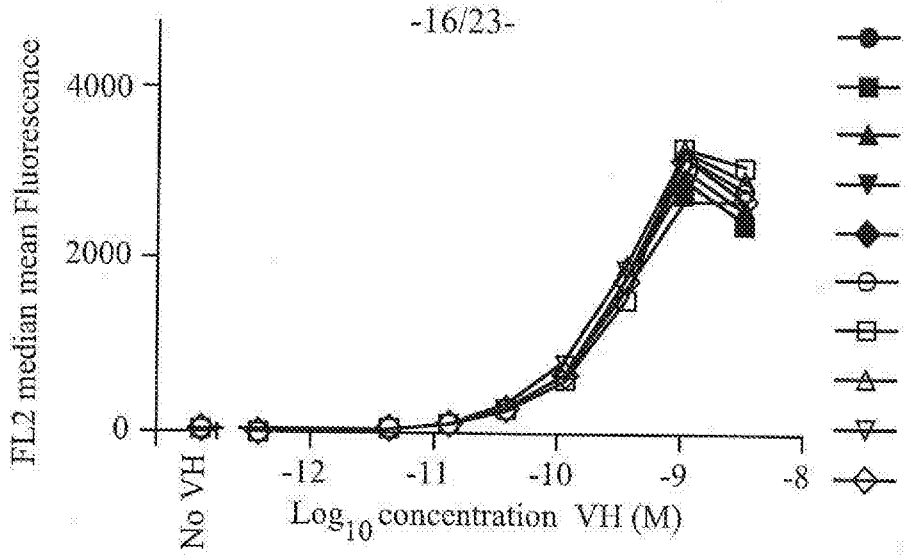

Figure 16d

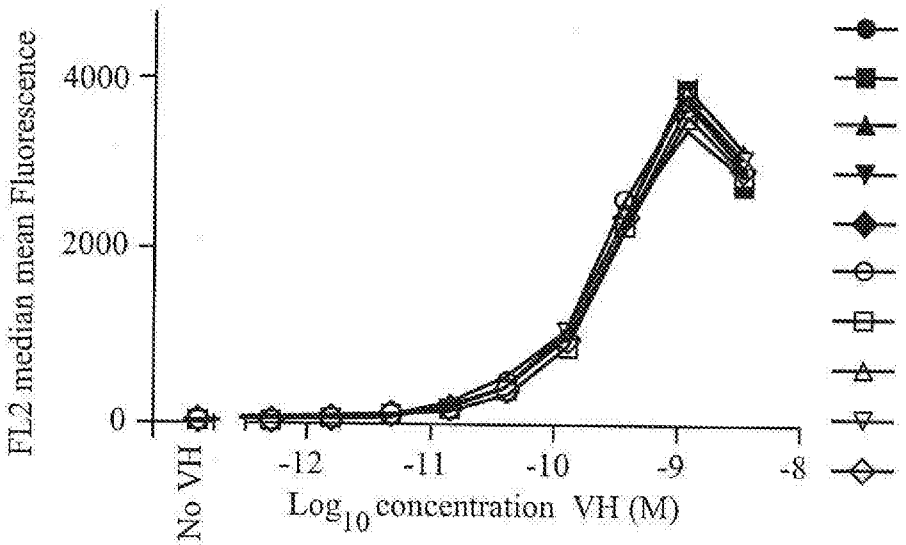

Figure 16e

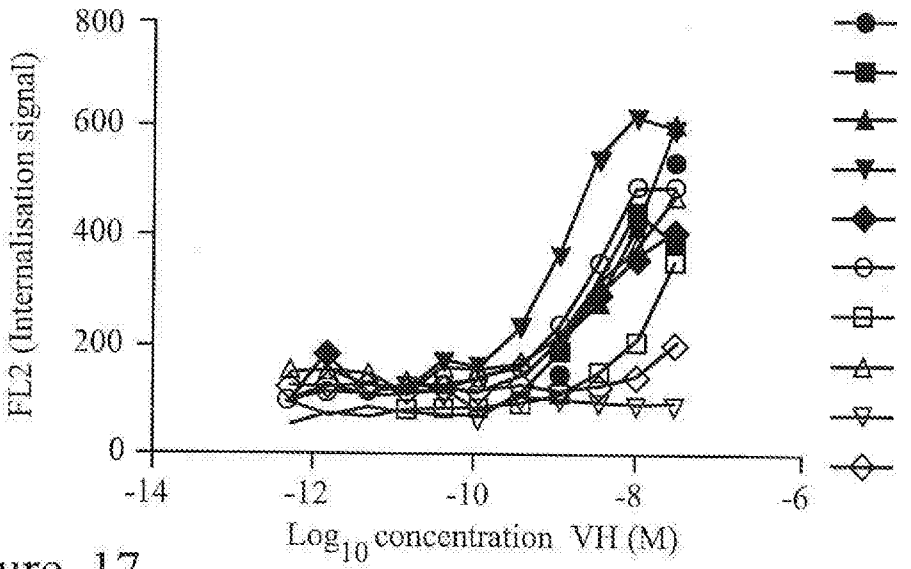

Figure 17

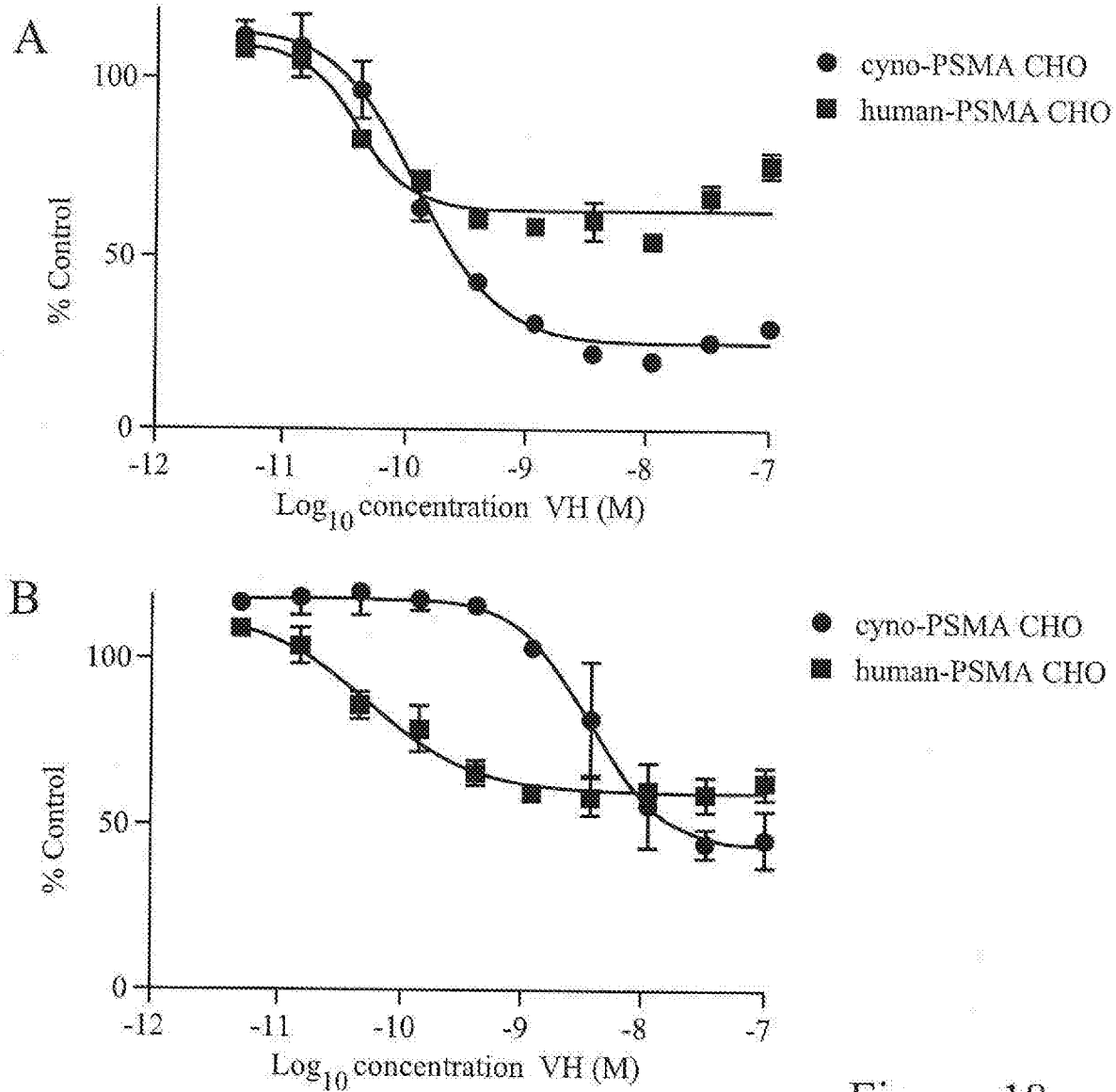

Figure 18

