(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 October 2022 (06.10.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/212831 A1

(51) International Patent Classification:
C07K 16/30 (2006.01)

(21) International Application Number:
PCT/US2022/023034

(22) International Filing Date:
01 April 2022 (01.04.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/170,050 02 April 2021 (02.04.2021) US

(71) Applicant: **AMGEN INC.** [US/US]; One Amgen Center Drive, Thousand Oaks, California 91320-1799 (US).

(72) Inventors: **KIELCZEWSKA, Agnieszka**; c/o Amgen Inc., One Amgen Center Drive, Law Department-Patent Operations, Mail Stop 28-5-A, THOUSAND OAKS, California 91320 (US). **TINBERG, Christine Elaine**; c/o Amgen Inc., One Amgen Center Drive, Law Department-Patent Operations, Mail Stop 28-5-A, THOUSAND OAKS, California 91320 (US).

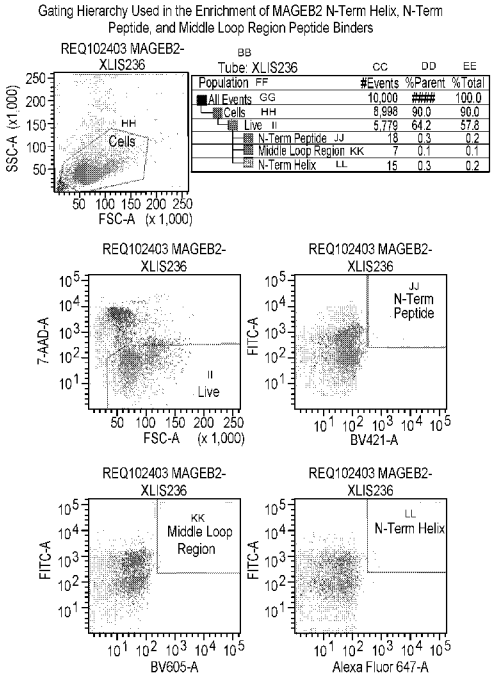
(74) Agent: **KONG, Lawrence B.**; 1120 Veterans Blvd., South San Francisco, California 94080 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) **Title:** MAGEB2 BINDING CONSTRUCTS

FIG. 1



(57) **Abstract:** The present invention relates to binding constructs comprising a domain which binds to MAGEB2. Moreover, the invention provides polynucleotides encoding the binding constructs, a vector comprising said polynucleotides and a host cell transformed or transfected with said polynucleotides or vectors. Furthermore, the invention provides processes for producing the binding constructs, methods of treatment using the binding constructs, diagnostic uses of the binding constructs, and kits comprising the binding constructs.

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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

MAGEB2 BINDING CONSTRUCTS

FIELD OF THE INVENTION

[0001] The field of this invention relates to compositions and methods related to cancer treatments and diagnostics, including binding constructs that bind to MAGEB2.

BACKGROUND OF THE INVENTION

The MAGE (melanoma antigen genes) family contains about 60 genes that are categorized into several subfamilies. The MAGE-A, -B, and -C subfamilies are expressed mainly in the testis and are aberrantly expressed in various cancer types. The MAGE-D, -E, -F, -G, -H, -L, and -N subfamilies are expressed in a wide variety of tissues. See, e.g., Lee and Potts, J. Mol. Biol., 2018. One of the MAGE family members, MAGEB2, is typically only expressed in normal testis. MAGEB2, which may function to enhance ubiquitin ligase activity of RING-type zinc finger containing E3 ubiquitin protein ligases, has been found to be aberrantly expressed in a variety of human tumors such as lung carcinoma, breast carcinoma, melanoma, and others. Given this aberrant expression, MAGEB2 is a potential target for new therapeutic agents.

Early detection and classification of cancer is a crucial factor in successful treatment of the disease. A sensitive and precise diagnostic assay that allows detection and quantification of tumor antigens, e.g., MAGEB2, would aid in earlier detection and classification of cancer in patients and could also predict clinical response and outcome for appropriate cancer therapeutics.

The ability to reliably detect these tumor antigens may provide early indication of the disease and/or the disease progression. Immunological diagnostic assays are an important tool for detecting a variety of disease conditions, including cancer. However, such assays may not always be sensitive and/or specific enough to reliably detect particular tumor antigens located on tumor cells, particularly if they are expressed in low levels and/or are not expressed on the surface of tumor cells.

In some instances, a molecular diagnostic assay may be desired and may provide the required specificity and sensitivity, and therefore be the best option to detect a particular tumor antigen. However, in other instances it may also be desired to confirm proper expression of the tumor antigen within the tumor tissue sample and thus an immunohistochemical assay may be better suited. Accordingly, there remains a need for sensitive and precise diagnostic assays to detect cancer antigens that are useful for detecting malignant cells and/or to help predict efficacy and to improve safety of the relevant therapeutic.

SUMMARY OF THE INVENTION

In one embodiment, the invention provides an isolated antigen binding construct that binds MAGEB2, wherein the binding construct binds to an epitope comprising a sequence selected from SEQ ID NOs: 2, 3, 4, or 388 - 554.

In another embodiment, the invention provides an isolated antigen binding construct, wherein the antigen binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 85; the CDRL2 comprises a sequence set forth in SEQ ID NO: 86; the CDRL3 comprises a sequence set forth in SEQ ID NO: 87; the CDRH1 comprises a sequence set forth in SEQ ID NO: 229; the CDRH2 comprises a sequence set forth in SEQ ID NO: 230; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 231.

In another embodiment, the invention provides an antigen binding construct, wherein the antigen binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 73; the CDRL2 comprises a sequence set forth in SEQ ID NO: 74; the CDRL3 comprises a sequence set forth in SEQ ID NO: 75; the CDRH1 comprises a sequence set forth in SEQ ID NO: 217; the CDRH2 comprises a sequence set forth in SEQ ID NO: 218; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 219.

In another embodiment, the invention provides an isolated antigen binding construct, wherein the antigen binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 91; the CDRL2 comprises a sequence set forth in SEQ ID NO: 92; the CDRL3 comprises a sequence set forth in SEQ ID NO: 93; the CDRH1 comprises a sequence set forth in SEQ ID NO: 235; the CDRH2 comprises a sequence set forth in SEQ ID NO: 236; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 237.

In a further embodiment, the invention provides an isolated antigen binding construct, wherein the antigen binding construct comprises a light chain variable region comprising a sequence set forth in SEQ ID NO: 346 and a heavy chain variable region comprising a sequence set forth in SEQ ID NO: 347.

In yet a further embodiment, the invention provides an isolated antigen binding construct, wherein the antigen binding construct comprises a light chain variable region comprising a sequence set forth in SEQ ID NO: 338 and a heavy chain variable region comprising a sequence set forth in SEQ ID NO: 339.

In another embodiment, the invention provides an antigen binding construct, wherein the antigen binding construct comprises a light chain variable region comprising a sequence set forth in SEQ ID NO: 350 and a heavy chain variable region comprising a sequence set forth in SEQ ID NO: 351.

In another embodiment, the invention provides a method of making an antibody that binds to MAGEB2 comprising immunizing an animal with a peptide comprising a sequence selected from SEQ ID NO: 2, 3, or 4, and isolating from said animal antibodies that bind to MAGEB2.

In another embodiment, the invention provides a method for treating a tumor in a subject, said method comprising: determining the subject as responsive to treatment with an anti-MAGEB2 therapeutic by obtaining a sample from the subject, wherein the sample comprises a cell from the tumor, measuring the level of MAGEB2 in the sample using an antigen binding construct provided herein, and determining the subject as responsive to treatment with an anti-MAGEB2 therapeutic, and administering to the subject an effective amount of the anti-MAGEB2 therapeutic.

In another embodiment, the invention provides a method of identifying a subject as needing an anti-MAGEB2 therapeutic comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct provided herein; and b) identifying the subject as needing the anti-MAGEB2 therapeutic when the level of MAGEB2 is increased relative to a control.

In another embodiment, the invention provides a method of determining treatment for a subject with a MAGEB2 positive tumor comprising: determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct provided herein; and determining the treatment as comprising an anti-MAGEB2 therapeutic when the level of MAGEB2 is increased, relative to a control.

In another embodiment, the invention provides a method of determining efficacy of treatment with an anti-MAGEB2 therapeutic in a subject comprising: determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct provided herein before treatment with an anti-MAGEB2 therapeutic and after treatment with an anti-MAGEB2 therapeutic; and determining the treatment as effective when the level of MAGEB2 positive tumor cells is decreased after treatment with the anti-MAGEB2 therapeutic.

In another embodiment, the invention provides a method of diagnosing a subject with a tumor, comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct provided herein; and b) diagnosing the subject as having a MAGEB2 positive tumor when the level of MAGEB2 is increased relative to a control.

In another embodiment, the invention provides a method of identifying a subject having a MAGEB2 positive tumor comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct provided herein; and b) identifying the subject as having a MAGEB2 positive tumor when the level of MAGEB2 is increased relative to a control.

In another embodiment, the invention provides a method of identifying a subject as needing an anti-MAGEB2 therapeutic comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct provided herein; and b) identifying the subject as needing the anti-MAGEB2 therapeutic when the level of MAGEB2 is increased relative to a control.

In another embodiment, the invention provides a method of determining treatment for a subject with a MAGEB2 positive tumor comprising: determining the level of MAGEB2 in a sample obtained from

the subject using an antigen binding construct provided herein; and determining the treatment as comprising an anti-MAGEB2 therapeutic when the level of MAGEB2 is increased, relative to a control.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. This figure shows the gating hierarchy used in the enrichment of MAGEB2 N-Term Helix, N-Term Peptide, and Middle Loop Region peptide binders as described in Example 2 herein.

Figure 2. This figure shows two plots that show binding by various anti-MAGEB2 antibodies binding to immunogen peptide and full length MAGEB2 protein. The MAGEB2 panel was run in a limited antigen style assay (only highest concentration shown) to identify highest affinity binders to full length MAGEB2 protein.

Figure 3. This figure shows immunohistochemical results for a MAGEB2 IHC assay that measured immunoreactivity in MAGEB2 transfected cells. Intense MAGEB2 IHC staining was observed in CHO-MAGE-B2+ cells with the four anti-MAGEB2 antibodies 4G17, 1J15, 1C3, 1I14, but not with IgG control.

Figure 4. This shows immunohistochemical results for a MAGEB2 IHC assay that measured immunoreactivity in MAGEB5 transfected cells. No MAGEB2 IHC staining was observed in CHO-MAGEB5+ cells with the four anti-MAGEB2 antibodies 4G17, 1J15, 1C3, 1I14, and with IgG control.

Figure 5. This figure shows immunohistochemical results for a MAGEB2 IHC assay that measured immunoreactivity in control testis tissues. Intense MAGEB2 IHC staining in spermatogonia cells was observed in testis with the four anti-MAGEB2 antibodies 4G17, 1J15, 1C3, 1I14, but not IgG control at 2 ug/ml. Some nuclear stain was seen with antibodies 4G17, 1J15, 1C3; Leydig cell stain was seen with antibody 1J15.

Figure 6. This figure shows immunohistochemical results for a MAGEB2 IHC Assay that measured immunoreactivity in normal human tissue. Non-specific particulate staining in hepatocytes was seen in liver tissue core (in normal human tissue with anti-MAGEB2 antibodies 4G17, 1J15, 1C3, but not with anti-MAGEB2 antibody 1I14, and IgG control at 5 ug/ml.

Figure 7. This figure shows immunohistochemical results for a MAGEB2 IHC assay that measured immunoreactivity in testis and liver tissue, with titration down of the 1C3 antibody. At 2 ug/ml Ab concentration, anti-MAGEB2 antibody 1C3 clone has weak to mild intensity specific staining in appropriate proportions of spermatogonia cells as previously observed. There is rare nuclear staining and the intensity is slightly less than that observed at higher concentrations. However, at this concentration there is no non-specific background staining in liver.

DETAILED DESCRIPTION

MAGEB2 is aberrantly expressed in a variety of human cancer types. MAGEB2 is not expressed on the surface of cells. MAGEB2 peptides, however, are displayed on the surface of tumor cells by the MHC class I molecule. In particular, the MAGEB2 peptide GVYDGEEHSV (SEQ ID NO: 1) is displayed on the surface of tumor cells by the MHC class I molecule as a peptide-MHC complex. See, for example, U.S. Patent Appl. Publ. No. US2016/0250307A1 (U.S. Patent Appl. No. 14/975,952) and US2017/0080070A1 (U.S. Patent Appl. No. 15/357,757). Although the MAGEB2 peptide-MHC complex is displayed on the surface of cells and would potentially be a target for a diagnostic agent, the number of copies on the cell surface of this peptide-MHC complex is far too low to be detected. Accordingly, binding constructs (e.g., antibodies) that are able to bind and detect MAGEB2 that is intracellularly expressed are described herein.

Binding Constructs

The present invention provides binding constructs comprising domains which bind to a MAGEB2 protein. These binding constructs are alternatively referred to as antigen binding constructs.

The term binding construct refers to a construct that is capable of binding to its specific target or antigen and comprises the variable heavy chain (VH) and/or variable light chain (VL) domains of an antibody or fragment thereof. Typically, a binding domain according to the present invention comprises the minimum structural requirements of an antibody which allow for the target binding. This minimum requirement may *e.g.* be defined by the presence of at least the three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL region) and/or the three heavy chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VH region), preferably of all six CDRs. An alternative approach to define the minimal structure requirements of an antibody is the definition of the epitope the antibody binds within the structure of a specific target, respectively, the protein domain of the target protein composing the epitope region (epitope cluster) or by reference to a specific antibody competing with the epitope of the defined antibody. Alternatively, the minimal structure requirements may be defined by the paratope sequences within the binding domain of the antibody.

Binding constructs of the present invention comprise at least one binding domain. The term "binding domain" characterizes in connection with the present invention a domain which specifically binds to, interacts with, or recognizes a given target epitope or a given target region on the target molecule, e.g., MAGEB2 or a specific region within MAGEB2. The structure and function of the binding domain is based on the structure and/or function of an antibody, e.g. of a full-length or whole immunoglobulin molecule, and is from the variable heavy chain (VH) and/or variable light chain (VL) domains of an antibody or fragment thereof. In certain embodiments, the binding domain comprises the presence of three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL region) and/or three heavy chain CDRs (i.e.

CDR1, CDR2 and CDR3 of the VH region). In certain embodiments, the binding domain is produced by or obtainable by phage-display or library screening methods rather than by grafting CDR sequences from a pre-existing (monoclonal) antibody into a scaffold.

The binding domain of a binding construct according to the invention may e.g. comprise the above referred groups of CDRs. Preferably, those CDRs are comprised in the framework of an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH); however, it does not have to comprise both. Fd fragments, for example, have two VH regions and often retain some antigen-binding function of the intact antigen-binding domain. Additional examples for the format of antibody fragments, antibody variants or binding domains include (1) a Fab fragment, a monovalent fragment having the VL, VH, CL and CH1 domains; (2) a $F(ab')_2$ fragment, a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; (3) an Fd fragment having the two VH and CH1 domains; (4) an Fv fragment having the VL and VH domains of a single arm of an antibody, (5) a dAb fragment (Ward et al., (1989) Nature 341 :544-546), which has a VH domain; (6) an isolated complementarity determining region (CDR), and (7) a single chain Fv (scFv) , the latter being preferred (for example, derived from an scFV-library). Examples for embodiments of binding constructs according to the invention are e.g. described in WO 00/006605, WO 2005/040220, WO 2008/119567, WO 2010/037838, WO 2013/026837, WO 2013/026833, US 2014/0308285, US 2014/0302037, WO 2014/144722, WO 2014/151910, and WO 2015/048272.

In a specific embodiment of the invention, the binding construct is a full-length antibody, as described herein below.

Also within the definition of "binding domain" or "domain which binds" are fragments of full-length antibodies, such as VH, VHH, VL, (s)dAb, Fv, light chain (VL-CL), Fd (VH-CH1), heavy chain, Fab, Fab', $F(ab')_2$ or "r IgG" ("half antibody" consisting of a heavy chain and a light chain). Binding constructs according to the invention may also comprise modified fragments of antibodies, also called antibody variants or antibody derivatives. Examples include, but are not limited to, scFv, di-scFv or bi(s)-scFv, scFv-Fc, scFv-zipper, scFab, Fab_2, Fab_3, diabodies, single chain diabodies, tandem diabodies (Tandab's), tandem di-scFv, tandem tri-scFv, „minibodies" exemplified by a structure which is as follows: $(VH\text{-}VL\text{-}CH3)_2$, $(scFv\text{-}CH3)_2$, $((scFv)_2\text{-}CH3 + CH3)$, $\{(scFv)_2\text{-}CH3\}$ or $(scFv\text{-}CH3\text{-}scFv)_2$, multibodies such as triabodies or tetrabodies, and single domain antibodies such as nanobodies or single variable domain antibodies comprising merely one variable region, which might be VHH, VH or VL, that specifically binds to an antigen or target independently of other variable regions or domains. Further possible formats of the binding constructs according to the invention are cross bodies, maxi bodies, hetero Fc constructs, mono Fc constructs and scFc constructs. Examples for those formats will be described herein below.

According to the present invention, binding domains are in the form of one or more polypeptides. Such polypeptides may include proteinaceous parts and non-proteinaceous parts (e.g. chemical linkers or chemical cross-linking agents such as glutaraldehyde). Proteins (including fragments thereof, preferably biologically active fragments, and peptides, usually having less than 30 amino acids) comprise two or more amino acids coupled to each other via a covalent peptide bond (resulting in a chain of amino acids).

The term "polypeptide" as used herein describes a group of molecules, which usually consist of more than 30 amino acids. Polypeptides may further form multimers such as dimers, trimers and higher oligomers, *i.e.*, consisting of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures of such multimers are, consequently, termed homo- or heterodimers, homo- or heterotrimers etc. An example for a heteromultimer is an antibody molecule, which, in its naturally occurring form, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms "peptide", "polypeptide" and "protein" also refer to naturally modified peptides / polypeptides / proteins wherein the modification is effected *e.g.* by post-translational modifications like glycosylation, acetylation, phosphorylation and the like. A "peptide", "polypeptide" or "protein" when referred to herein may also be chemically modified such as pegylated. Such modifications are well known in the art and described herein below.

The definition of "antibody" according to the invention comprises full-length antibodies, also including camelid antibodies and other immunoglobulins generated by biotechnological or protein engineering methods or processes. These full-length antibodies may be for example monoclonal, recombinant, chimeric, deimmunized, humanized and human antibodies, as well as antibodies from other species such as mouse, hamster, rabbit, rat, goat, or non-human primates.