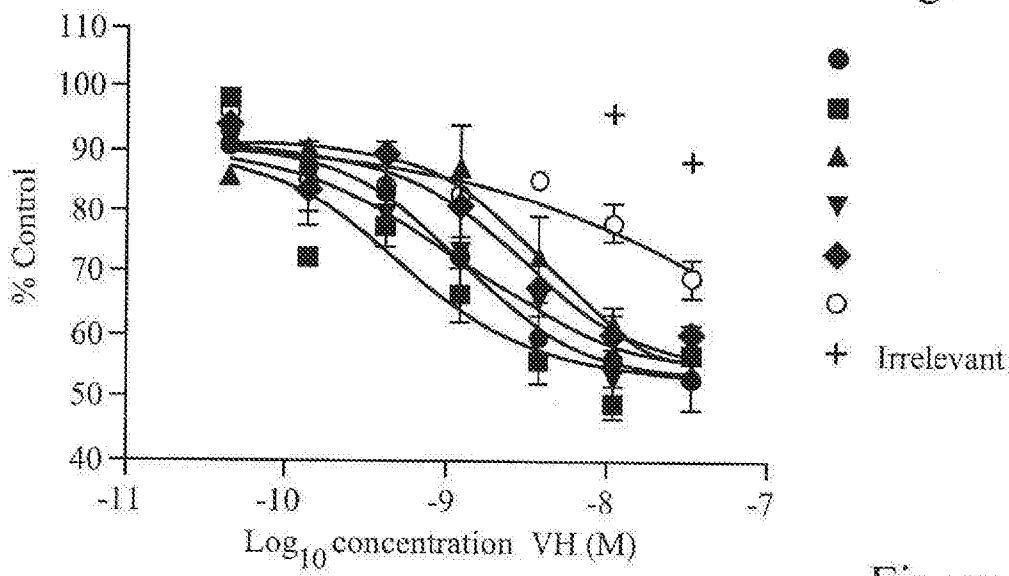

Figure 19

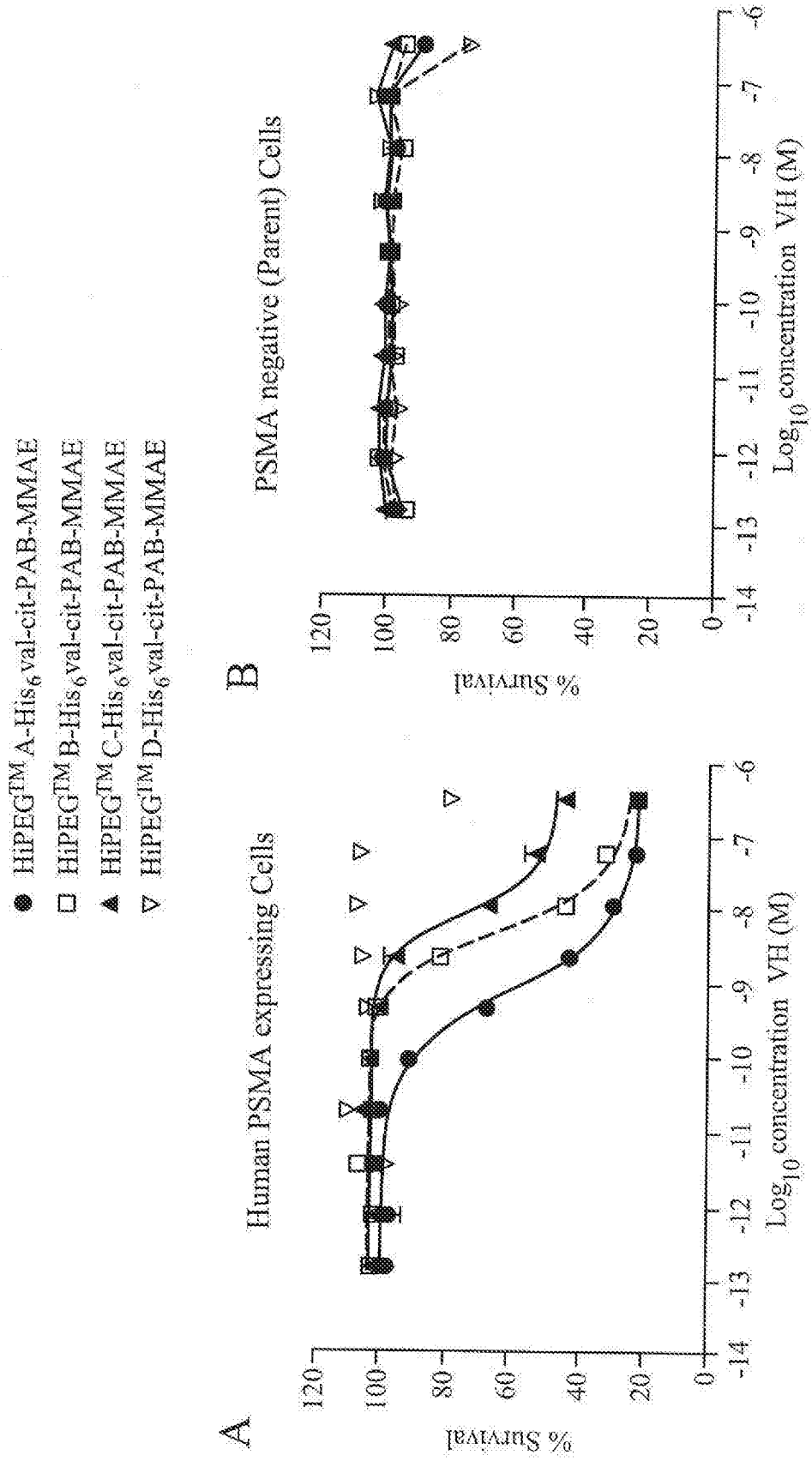
HiPEG™ A-His6 val-cit-PAB-MMAE
HiPEG™ B-His6 val-cit-PAB-MMAE
HiPEG™ C-His6 val-cit-PAB-MMAE
HiPEG™ D-His6 val-cit-PAB-MMAE
A
Human PSMA expressing Cells
% Survival
120
100
80
60
40
20
0
-14 -13 -12 -11 -10 -9 -8 -7 -6
Log10 concentration VH (M)
B
PSMA negative (Parent) Cells
% Survival
120
100
80
60
40
20
0
-14 -13 -12 -11 -10 -9 -8 -7 -6
Log10 concentration VH (M)