For the antibodies provided herein, the variable regions of immunoglobulin chains generally exhibit the same overall structure, comprising relatively conserved framework regions (FR) joined by three hypervariable regions, more often called "complementarity determining regions" or CDRs. The CDRs from the two chains of each heavy chain/light chain pair mentioned above typically are aligned by the framework regions to form a structure that binds specifically to the target epitope. From N-terminal to C-terminal, naturally-occurring light and heavy chain variable regions both typically conform with the following order of these elements: FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. A numbering system has been devised for assigning numbers to amino acids that occupy positions in each of these domains. This numbering system is defined in Kabat Sequences of Proteins of Immunological Interest (1987 and 1991, NIH, Bethesda, Md.), or Chothia & Lesk, 1987, J. Mol. Biol. 196:901-917; Chothia et al., 1989, Nature 342:878-883.

The term "variable" refers to the portions of the antibody or immunoglobulin domains that exhibit variability in their sequence and that are involved in determining the specificity and binding affinity of a particular antibody (i.e., the "variable domain(s)"). The pairing of a variable heavy chain (VH) and a variable light chain (VL) together forms an antigen-binding domain.

Variability is not evenly distributed throughout the variable domains of antibodies; it is concentrated in sub-domains of each of the heavy and light chain variable regions. These sub-domains are called "hypervariable regions" or "complementarity determining regions" (CDRs). The more conserved (i.e., non-hypervariable) portions of the variable domains are called the "framework" regions (FRM or FR) and provide a scaffold for the six CDRs in three-dimensional space to form an antigen-binding surface. The variable domains of naturally occurring heavy and light chains each comprise four framework (FRM) regions (FR1, FR2, FR3, and FR4), largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the framework regions and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site.

The terms "CDR", and its plural "CDRs", refer to the complementarity determining region of which three make up the binding character of a light chain variable region (CDR-L1, CDR-L2 and CDR-L3) and three make up the binding character of a heavy chain variable region (CDR-H1, CDR-H2 and CDR-H3). CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen and hence contribute to the functional activity of an antibody molecule, i.e., they are the main determinants of binding specificity to a particular target.

The exact definitional CDR boundaries and lengths are subject to different classification and numbering systems. CDRs may therefore be referred to by Kabat, Chothia, contact or any other boundary definitions, including the numbering system described herein. Despite differing boundaries, each of these systems has some degree of overlap in what constitutes the so called "hypervariable regions" within the variable sequences. CDR definitions according to these systems may therefore differ in length and boundary areas with respect to the adjacent framework region. See for example Kabat (an approach based on cross-species sequence variability), Chothia (an approach based on crystallographic studies of antigen-antibody complexes), and/or MacCallum (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991; Chothia et al., J. Mol. Biol, 1987, 196: 901-917; and MacCallum et al., J. Mol. Biol, 1996, 262: 732). Still another standard for characterizing the antigen binding side is the AbM definition used by Oxford Molecular's AbM antibody modeling software. See, e.g., Protein Sequence and Structure Analysis of Antibody Variable Domains, Antibody Engineering Lab Manual (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg). To the extent that two residue identification

techniques define regions of overlapping, but not identical regions, they can be combined to define a hybrid CDR. However, the numbering in accordance with the Kabat system is preferred.

Typically, CDRs form a loop structure that can be classified as a canonical structure. The term "canonical structure" refers to the main chain conformation that is adopted by the antigen binding (CDR) loops. Each canonical structure can be characterized by the torsion angles of the polypeptide backbone. Correspondent loops between antibodies may, therefore, have very similar three dimensional structures, despite high amino acid sequence variability in most parts of the loops (Chothia and Lesk, J. Mol. Biol., 1987, 196: 901; Chothia et al., Nature, 1989, 342: 877; Martin and Thornton, J. Mol. Biol, 1996, 263: 800). Furthermore, there is a relationship between the adopted loop structure and the amino acid sequences surrounding it. The conformation of a particular canonical class is determined by the length of the loop and the amino acid residues residing at key positions within the loop, as well as within the conserved framework (i.e., outside of the loop). Assignment to a particular canonical class can therefore be made based on the presence of these key amino acid residues.

The term "canonical structure" may also include considerations as to the linear sequence of the antibody, for example, as catalogued by Kabat (Kabat et al.). The Kabat numbering scheme (system) is a widely adopted standard for numbering the amino acid residues of an antibody variable domain in a consistent manner and is the preferred scheme applied in the present invention as also mentioned elsewhere herein. Additional structural considerations can also be used to determine the canonical structure of an antibody. For example, those differences not fully reflected by Kabat numbering can be described by the numbering system of Chothia et al. and/or revealed by other techniques, for example, crystallography and two- or three-dimensional computational modeling. Accordingly, a given antibody sequence may be placed into a canonical class which allows for, among other things, identifying appropriate chassis sequences (e.g., based on a desire to include a variety of canonical structures in a library). Kabat numbering of antibody amino acid sequences and structural considerations as described by Chothia et al., loc. cit. and their implications for construing canonical aspects of antibody structure, are described in the literature. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in the art. For a review of the antibody structure, see Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, eds. Harlow et al., 1988.

As used herein, the terms "single-chain Fv," "single-chain antibodies" or "scFv" refer to single polypeptide chain antibody fragments that comprise the variable regions from both the heavy and light chains, but lack the constant regions. Generally, a single-chain antibody further comprises a polypeptide linker between the VH and VL domains which enables it to form the desired structure which would allow for antigen binding. Single chain antibodies are discussed in detail by Pluckthun in

The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994). Various methods of generating single chain antibodies are known, including those described in U.S. Pat. Nos. 4,694,778 and 5,260,203; International Patent Application Publication No. WO 88/01649; Bird (1988) Science 242:423-442; Huston *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883; Ward *et al.* (1989) Nature 334:54454; Skerra *et al.* (1988) Science 242:1038-1041. In specific embodiments, single-chain antibodies can also be bispecific, multispecific, human, and/or humanized and/or synthetic.

In some embodiments, the binding constructs of the present invention are "*in vitro* generated binding constructs". This term refers to a binding construct according to the above definition where all or part of the variable region (e.g., at least one CDR) is generated in a non-immune cell selection, *e.g.*, an *in vitro* phage display, protein chip or any other method in which candidate sequences can be tested for their ability to bind to an antigen. In other embodiments, the binding construct sequences are generated by genomic rearrangement in an immune cell in an animal. This term thus preferably excludes sequences generated solely by genomic rearrangement in an immune cell in an animal. It is envisaged that the first and/or second domain of the binding construct is produced by or obtainable by phage display or library screening methods rather than by grafting CDR sequences from a pre-existing (monoclonal) antibody into a scaffold.

A "recombinant antibody" is an antibody made through the use of recombinant DNA technology or genetic engineering.

The term "monoclonal antibody" (mAb) or monoclonal antibody construct as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site or epitope on the antigen, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different antigenic sites or epitopes. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by clonal cell culture and are uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

For the preparation of monoclonal antibodies, any technique providing antibodies produced by continuous cell line cultures can be used. For example, monoclonal antibodies to be used may be made by the hybridoma method first described by Koehler *et al.*, Nature, 256: 495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). Examples for further

techniques to produce human monoclonal antibodies include the trioma technique, the human B-cell hybridoma technique (Kozbor, Immunology Today 4 (1983), 72) and the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985), 77-96).

Hybridomas can then be screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance analysis, e.g. Biacore™ to identify one or more hybridomas that produce an antibody that specifically binds with a specified antigen. Any form of the relevant antigen may be used as the immunogen, *e.g.*, recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as an antigenic peptide thereof. Surface plasmon resonance as employed in the Biacore system can be used to increase the efficiency of phage antibodies which bind to an epitope of a target cell surface antigen (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmborg, J. Immunol. Methods 183 (1995), 7-13).

Another exemplary method of making monoclonal antibodies includes screening protein expression libraries, e.g., phage display or ribosome display libraries. Phage display is described, for example, in Ladner *et al.*, U.S. Patent No. 5,223,409; Smith (1985) Science 228:1315-1317, Clackson *et al.*, Nature, 352: 624-628 (1991) and Marks *et al.*, J. Mol. Biol., 222: 581-597 (1991).

In addition to the use of display libraries, the relevant antigen can be used to immunize a non-human animal, *e.g.*, a rodent (such as a mouse, hamster, rabbit or rat). In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig (immunoglobulin) loci. Using the hybridoma technology, antigen-specific monoclonal antibodies derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green *et al.* (1994) Nature Genetics 7:13-21, US 2003-0070185, WO 96/34096, and WO 96/33735.

A monoclonal antibody can also be obtained from a non-human animal, and then modified, *e.g.*, humanized, deimmunized, rendered chimeric etc., using recombinant DNA techniques known in the art. Examples of modified binding constructs include humanized variants of non-human antibodies, "affinity matured" antibodies (see, *e.g.* Hawkins et al. J. Mol. Biol. 254, 889-896 (1992) and Lowman *et al.*, Biochemistry 30, 10832- 10837 (1991)) and antibody mutants with altered effector function(s) (see, *e.g.*, US Patent 5,648,260, Kontermann and Dübel (2010), *loc. cit.* and Little (2009), *loc. cit.*).

In immunology, affinity maturation is the process by which B cells produce antibodies with increased affinity for antigen during the course of an immune response. With repeated exposures to the same antigen, a host will produce antibodies of successively greater affinities. Like the natural prototype, the in vitro affinity maturation is based on the principles of mutation and selection. The in vitro affinity maturation has successfully been used to optimize binding constructs, e.g., antibodies or antibody fragments. Random mutations inside the CDRs are introduced using radiation, chemical mutagens or

error-prone PCR. In addition, the genetic diversity can be increased by chain shuffling. Two or three rounds of mutation and selection using display methods like phage display usually results in antibody fragments with affinities in the low nanomolar range.

A preferred type of an amino acid substitutional variation of the binding constructs involves substituting one or more hypervariable region residues of a parent antibody (e. g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sides (e. g. 6-7 sides) are mutated to generate all possible amino acid substitutions at each side. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e. g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sides for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the binding domain and, *e.g.*, the MAGEB2. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

The antibodies of the present invention specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. USA, 81: 6851-6855 (1984)). Chimeric antibodies of interest herein include "primitized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc.) and human constant region sequences. A variety of approaches for making chimeric antibodies have been described. See *e.g.*, Morrison *et al.*, Proc. Natl. Acad. ScL U.S.A. 81:6851 , 1985; Takeda *et al.*, Nature 314:452, 1985, Cabilly *et al.*, U.S. Patent No. 4,816,567; Boss *et al.*, U.S. Patent No. 4,816,397; Tanaguchi *et al.*, EP 0171496; EP 0173494; and GB 2177096.

"Humanized" antibodies, variants or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) are antibodies or immunoglobulins of mostly human sequences,

which contain (a) minimal sequence(s) derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (also CDR) of the recipient are replaced by residues from a hypervariable region of a non-human (*e.g.*, rodent) species (donor antibody) such as mouse, rat, hamster or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, "humanized antibodies" as used herein may also comprise residues which are found neither in the recipient antibody nor the donor antibody. These modifications are made to further refine and optimize antibody performance. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, Nature, 321: 522-525 (1986); Reichmann *et al.*, Nature, 332: 323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2: 593-596 (1992).

Humanized antibodies or fragments thereof can be generated by replacing sequences of the Fv variable domain that are not directly involved in antigen binding with equivalent sequences from human Fv variable domains. Exemplary methods for generating humanized antibodies or fragments thereof are provided by Morrison (1985) Science 229:1202-1207; by Oi *et al.* (1986) BioTechniques 4:214; and by US 5,585,089; US 5,693,761; US 5,693,762; US 5,859,205; and US 6,407,213. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable domains from at least one of a heavy or light chain. Such nucleic acids may be obtained from a hybridoma producing an antibody against a predetermined target, as described above, as well as from other sources. The recombinant DNA encoding the humanized antibody molecule can then be cloned into an appropriate expression vector.

Humanized antibodies may also be produced using transgenic animals such as mice that express human heavy and light chain genes, but are incapable of expressing the endogenous mouse immunoglobulin heavy and light chain genes. Winter describes an exemplary CDR grafting method that may be used to prepare the humanized antibodies described herein (U.S. Patent No. 5,225,539). All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR, or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

A humanized antibody can be optimized by the introduction of conservative substitutions, consensus sequence substitutions, germline substitutions and/or back mutations. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, (*e.g.*, Teng *et al.*, Proc. Natl. Acad. Sci. U.S.A., 80: 7308-7312, 1983; Kozbor *et al.*, Immunology Today, 4: 7279, 1983; Olsson *et al.*, Meth. Enzymol., 92: 3-16, 1982, and EP 239 400).

The term "human antibody", "human antibody construct" and "human binding domain" includes antibodies, antibody fragments, and binding domains having antibody regions such as variable and constant regions or domains which correspond substantially to human germline immunoglobulin sequences known in the art, including, for example, those described by Kabat *et al.* (1991) (*loc. cit.*). The human antibodies, antibody fragments or binding domains of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or side-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs, and in particular, in CDR3. The human antibodies, antibody fragments or binding domains can have at least one, two, three, four, five, or more positions replaced with an amino acid residue that is not encoded by the human germline immunoglobulin sequence. The definition of human antibodies, antibody fragments and binding domains as used herein also contemplates fully human antibodies, which include only non-artificially and/or genetically altered human sequences of antibodies as those can be derived by using technologies or systems such as the Xenomouse. Preferably, a "fully human antibody" does not include amino acid residues not encoded by human germline immunoglobulin sequences.

The ability to clone and reconstruct megabase-sized human loci in yeast artificial chromosomes YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the use of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (mAbs) – an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized mAbs and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, and cancer, which require repeated compound administrations.

One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large

repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human mAbs with the desired specificity could be readily produced and selected. This general strategy was demonstrated in connection with the generation of the first XenoMouse mouse strains (see Green et al. Nature Genetics 7:13-21 (1994)). The XenoMouse strains were engineered with YACs containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human mAbs. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions may recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively. See Mendez *et al.* Nature Genetics 15:146-156 (1997) and U.S. patent application Ser. No. 08/759,620.

The production of the XenoMouse® animals is further discussed and delineated in U.S. patent applications Ser. No. 07/466,008, Ser. No. 07/610,515, Ser. No. 07/919,297, Ser. No. 07/922,649, Ser. No. 08/031,801, Ser. No. 08/112,848, Ser. No. 08/234,145, Ser. No. 08/376,279, Ser. No. 08/430,938, Ser. No. 08/464,584, Ser. No. 08/464,582, Ser. No. 08/463,191, Ser. No. 08/462,837, Ser. No. 08/486,853, Ser. No. 08/486,857, Ser. No. 08/486,859, Ser. No. 08/462,513, Ser. No. 08/724,752, and Ser. No. 08/759,620; and U.S. Pat. Nos. 6,162,963; 6,150,584; 6,114,598; 6,075,181, and 5,939,598 and Japanese Patent Nos. 3 068 180 B2, 3 068 506 B2, and 3 068 507 B2. See also Mendez *et al.* Nature Genetics 15:146-156 (1997) and Green and Jakobovits J. Exp. Med. 188:483-495 (1998), EP 0 463 151 B1, WO 94/02602, WO 96/34096, WO 98/24893, WO 00/76310, and WO 03/47336.

In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more VH genes, one or more DH genes, one or

more JH genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Pat. No. 5,545,807 to Surani *et al.* and U.S. Pat. Nos. 5,545,806; 5,625,825; 5,625,126; 5,633,425; 5,661,016; 5,770,429; 5,789,650; 5,814,318; 5,877,397; 5,874,299; and 6,255,458 each to Lonberg and Kay, U.S. Pat. Nos. 5,591,669 and 6,023.010 to Krimpenfort and Berns, U.S. Pat. Nos. 5,612,205; 5,721,367; and 5,789,215 to Berns *et al.*, and U.S. Pat. No. 5,643,763 to Choi and Dunn, and GenPharm International U.S. patent application Ser. No. 07/574,748, Ser. No. 07/575,962, Ser. No. 07/810,279, Ser. No. 07/853,408, Ser. No. 07/904,068, Ser. No. 07/990,860, Ser. No. 08/053,131, Ser. No. 08/096,762, Ser. No. 08/155,301, Ser. No. 08/161,739, Ser. No. 08/165,699, Ser. No. 08/209,741. See also EP 0 546 073 B1, WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884 and U.S. Pat. No. 5,981,175. See further Taylor *et al.* (1992), Chen *et al.* (1993), Tuaillon *et al.* (1993), Choi *et al.* (1993), Lonberg *et al.* (1994), Taylor *et al.* (1994), and Tuaillon *et al.* (1995), Fishwild *et al.* (1996).

Kirin has also demonstrated the generation of human antibodies from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced. See European Patent Application Nos. 773 288 and 843 961. Xenerex Biosciences is developing a technology for the potential generation of human antibodies. In this technology, SCID mice are reconstituted with human lymphatic cells, e.g., B and/or T cells. Mice are then immunized with an antigen and can generate an immune response against the antigen. See U.S. Pat. Nos. 5,476,996; 5,698,767; and 5,958,765.

In some embodiments, the binding constructs of the invention are "isolated" or "substantially pure" binding constructs. "Isolated" or "substantially pure", when used to describe the binding constructs disclosed herein, means a binding construct that has been identified, separated and/or recovered from a component of its production environment. Preferably, the binding construct is free or substantially free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. It is understood that the isolated protein may constitute a wide range of percent concentration, e.g., from 5% to 99.9% by weight of the total protein content, depending on the circumstances. The polypeptide may be made at a significantly higher concentration through the use of an inducible promoter or high expression promoter, such that it is made at increased concentration levels. The definition includes the production of a binding construct in a wide variety of organisms and/or host cells that are known in the art. In preferred embodiments, the binding construct will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup

sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Ordinarily, however, an isolated binding construct will be prepared by at least one purification step.

Peptides are short chains of amino acid monomers linked by covalent peptide (amide) bonds. Hence, peptides fall under the broad chemical classes of biological oligomers and polymers. Amino acids that are part of a peptide or polypeptide chain are termed "residues" and can be consecutively numbered. All peptides except cyclic peptides have an N-terminal residue at one end and a C-terminal residue at the other end of the peptide. An oligopeptide consists of only a few amino acids (usually between two and twenty). A polypeptide is a longer, continuous, and unbranched peptide chain. Peptides are distinguished from proteins on the basis of size, and as an arbitrary benchmark can be understood to contain approximately 50 or fewer amino acids. Proteins consist of one or more polypeptides, usually arranged in a biologically functional way. While aspects of the lab techniques applied to peptides versus polypeptides and proteins differ (e.g., the specifics of electrophoresis, chromatography, etc.), the size boundaries that distinguish peptides from polypeptides and proteins are not absolute. Therefore, in the context of the present invention, the terms "peptide", "polypeptide" and "protein" may be used interchangeably, and the term "polypeptide" is often preferred.