Figure 20

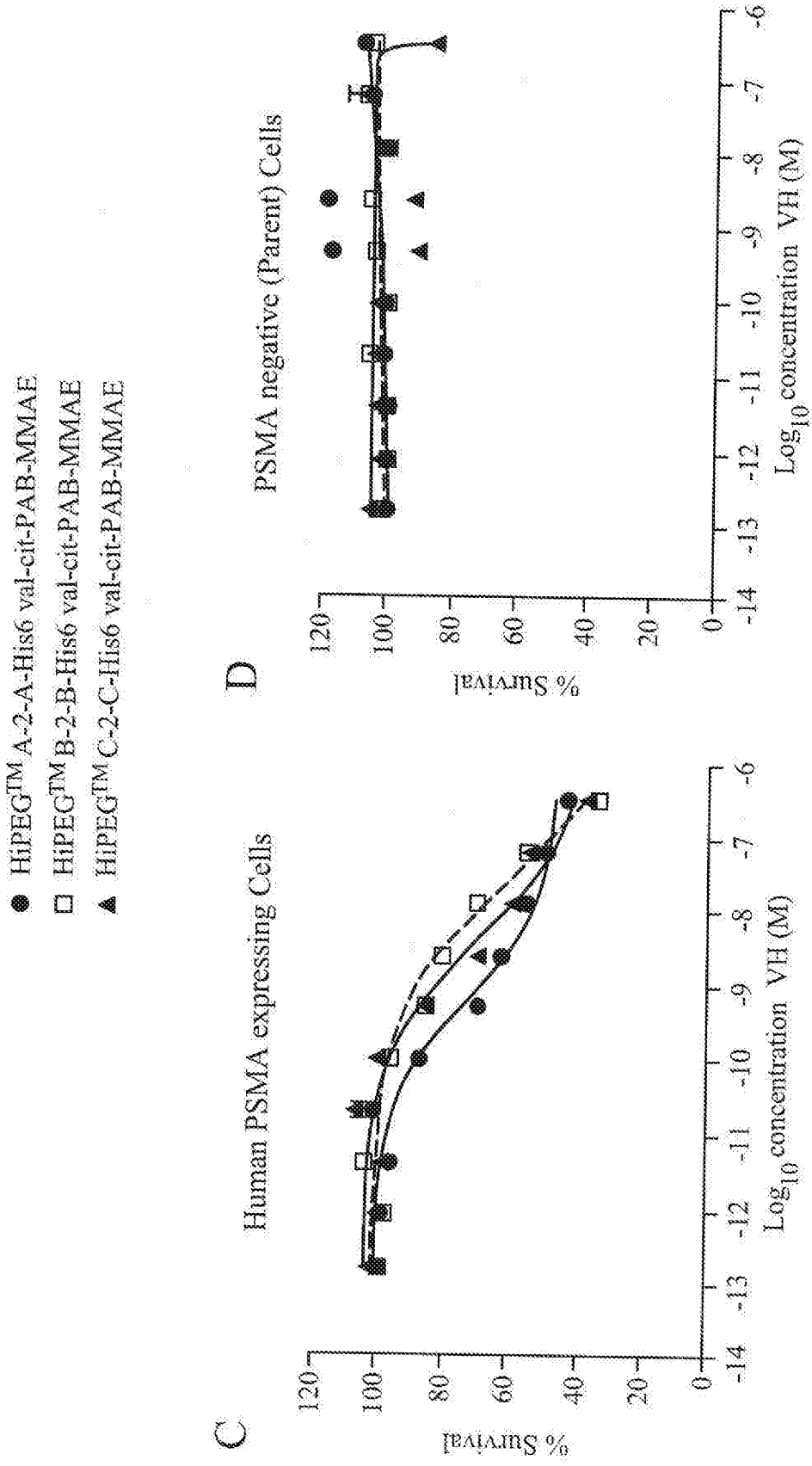
HiPEG™ A-2-A-His6 val-cit-PAB-MMAE
HiPEG™ B-2-B-His6 val-cit-PAB-MMAE
HiPEG™ C-2-C-His6 val-cit-PAB-MMAE
C
Human PSMA expressing Cells
% Survival
Log10 concentration VH (M)
D
PSMA negative (Parent) Cells
% Survival
Log10 concentration VH (M)