Polypeptides may further form multimers such as dimers, trimers and higher oligomers, which consist of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding structures of higher order of such multimers are, consequently, termed homo- or heterodimers, homo- or heterotrimers etc. An example for a hereteromultimer is a full-length antibody or immunoglobulin molecule, which, in its naturally occurring form, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms "peptide", "polypeptide" and "protein" also refer to naturally modified peptides / polypeptides / proteins wherein the modification is accomplished *e.g.* by post-translational modifications like glycosylation, acetylation, phosphorylation and the like. A "peptide", "polypeptide" or "protein" when referred to herein may also be chemically modified such as pegylated. Such modifications are well known in the art and described herein below.

A binding construct is said to "specifically bind" or "immunospecifically bind" to its antigen when the binding construct binds its antigen with a dissociation constant (KD) is ≤10-7 M as measured via a surface plasma resonance technique (e.g., BIACore, GE-Healthcare Uppsala, Sweden) or Kinetic Exclusion Assay (KinExA, Sapidyne, Boise, Idaho). In accordance with this invention a binding construct specifically binds or immunospecifically binds to MAGEB2.

Because of the sequence similarity between homologous proteins in different species, a binding construct or a binding domain that specifically binds to its target (such as a human target) may, however, cross-react with homologous target molecules from different species (such as, from non-

human primates). The term "specific / immunospecific binding" can hence include the binding of a binding construct or binding domain to epitopes or structurally related epitopes in more than one species.

The term "epitope" refers to a region on an antigen, or specific amino acid residues, to which a binding domain, such as an antibody or immunoglobulin, or a derivative, fragment or variant of an antibody or an immunoglobulin, specifically binds. An "epitope" is antigenic and thus the term epitope is sometimes also referred to herein as "antigenic structure" or "antigenic determinant". Thus, the binding domain is an "antigen interaction site". Said binding/interaction is also understood to define a "specific recognition".

"Epitopes" can be formed both by contiguous amino acids or non-contiguous amino acids juxtaposed by tertiary folding of a protein. A "linear epitope" is an epitope where a contiguous amino acid primary sequence comprises the recognized epitope. A linear epitope typically includes at least 3 or at least 4, and more typically, at least 5 or at least 6 or at least 7, and frequently, about 8 to about 10 amino acids in a unique sequence, or even longer than 10 amino acids.

A "conformational epitope", in contrast to a linear epitope, is an epitope wherein the primary sequence of the amino acids comprising the epitope is not the sole defining component of the epitope recognized (*e.g.*, an epitope wherein the primary sequence of amino acids is not necessarily recognized by the binding domain). Typically, a conformational epitope comprises an increased number of amino acids relative to a linear epitope, and comprises noncontiguous amino acid sequences. With regard to recognition of conformational epitopes, the binding domain paratope recognizes a three-dimensional structure of the antigen, preferably a peptide or protein or fragment thereof (in the context of the present invention, the antigenic structure for one of the binding domains is comprised within the target cell surface antigen protein). For example, when a protein molecule folds to form a three-dimensional structure, certain amino acids and/or the polypeptide backbone forming the conformational epitope become juxtaposed enabling the antibody to recognize the epitope. Methods of determining the conformation of epitopes include, but are not limited to, x-ray crystallography, two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy and site-directed spin labelling and electron paramagnetic resonance (EPR) spectroscopy.

Various methods for identifying proteins, regions of proteins, or peptides that are useful as immunogens or for screening assays are known in the art. In one embodiment, and as described herein in the Examples, a Shannon entropy analysis of the MAGEB2 protein amino acid sequence can identify particular peptide sequences that can be used as immunogens.

Exemplary MAGEB2 peptides that were identified to be used as immunogens included: MAGEB2 peptide a.a.43-76: SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV (N-terminal region peptide) (SEQ ID NO: 2), MAGEB2 peptide a.a.95-125: SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK (MHD N-terminal helix

peptide) (SEQ ID NO: 3), MAGEB2 peptide a.a.185-200: DLTDEESLLSSWDFPR (MHD middle loop peptide) (SEQ ID NO: 4).

Accordingly, in one embodiment of the present invention, provided is a method of immunizing an animal comprising administration to the animal any of the peptides of SEQ ID NOs: 2, 3, or 4.

In another embodiment, provided is a method of generating an isolated antibody comprising immunizing an animal with any of the peptides of SEQ ID NOs: 2, 3, or 4 and isolating said antibody. In further embodiments, provided is a method of generating an isolated monoclonal antibody comprising immunizing an animal with any of the peptides of SEQ ID NOs: 2, 3, or 4 and further generating a monoclonal antibody using art recognized steps described herein.

In yet another embodiment, provided is an isolated antibody generated by a process comprising immunizing an animal with any of the peptides of SEQ ID NOs: 2, 3, or 4.

In yet other embodiments, fragments of the peptides of SEQ ID NOs: 2, 3, or 4 can be used as the immunogen. Accordingly, in one embodiment of the present invention, the peptide immunogen comprises a fragment of the MAGEB2 peptide a.a.43-76: SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV (N-terminal region peptide) (SEQ ID NO: 2) that is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 amino acids long.

In another embodiment of the present invention, the peptide immunogen comprises a fragment of the MAGEB2 peptide a.a.95-125: SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK (MHD N-terminal helix peptide) (SEQ ID NO: 3) that is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, amino acids long.

In another embodiment of the present invention, the peptide immunogen comprises a fragment of the MAGEB2 peptide a.a.185-200: DLTDEESLLSSWDFPR (MHD middle loop peptide) (SEQ ID NO: 4) that is 10, 11, 12, 13, 14, or 15 amino acids long.

In further embodiments of the present invention, provided are binding constructs that compete for binding with any of the binding constructs described in the present invention. Competition assays are well known in the art and exemplary assays are described further herein.

In other embodiments, alternative peptides that can be used as immunogens for screening are provided in Table 1 below. Accordingly, the invention provides

Table 1.

MAGEB2 Peptide Amino Acid Sequence	SEQ ID NO:
SVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV	388
VSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV	389
SGGAASSSPAAGIPQEPQRAPTTAAAAAAGV	390
SGGAASSSPAAGIPQEPQRAPTTAAAAAAGV	391
GGAASSSPAAGIPQEPQRAPTTAAAAAAGV	392
GAASSSPAAGIPQEPQRAPTTAAAAAAGV	393
AASSSPAAGIPQEPQRAPTTAAAAAAGV	394
ASSSPAAGIPQEPQRAPTTAAAAAAGV	395
SSSPAAGIPQEPQRAPTTAAAAAAGV	396
SSPAAGIPQEPQRAPTTAAAAAAGV	397
SPAAGIPQEPQRAPTTAAAAAAGV	398
PAAGIPQEPQRAPTTAAAAAAGV	399
AAGIPQEPQRAPTTAAAAAAGV	400
AGIPQEPQRAPTTAAAAAAGV	401
GIPQEPQRAPTTAAAAAAGV	402
IPQEPQRAPTTAAAAAAGV	403
PQEPQRAPTTAAAAAAGV	404
QEPQRAPTTAAAAAAGV	405
EPQRAPTTAAAAAAGV	406
PQRAPTTAAAAAAGV	407
QRAPTTAAAAAAGV	408
RAPTTAAAAAAGV	409
APTTAAAAAAGV	410
PTTAAAAAAGV	411
TTAAAAAAGV	412
TAAAAAAGV	413
AAAAAAGV	414
AAAAAGV	415
AAAAGV	416
AAAGV	417
SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAG	418
SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAA	419
SSVSGGAASSSPAAGIPQEPQRAPTTAAAAA	420

SSVSGGAASSSPAAG|PQEPQRAPTTAAAA	421
SSVSGGAASSSPAAG|PQEPQRAPTTAAA	422
SSVSGGAASSSPAAG|PQEPQRAPTTAA	423
SSVSGGAASSSPAAG|PQEPQRAPTTA	424
SSVSGGAASSSPAAG|PQEPQRAPTT	425
SSVSGGAASSSPAAG|PQEPQRAPT	426
SSVSGGAASSSPAAG|PQEPQRAP	427
SSVSGGAASSSPAAG|PQEPQRA	428
SSVSGGAASSSPAAG|PQEPQR	429
SSVSGGAASSSPAAG|PQEPQ	430
SSVSGGAASSSPAAG|PQEP	431
SSVSGGAASSSPAAG|PQE	432
SSVSGGAASSSPAAG|PQ	433
SSVSGGAASSSPAAG|P	434
SSVSGGAASSSPAAG|	435
SSVSGGAASSSPAAG	436
SSVSGGAASSSPAA	437
SSVSGGAASSSPA	438
SSVSGGAASSSP	439
SSVSGGAASSS	440
SSVSGGAASS	441
SSVSGGAAS	442
SSVSGGAA	443
SSVSGGA	444
SSVSGG	445
SSVSG	446
SVSGGAASSSPAAG|PQEPQRAPTTAAAAAAG	447
VSGGAASSSPAAG|PQEPQRAPTTAAAAAA	448
SGGAASSSPAAG|PQEPQRAPTTAAAAA	449
GGAASSSPAAG|PQEPQRAPTTAAAA	450
GAASSSPAAG|PQEPQRAPTTAAA	451
AASSSPAAG|PQEPQRAPTTAA	452
ASSSPAAG|PQEPQRAPTTA	453
SSSPAAG|PQEPQRAPTT	454

SSPAAGIPQEPQRAPT	455
SPAAGIPQEPQRAP	456
PAAGIPQEPQRA	457
AAGIPQEPQR	458
AGIPQEPQ	459
GIPQEP	460
IPQE	461
SSQASTSTKSPSEDPLTRKSGSLVQFLLYK	462
SQASTSTKSPSEDPLTRKSGSLVQFLLYK	463
QASTSTKSPSEDPLTRKSGSLVQFLLYK	464
ASTSTKSPSEDPLTRKSGSLVQFLLYK	465
STSTKSPSEDPLTRKSGSLVQFLLYK	466
TSTKSPSEDPLTRKSGSLVQFLLYK	467
STKSPSEDPLTRKSGSLVQFLLYK	468
TKSPSEDPLTRKSGSLVQFLLYK	469
KSPSEDPLTRKSGSLVQFLLYK	470
SPSEDPLTRKSGSLVQFLLYK	471
PSEDPLTRKSGSLVQFLLYK	472
SEDPLTRKSGSLVQFLLYK	473
EDPLTRKSGSLVQFLLYK	474
DPLTRKSGSLVQFLLYK	475
PLTRKSGSLVQFLLYK	476
LTRKSGSLVQFLLYK	477
TRKSGSLVQFLLYK	478
RKSGSLVQFLLYK	479
KSGSLVQFLLYK	480
SGSLVQFLLYK	481
GSLVQFLLYK	482
SLVQFLLYK	483
LVQFLLYK	484
VQFLLYK	485
QFLLYK	486
FLLYK	487
SSSQASTSTKSPSEDPLTRKSGSLVQFLLY	488

SSSQASTSTKSPSEDPLTRKSGSLVQFLL	489
SSSQASTSTKSPSEDPLTRKSGSLVQFL	490
SSSQASTSTKSPSEDPLTRKSGSLVQF	491
SSSQASTSTKSPSEDPLTRKSGSLVQ	492
SSSQASTSTKSPSEDPLTRKSGSLV	493
SSSQASTSTKSPSEDPLTRKSGSL	494
SSSQASTSTKSPSEDPLTRKSGS	495
SSSQASTSTKSPSEDPLTRKSG	496
SSSQASTSTKSPSEDPLTRKS	497
SSSQASTSTKSPSEDPLTRK	498
SSSQASTSTKSPSEDPLTR	499
SSSQASTSTKSPSEDPLT	500
SSSQASTSTKSPSEDPL	501
SSSQASTSTKSPSEDP	502
SSSQASTSTKSPSED	503
SSSQASTSTKSPSE	504
SSSQASTSTKSPS	505
SSSQASTSTKSP	506
SSSQASTSTKS	507
SSSQASTSTK	508
SSSQASTST	509
SSSQASTS	510
SSSQAST	511
SSSQAS	512
SSSQA	513
SSQASTSTKSPSEDPLTRKSGSLVQFLLY	514
SQASTSTKSPSEDPLTRKSGSLVQFLL	515
QASTSTKSPSEDPLTRKSGSLVQFL	516
ASTSTKSPSEDPLTRKSGSLVQF	517
STSTKSPSEDPLTRKSGSLVQ	518
TSTKSPSEDPLTRKSGSLV	519
STKSPSEDPLTRKSGSL	520
TKSPSEDPLTRKSGS	521
KSPSEDPLTRKSG	522

SPSEDPLTRKS	523
PSEDPLTRK	524
SEDPLTR	525
EDPLT	526
LTDEESLLSSWDFPR	527
TDEESLLSSWDFPR	528
DEESLLSSWDFPR	529
EESLLSSWDFPR	530
ESLLSSWDFPR	531
SLLSSWDFPR	532
LLSSWDFPR	533
LSSWDFPR	534
SSWDFPR	535
SWDFPR	536
WDFPR	537
DLTDEESLLSSWDFP	538
DLTDEESLLSSWDF	539
DLTDEESLLSSWD	540
DLTDEESLLSSW	541
DLTDEESLLSS	542
DLTDEESLLS	543
DLTDEESLL	544
DLTDEESL	545
DLTDEES	546
DLTDEE	547
DLTDE	548
LTDEESLLSSWDFP	549
TDEESLLSSWDF	550
DEESLLSSWD	551
EESLLSSW	552
ESLLSS	553
SLLS	554

Affinity

The interaction between the binding domain and the epitope or the region comprising the epitope implies that a binding domain exhibits appreciable affinity for the epitope and/or the region comprising the epitope on a particular protein or antigen, and unless otherwise specified, does not exhibit significant binding or reactivity with proteins or antigens other than MAGEB2. This affinity can be measured by various techniques known to one skilled in the art, such as in a surface plasmon resonance assay, such as a Biacore assay, or in a cell based assay.

"Appreciable affinity" includes binding with an affinity of about 10^{-6} M (KD) or stronger. Preferably, binding is considered specific when the binding affinity is about 10^{-12} to 10^{-8} M, 10^{-12} to 10^{-9} M, 10^{-12} to 10^{-10} M, 10^{-11} to 10^{-8} M, preferably of about 10^{-11} to 10^{-9} M. Whether a binding domain specifically reacts with or binds to a target can be tested readily by, *inter alia*, comparing the reaction of said binding domain with a target protein or antigen with the reaction of said binding domain with proteins or antigens other than the MAGEB2. Preferably, a binding domain of the invention does not essentially or substantially bind to proteins or antigens other than MAGEB2.

Specificity

The term "does not significantly bind" means that a binding construct or binding domain of the present invention does not bind to a protein or antigen other than MAGEB2. For example, a binding construct or binding domain that exhibits binding to proteins with similar amino acid sequences, e.g., MAGEA4 or MAGEA8, would not be a desirable binding construct.

In the present invention, the MAGEB2 binding constructs possess surprising levels of specificity and selectivity to their target, as evidenced by its lack of binding to target negative cells. See, e.g., Examples 2 and 3 herein.

This specificity and selectivity are highly desired, yet difficult to achieve, properties for a binding construct in a diagnostic assay as it limits, reduces, or eliminates off-target binding and any potential false readouts.

Specific binding is believed to be effected by specific motifs in the amino acid sequence of the binding domain and the antigen. Thus, binding is achieved as a result of their primary, secondary and/or tertiary structure as well as the result of secondary modifications of said structures. The specific interaction of the antigen-interaction-side with its specific antigen may result in a simple binding of said side to the antigen. Moreover, the specific interaction of the antigen-interaction-side with its specific antigen may alternatively or additionally result in the initiation of a signal, *e.g.* due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc.

MAGEB2 Binding Constructs

The invention provides binding constructs comprising a domain which binds to MAGEB2, and in specific embodiments, to the MAGEB2 peptides SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV (N-terminal region peptide) (SEQ ID NO: 2), a.a.95-125: SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK (MHD N-terminal helix peptide) (SEQ ID NO: 3), or a.a.185-200: DLTDEESLLSSWDFPR (SEQ ID NO: 4).
Table 2 below provides amino acid sequences of exemplary MAGEB2 binding constructs VH-CDRs and VL-CDRs. Table 3 below provides amino acid sequences of exemplary MAGEB2 binding construct VH and VL domains.

Table 2. Exemplary Light Chain CDR and Heavy Chain CDR Sequences

Molecule	CDR-L1	CDR-L2	CDR-L3	CDR-H1	CDR-H2	CDR-H3
1I14	QSSQSVYDNNAL A (SEQ ID NO: 85)	GASTLAS (SEQ ID NO: 86)	QCTYYVSSYQND (SEQ ID NO: 87)	SYAMS (SEQ ID NO: 229)	SIGGGGSAVYASW AKG (SEQ ID NO: 230)	GFYSIDL (SEQ ID NO: 231)
1C3	QASQNISSYLA (SEQ ID NO: 73)	RASTLAS (SEQ ID NO: 74)	QSYDDSRSSNFFY A (SEQ ID NO: 75)	NYYIC (SEQ ID NO: 217)	CIDNANGRTYYAS WAKG (SEQ ID NO: 218)	SLATPL (SEQ ID NO: 219)
1H17	QSSKSVYNKNWL S (SEQ ID NO: 91)	GASTLAS (SEQ ID NO: 92)	AGGYSSSSDTFA (SEQ ID NO: 93)	SGQLMC (SEQ ID NO: 235)	CIGSGSNAISTFYAS WAQG (SEQ ID NO: 236)	VGSDDYGDSDVFDP (SEQ ID NO: 237)