Figure 20

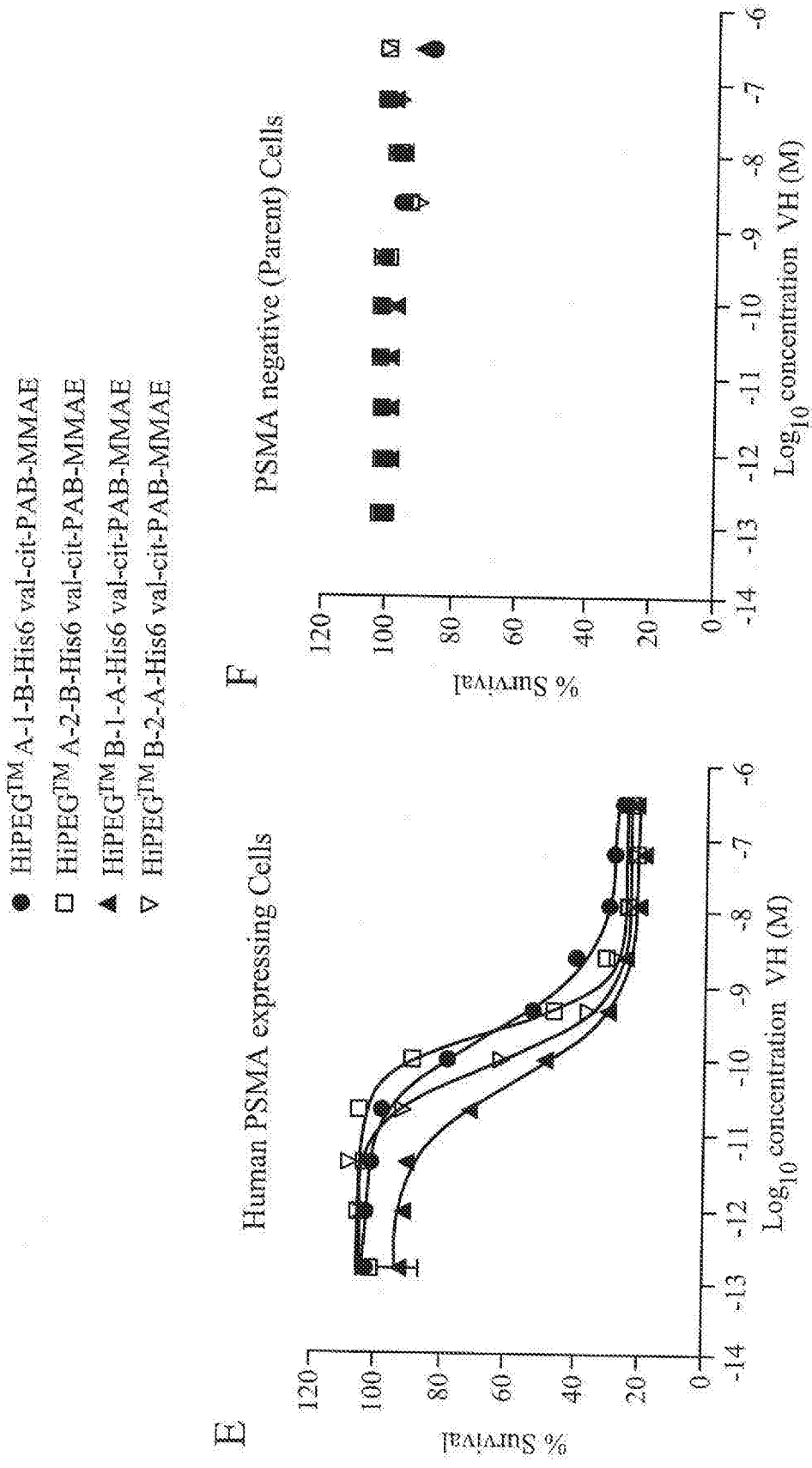
HiPEG™ A-1-B-His6 val-cit-PAB-MMAE
HiPEG™ A-2-B-His6 val-cit-PAB-MMAE
HiPEG™ B-1-A-His6 val-cit-PAB-MMAE
HiPEG™ B-2-A-His6 val-cit-PAB-MMAE
E
Human PSMA expressing Cells
% Survival
120
100
80
60
40
20
0
-14 -13 -12 -11 -10 -9 -8 -7 -6
Log10 concentration VH (M)
F
PSMA negative (Parent) Cells
% Survival
120
100
80
60
40
20
0
-14 -13 -12 -11 -10 -9 -8 -7 -6
Log10 concentration VH (M)