Table 3. Exemplary Variable Light Chain and Variable Heavy Chain Sequences

Molecule	VL	VH
1I14	DVVMTQTPASVEATVGGTVTIKCQSSQSVYDNNALA WYQQNAGQRPRLLIYGASTLASGVPSRFSASGSGTEF TLTISDLECADAATYYCQCTYYVSSYQNDFGGGTEVVV K (SEQ ID NO: 346)	QSVEESGGRLVTPGTPLTLTCTISGFSLSSYAMSWVRQAPGKGL EWIGSIGGGGSAVYASWAKGRFTISKTSTTVDLRITSPTTEDTAM YFCGRGFYSIDLWGPGTLVTVSS (SEQ ID NO: 347)
1C3	DIVMTQTPSSVEAAVGGTVTIKCQASQNISSYLAWYQ QKPGQPPKLLIYRASTLASGVPSRFKGSGSGTQFTLTIS DLECADAATYYCQSYDDSRSSNFFYAFGGGTEVVVK (SEQ ID NO: 338)	QSLEESGGGLVQPEGSLTLTCTAFGVTLTNYYICWVRQAPGKGL EWVGCIDNANGRTYYASWAKGRFTISKTSSTTGTLQMTSLTAA DTATYFCARSLATPLWGPGTLVTVSS (SEQ ID NO: 339)
1H17	AAVLTQTPSPVSAAVGGTVSASCQSSKSVYNKNWLS WFQQKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQF TLTISDVQCDDAATYYCAGGYSSSSDTFAFGGGTEVVV K (SEQ ID NO: 350)	QEQLVESGGGLVKPGASLTLTCKASGFSFSSGQLMCWVRQAPG KGLEWIACIGSGSNAISTFYASWAQGRFTISKSSSTTVTLQLTSLT AADTATYFCARVGSDDYGDSDVFDPWGPGTLVTVSS (SEQ ID NO: 351)

In one embodiment, the invention provides an isolated binding domain that binds to MAGEB2, wherein the binding domain comprises:

a) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 229, CDR-H2 as depicted in SEQ ID NO: 230, and CDR-H3 as depicted in SEQ ID NO: 231, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 85, CDR-L2 as depicted in SEQ ID NO: 86 and CDR-L3 as depicted in SEQ ID NO: 87; or

b) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 217, CDR-H2 as depicted in SEQ ID NO: 218, and CDR-H3 as depicted in SEQ ID NO: 219, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 73, CDR-L2 as depicted in SEQ ID NO: 74 and CDR-L3 as depicted in SEQ ID NO: 75; or

c) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 235, CDR-H2 as depicted in SEQ ID NO: 236, and CDR-H3 as depicted in SEQ ID NO: 237, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 91, CDR-L2 as depicted in SEQ ID NO: 92 and CDR-L3 as depicted in SEQ ID NO: 93.

In one embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain comprises:

a) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 229, CDR-H2 as depicted in SEQ ID NO: 230, and CDR-H3 as depicted in SEQ ID NO: 231, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 85, CDR-L2 as depicted in SEQ ID NO: 86 and CDR-L3 as depicted in SEQ ID NO: 87; or

b) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 217, CDR-H2 as depicted in SEQ ID NO: 218, and CDR-H3 as depicted in SEQ ID NO: 219, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 73, CDR-L2 as depicted in SEQ ID NO: 74 and CDR-L3 as depicted in SEQ ID NO: 75; or

c) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 235, CDR-H2 as depicted in SEQ ID NO: 236, and CDR-H3 as depicted in SEQ ID NO: 237, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 91, CDR-L2 as depicted in SEQ ID NO: 92 and CDR-L3 as depicted in SEQ ID NO: 93.

In one embodiment, the invention provides an isolated binding domain that binds to MAGEB2, wherein the binding domain binds to the same epitope as an antibody or binding construct that comprises:

a) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 229, CDR-H2 as depicted in SEQ ID NO: 230, and CDR-H3 as depicted in SEQ ID NO: 231, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 85, CDR-L2 as depicted in SEQ ID NO: 86 and CDR-L3 as depicted in SEQ ID NO: 87; or

b) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 217, CDR-H2 as depicted in SEQ ID NO: 218, and CDR-H3 as depicted in SEQ ID NO: 219, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 73, CDR-L2 as depicted in SEQ ID NO: 74 and CDR-L3 as depicted in SEQ ID NO: 75; or

c) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 235, CDR-H2 as depicted in SEQ ID NO: 236, and CDR-H3 as depicted in SEQ ID NO: 237, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 91, CDR-L2 as depicted in SEQ ID NO: 92 and CDR-L3 as depicted in SEQ ID NO: 93.

In one embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain binds to the same epitope as an antibody or binding construct that comprises:

a) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 229, CDR-H2 as depicted in SEQ ID NO: 230, and CDR-H3 as depicted in SEQ ID NO: 231, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 85, CDR-L2 as depicted in SEQ ID NO: 86 and CDR-L3 as depicted in SEQ ID NO: 87; or

b) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 217, CDR-H2 as depicted in SEQ ID NO: 218, and CDR-H3 as depicted in SEQ ID NO: 219, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 73, CDR-L2 as depicted in SEQ ID NO: 74 and CDR-L3 as depicted in SEQ ID NO: 75; or

c) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 235, CDR-H2 as depicted in SEQ ID NO: 236, and CDR-H3 as depicted in SEQ ID NO: 237, and a VL region comprising CDR-L1 as depicted in SEQ ID NO: 91, CDR-L2 as depicted in SEQ ID NO: 92 and CDR-L3 as depicted in SEQ ID NO: 93.

In one embodiment, the invention provides an isolated binding domain that binds to MAGEB2, wherein the binding domain comprises:

a) a VH region comprising SEQ ID NO: 347 and a VL region comprising SEQ ID NO: 346; or

b) a VH region comprising SEQ ID NO: 339 and a VL region comprising SEQ ID NO: 338; or

c) a VH region comprising SEQ ID NO: 351 and a VL region comprising SEQ ID NO: 350.

In one embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain comprises:

a) a VH region comprising SEQ ID NO: 347 and a VL region comprising SEQ ID NO: 346; or

b) a VH region comprising SEQ ID NO: 339 and a VL region comprising SEQ ID NO: 338; or

c) a VH region comprising SEQ ID NO: 351 and a VL region comprising SEQ ID NO: 350.

In one embodiment, the invention provides an isolated binding domain that binds to MAGEB2, wherein the binding domain binds to the same epitope as an antibody or binding construct that comprises:

a) a VH region comprising SEQ ID NO: 347 and a VL region comprising SEQ ID NO: 346; or

b) a VH region comprising SEQ ID NO: 339 and a VL region comprising SEQ ID NO: 338; or

c) a VH region comprising SEQ ID NO: 351 and a VL region comprising SEQ ID NO: 350.

In one embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain binds to the same epitope as an antibody or binding construct that comprises:

a) a VH region comprising SEQ ID NO: 347 and a VL region comprising SEQ ID NO: 346; or

b) a VH region comprising SEQ ID NO: 339 and a VL region comprising SEQ ID NO: 338; or

c) a VH region comprising SEQ ID NO: 351 and a VL region comprising SEQ ID NO: 350.

In another embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain comprises a VH region selected from the group consisting of any VH region and a corresponding VL region as depicted in any of the sequences in Tables 21-25 herein.

In another embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain binds to the same epitope as an antibody or binding construct that comprises a VH region selected from the group consisting of any VH region and a corresponding VL region as depicted in any of the sequences in Table 23 herein.

In another embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain comprises a CDR-H1, CDR-H2, CDR-H3 from a VH region selected from the group consisting of any VH region as depicted in any of the sequences in Table 22 herein and further comprises a corresponding CDR-L1, CDR-L2, CDR-L3 from a VL region selected from the group consisting of any VL region as depicted in any of the sequences in Table 21 herein.

In another embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain binds to the same epitope as an antibody or binding construct that comprises a CDR-H1, CDR-H2, CDR-H3 from a VH region selected from the group consisting of any VH region as depicted in any of the sequences in Table 22 herein and further comprises a corresponding CDR-L1, CDR-L2, CDR-L3 from a VL region selected from the group consisting of any VL region as depicted in any of the sequences in Table 21 herein.

In another embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain comprises a VH and a VL region consensus sequence selected from any of the sequences in Table 25 herein.

In another embodiment, the invention provides an isolated binding construct comprising a binding domain that binds to MAGEB2, wherein the binding domain comprises a CDR-H1, CDR-H2, CDR-H3 from a VH region consensus sequence selected from the group consisting of any VH region as depicted in any of the sequences in Table 25 herein and further comprises a corresponding CDR-L1, CDR-L2, CDR-L3 from a VL region consensus sequence selected from the group consisting of any VL region as depicted in any of the sequences in Table 25 herein.

Exemplary binding construct full sequences are presented below:

1C3_LC (SEQ ID NO: 555)

MDMRVPAQLLGLLLLWLRGARCDIVMTQTPSSVEAAVGGTVTIKCQASQNISSYLAWYQQKPGQPPKLLIYRASTLA
SGVPSRFKGSGSGTQFTLTISDLECADAATYYCQSYDDSRSSNFFYAFGGGTEVVVKGDPVAPTVLLFPPSSDEVATGT
VTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSFSR
KNC

1C3_HC (SEQ ID NO: 556)
MDMRVPAQLLGLLLLWLRGARCQSLEESGGGLVQPEGSLTLTCTAFGVTLTNYYICWVRQAPGKGLEWVGCIDNAN
GRTYYASWAKGRFTISKTSSTTGTLQMTSLTAADTATYFCARSLATPLWGPGTLVTVSSGQPKAPSVFPLAPCCGDTP
SSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPS
TCSKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIR
VVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVE
WEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK

1I14_LC (SEQ ID NO: 557)
MDMRVPAQLLGLLLLWLRGARCDVVMTQTPASVEATVGGTVTIKCQSSQSVYDNNALAWYQQNAGQRPRLLIYG
ASTLASGVPSRFSASGSGTEFTLTISDLECADAATYYCQCTYYVSSYQNDFGGGTEVVVKGDPVAPTVLLFPPSSDEVA
TGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQ
SFSRKNC