Figure 20

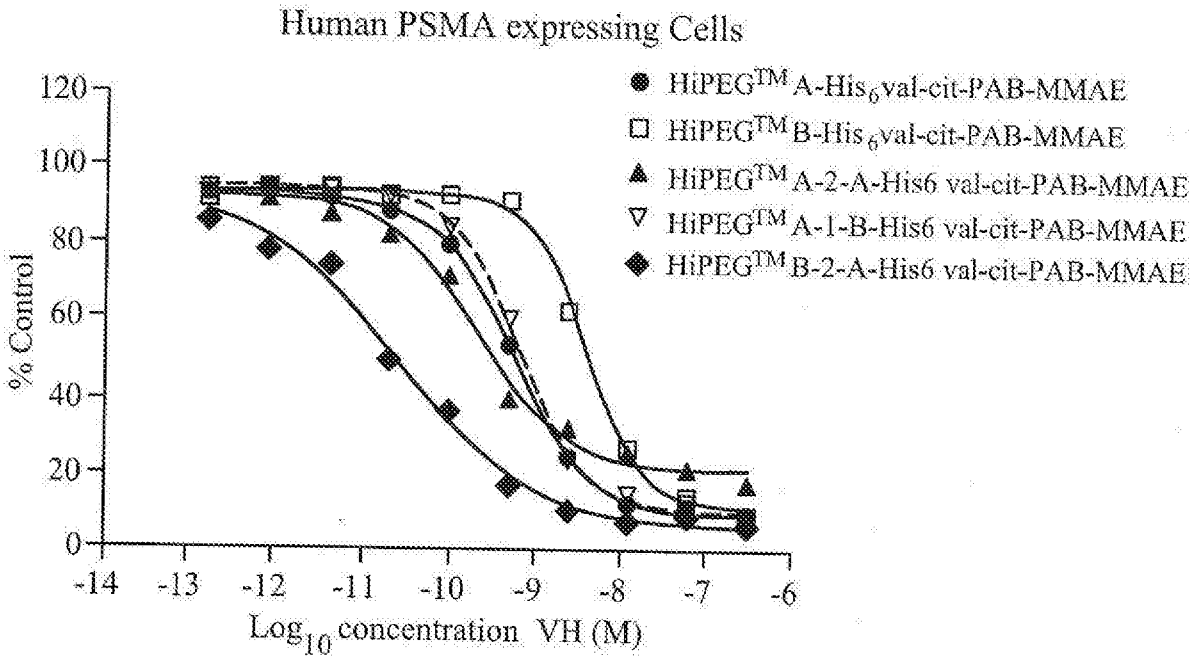
Human PSMA expressing Cells
HiPEG™ A-His6val-cit-PAB-MMAE
HiPEG™ B-His6val-cit-PAB-MMAE
HiPEG™ A-2-A-His6 val-cit-PAB-MMAE
HiPEG™ A-1-B-His6 val-cit-PAB-MMAE
HiPEG™ B-2-A-His6 val-cit-PAB-MMAE
% Control
120
100
80
60
40
20
0
-14
-13
-12
-11
-10
-9
-8
-7
-6
Log10 concentration VH (M)

Figure 21

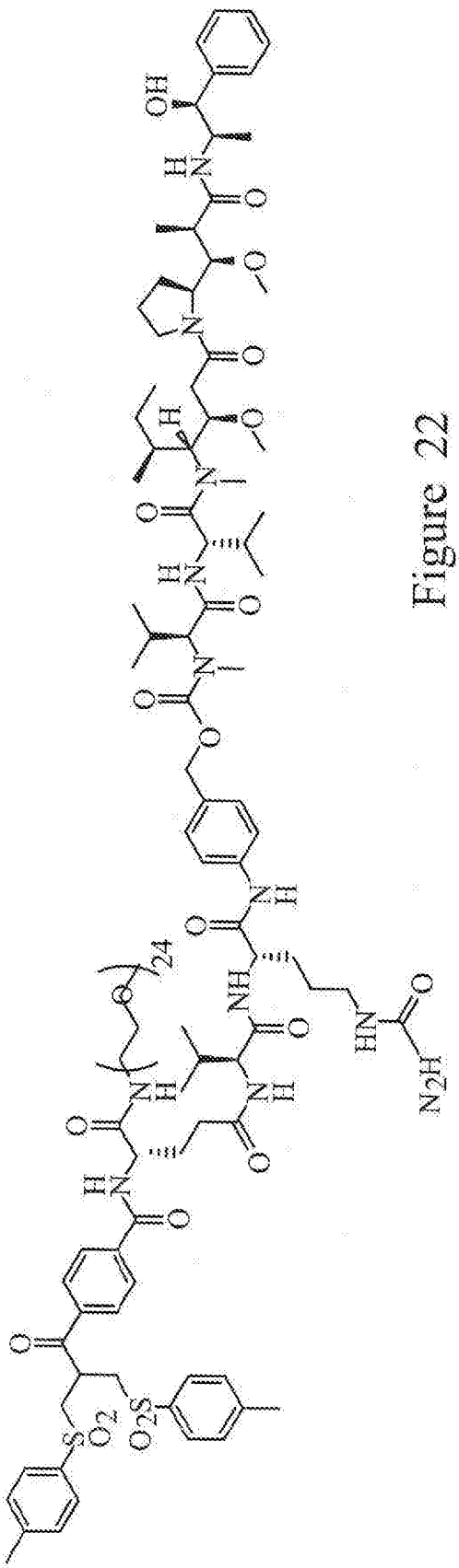

Figure 22

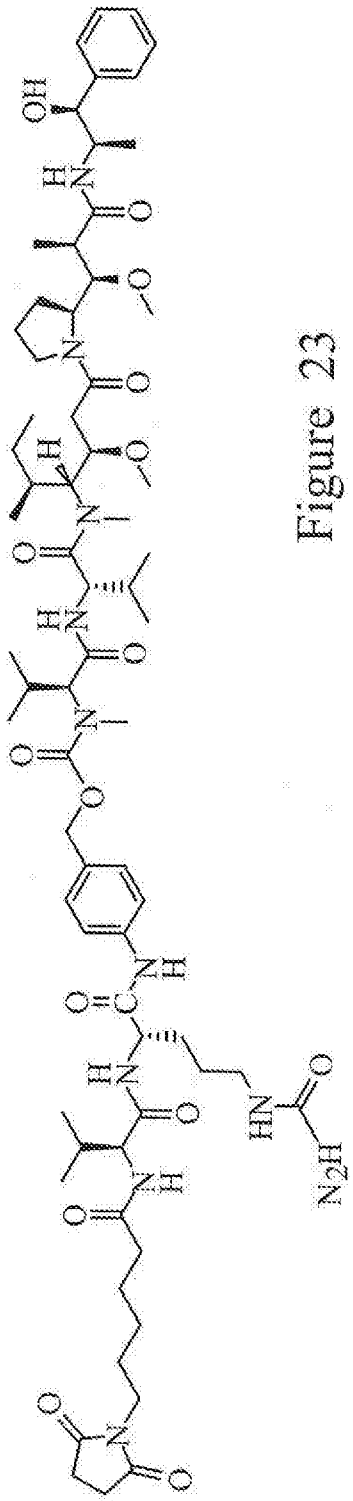

Figure 23

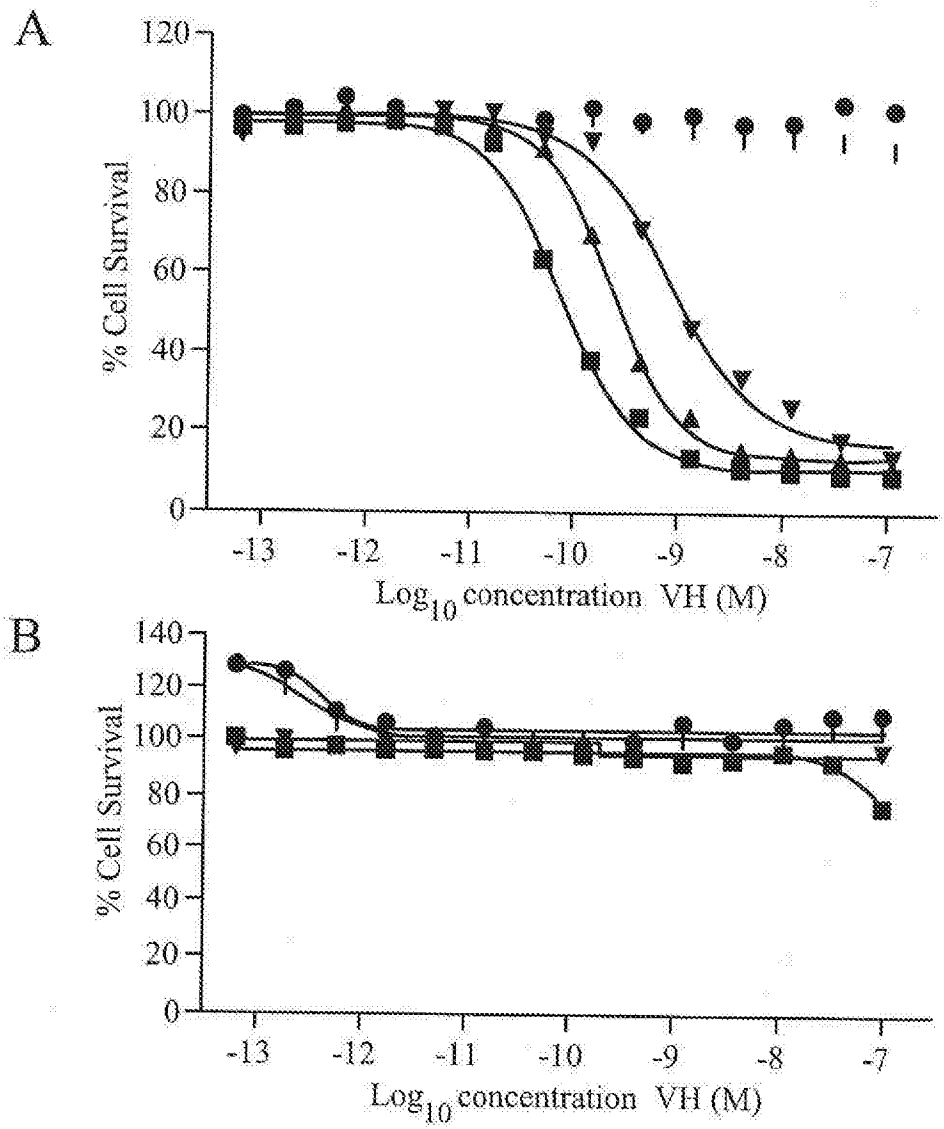
A
120
100
80
60
40
20
0
% Cell Survival
-13
-12
-11
-10
-9
-8
-7
Log10 concentration VH (M)
B
140
120
100
80
60
40
20
0
% Cell Survival
-13
-12
-11
-10
-9
-8
-7
Log10 concentration VH (M)

Figure 24

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2017/050076

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/30 G01N33/53
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MATTHIAS D'HUYVETTER ET AL: "Radiolabeled nanobodies as theranostic tools in targeted radionuclide therapy of cancer", EXPERT OPINION ON DRUG DELIVERY, vol. 1-6, 49-8111, no. 12, 18 July 2014 (2014-07-18), pages 1939-1954, XP055357047, GB ISSN: 1742-5247, DOI: 10.1517/17425247.2014.941803 page 1945, Figure 1, Table 3 ----- -/--	1-11, 54-88

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 March 2017	26/05/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Heder, Andreas

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HOLT L J ET AL: "Domain antibodies: proteins for therapy", TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 21, no. 11, 1 November 2003 (2003-11-01), pages 484-490, XP004467495, ISSN: 0167-7799, DOI: 10.1016/J.TIBTECH.2003.08.007 page 486	1-11, 54-88
Y	ROB C. ROOVERS ET AL: "A biparatopic anti-EGFR nanobody efficiently inhibits solid tumour growth", INTERNATIONAL JOURNAL OF CANCER, vol. 129, no. 8, 15 October 2011 (2011-10-15), pages 2013-2024, XP055086969, ISSN: 0020-7136, DOI: 10.1002/ijc.26145 pages 2013/14, and 2022/23, Tab. 2	1-11, 54-88
Y	DANGSHE MA ET AL: "Potent antitumor activity of an auristatin-conjugated, fully human monoclonal antibody to prostate-specific membrane antigen", CLINICAL CANCER RESEARCH, THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, vol. 12, no. 8, 15 April 2006 (2006-04-15) , pages 2591-2596, XP002623984, ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-05-2107 Tab. 1, page 2591, page 2595 right col.	1-11, 54-88
Y	DORONINA S O ET AL: "Development of potent monoclonal antibody auristatin conjugates for cancer therapy", NATURE BIOTECHNOLOGY, GALE GROUP INC, US, vol. 21, no. 7, 1 July 2003 (2003-07-01), pages 778-784, XP002280966, ISSN: 1087-0156, DOI: 10.1038/NBT832 pages 778, 782; figure 1	1-11, 54-88
Y	ZHU YANNI ET AL: "Multifunctional receptor-targeting antibodies for cancer therapy.", November 2015 (2015-11), THE LANCET. ONCOLOGY NOV 2015, VOL. 16, NR. 15, PAGE(S) E543 - E554, XP055357767, ISSN: 1474-5488 page e547 - page e548; figure 1	1-11, 54-88

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2017/050076

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-11, 54-88(all partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-11, 54-88(all partially)

Immunoconjugate comprising one or more antigen-binding moieties comprising a single human heavy chain variable domain antibody, one or more linkers, and an auristatin, wherein said antigen-binding moiety is specific for human PSMA, having a CDR3 of SEQ ID NO: 3, and having the additional features mentioned under subinventions 1.1, and 1.2, and uses thereof

1.1. claims: 1-11, 54-88(all partially)

see invention 1, wherein CDR1 has SEQ ID NO: 1, and CDR 2 has SEQ ID NO: 2

1.2. claims: 1-11, 54-88(all partially)

see invention 1, wherein the CDR1, 2, and 3 sequences are those shown in Figure 1 for single domain antibodies 1.1, 1.8, 1.10, 1.11, 1.12, 1.14, 1.16, 1.17, and 1.18

---

2. claims: 1-11, 54-88(all partially)

Immunoconjugate comprising one or more antigen-binding moieties comprising a single human heavy chain variable domain antibody, one or more linkers, and an auristatin, wherein said antigen-binding moiety is specific for human PSMA, having a CDR3 of SEQ ID NO: 3, and CDR1 and 2 other than those belonging to invention 1, and uses thereof

---

3-16. claims: 1-8, 12-88(all partially)

14 groups of inventions, each comprising further subgroups, relating to immunoconjugates comprising one or more antigen-binding moieties comprising a single human heavy chain variable domain antibody, one or more linkers, and an auristatin, wherein said antigen-binding moiety is specific for human PSMA, having a CDR3 of SEQ ID NO: 83, 183, 279, 295, 303, 331, 363, 367, 371, 375, 379, 383, 387, or 391, respectively, and uses thereof

---

17. claims: 1-88(partially)

Immunoconjugate comprising one or more antigen-binding moieties comprising a single human heavy chain variable domain antibody, one or more linkers, and an auristatin, wherein said antigen-binding moiety is specific for human PSMA, having a CDR3 different from inventions 1-16 above, and uses thereof

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210