1I14_HC (SEQ ID NO: 558)
MDMRVPAQLLGLLLLWLRGARCQSVEESGGRLVTPGTPLTLTCTISGFSLSSYAMSWVRQAPGKGLEWIGSIGGGGS
AVYASWAKGRFTISKTSTTVDLRITSPTTEDTAMYFCGRGFYSIDLWGPGTLVTVSSGQPKAPSVFPLAPCCGDTPSST
VTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTC
SKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVV
STLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWE
KNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK

1H17_LC (SEQ ID NO: 559)
MDMRVPAQLLGLLLLWLRGARCAAVLTQTPSPVSAAVGGTVSASCQSSKSVYNKNWLSWFQQKPGQPPKLLIYGA
STLASGVPSRFKGSGSGTQFTLTISDVQCDDAATYYCAGGYSSSSDTFAFGGGTEVVVKGDPVAPTVLLFPPSSDEVAT
GTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQS
FSRKNC

1H17_HC (SEQ ID NO: 560)
MDMRVPAQLLGLLLLWLRGARCQEQLVESGGGLVKPGASLTLTCKASGFSFSSGQLMCWVRQAPGKGLEWIACIGS
GSNAISTFYASWAQGRFTISKSSSTTVTLQLTSLTAADTATYFCARVGSDDYGDSDVFDPWGPGTLVTVSSGQPKAPS
VFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPA
TNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTAR
PPLREQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTC
MINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRS
PGK

Competitive Binding

Whether or not an antibody or binding construct competes for binding to an antigen (such as MAGEB2) with another given antibody or binding construct can be measured in a competition assay such as a competitive ELISA. Avidin-coupled microparticles (beads) can also be used. Similar to an avidin-coated ELISA plate, when reacted with a biotinylated protein, each of these beads can be used as a substrate on which an assay can be performed. Antigen is coated onto a bead and then precoated

with the first antibody. The second antibody is added, and any additional binding is determined. Read-out occurs via flow cytometry. Preferably a cell-based competition assay that allows binding to intracellularly expressed MAGEB2 is used, using either cells that naturally express MAGEB2, or cells that were stably or transiently transformed with MAGEB2. The term "competes for binding", in the present context, means that competition occurs between the two tested antibodies of at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90%, as determined by any one of the assays disclosed above, preferably the cell-based assay.

Competitive antibody binding assays include assays determining the competitive binding of two antibodies/ binding constructs to a cell surface bound antigen. Common methods aim to detect binding of two antibodies/ binding constructs, A and B, to the same antigen expressed by a cell may include steps of:

blocking of the antigen by pre-incubation of cells with antibody/ binding construct A followed by a sub-maximal addition of labeled antibody/ binding construct B and detecting the binding of B compared with binding in the absence of A;

titration (i.e. adding different amounts) of antibody/ binding construct A in the presence of sub-maximal amounts of labeled antibody/ binding construct B and detecting the effect on binding of B; or co-titration of A and B, wherein both antibodies/ binding constructs are incubated together at maximal concentration and detecting whether the total binding equals or exceeds that of either A or B alone, i.e. a method which cannot be affected by the order of addition or relative amounts of the antibodies/ binding constructs.

When two antibodies/ binding constructs A and B compete for a antigen, the antibodies will very often compete with each other in blocking assays independently from the order of the addition of the antibodies. In other words, competition is detected if the assay is carried out in either direction. However, this is not always the case, and under certain circumstances the order of the addition of the antibodies or the direction of the assay may have an effect on the signal generated. This may be due to differences in affinities or avidities of the potentially competing antibodies/ binding constructs. If the order of the addition has a significant effect on the signal generated, it is concluded that the two antibodies/binding constructs do compete if competition is detected in at least one order.

Epitope Amino Acid Residues

Structural analysis of the interaction between the target polypeptide and binding domain of, for example, a construct contemplated herein can provide amino acid residues of the target epitope involved with the binding interaction. In certain embodiments, the crystal structure of the target-binder interaction can provide these amino acid residues. In other embodiments, various other analyses can be performed to ascertain amino acid residues involved in the binding interaction. For

example, Xscan, alanine scanning, arginine scanning, and other techniques known to those skilled in the art. In the present invention, the binding constructs were generated using specific peptide immunogens, and must therefore bind to these specific peptide sequences.

In embodiments where a peptide is used as the immunogen, such as those provided herein in SEQ ID NOs 2, 3, or 4, antibodies that are isolated will be expected to bind an epitope that comprises all of, or a fragment of, the peptide immunogen. This epitope can be a linear epitope with that comprises all of, or a fragment of, the peptide immunogen amino acid sequences. Alternatively, and more likely in the case of longer peptide immunogens, this epitope can be a conformational epitope that comprises discontinuous amino acid sequences from within the peptide immunogen. Accordingly, in one embodiment of the present invention, the epitope that the binding constructs (e.g., antibodies) bind to comprises the MAGEB2 peptide a.a.43-76: SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV (N-terminal region peptide) (SEQ ID NO: 2).

In another embodiment, the epitope that the binding constructs (e.g., antibodies) bind to comprises a fragment of the MAGEB2 peptide a.a.43-76: SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV (N-terminal region peptide) (SEQ ID NO: 2) that is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, or 33 amino acids long.

In another embodiment, the epitope that the binding constructs (e.g., antibodies) bind to comprises at least two, at least three, at least four, or at least five discontinuous amino acid sequence fragments of the MAGEB2 peptide a.a.43-76: SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV (N-terminal region peptide) (SEQ ID NO: 2), wherein each discontinuous amino acid sequence fragment is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids long

In another embodiment of the present invention, the epitope that the binding constructs of the invention bind to comprises the MAGEB2 peptide a.a.95-125: SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK (MHD N-terminal helix peptide) (SEQ ID NO: 3).

In another embodiment, the epitope that the binding constructs (e.g., antibodies) bind to comprises a fragment of the MAGEB2 peptide a.a.95-125: SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK (MHD N-terminal helix peptide) (SEQ ID NO: 3) that is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30, amino acids long.

In another embodiment, the epitope that the binding constructs (e.g., antibodies) bind to comprises at least two, at least three, at least four, or at least five discontinuous amino acid sequence fragments of the MAGEB2 peptide a.a.95-125: SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK (MHD N-terminal helix peptide) (SEQ ID NO: 3), wherein each discontinuous amino acid sequence fragment is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids long.

In another embodiment of the present invention, the epitope that the binding constructs of the invention bind to comprises the MAGEB2 peptide a.a.185-200: DLTDEESLLSSWDFPR (MHD middle loop peptide) (SEQ ID NO: 4).

In another embodiment, the epitope that the binding constructs (e.g., antibodies) bind to comprises a fragment of the MAGEB2 peptide a.a.185-200: DLTDEESLLSSWDFPR (MHD middle loop peptide) (SEQ ID NO: 4) that is 10, 11, 12, 13, 14, or 15 amino acids long.

In another embodiment, the epitope that the binding constructs (e.g., antibodies) bind to comprises at least two, at least three, at least four, or at least five discontinuous amino acid sequence fragments of the MAGEB2 peptide a.a.185-200: DLTDEESLLSSWDFPR (MHD middle loop peptide) (SEQ ID NO: 4), wherein each discontinuous amino acid sequence fragment is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids long.

In one embodiment, the invention provides a binding construct that binds to an epitope comprising the peptides of any of SEQ ID NOs: 2, 3, 4, or 388 - 554.

The region of the binding domain that binds to the epitope is called a "paratope." Specific binding is believed to be accomplished by specific motifs in the amino acid sequence of the binding domain and the antigen. Thus, binding is achieved as a result of their primary, secondary and/or tertiary structure as well as the result of potential secondary modifications of said structures.

The term "Fc portion" or "Fc monomer" means in connection with this invention a polypeptide comprising at least one domain having the function of a CH2 domain and at least one domain having the function of a CH3 domain of an immunoglobulin molecule. As apparent from the term "Fc monomer", the polypeptide comprising those CH domains is a "polypeptide monomer". An Fc monomer can be a polypeptide comprising at least a fragment of the constant region of an immunoglobulin excluding the first constant region immunoglobulin domain of the heavy chain (CH1), but maintaining at least a functional part of one CH2 domain and a functional part of one CH3 domain, wherein the CH2 domain is amino terminal to the CH3 domain.

In one embodiment of this definition, an Fc monomer can be a polypeptide constant region comprising a portion of the Ig-Fc hinge region, a CH2 region and a CH3 region, wherein the hinge region is amino terminal to the CH2 domain. It is envisaged that the hinge region of the present invention promotes dimerization. Such Fc polypeptide molecules can be obtained by papain digestion of an immunoglobulin region (of course resulting in a dimer of two Fc polypeptide), for example and not limitation. In another aspect of this definition, an Fc monomer can be a polypeptide region comprising a portion of a CH2 region and a CH3 region. Such Fc polypeptide molecules can be obtained by pepsin digestion of an immunoglobulin molecule, for example and not limitation.

In one embodiment, the polypeptide sequence of an Fc monomer is substantially similar to an Fc polypeptide sequence of: an IgG1 Fc region, an IgG2 Fc region, an IgG3 Fc region, an IgG4 Fc region, an

IgM Fc region, an IgA Fc region, an IgD Fc region and an IgE Fc region. (See, e.g., Padlan, Molecular Immunology, 31(3), 169-217 (1993)). Because there is some variation between immunoglobulins, and solely for clarity, Fc monomer refers to the last two heavy chain constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three heavy chain constant region immunoglobulin domains of IgE and IgM. As mentioned, the Fc monomer can also include the flexible hinge N-terminal to these domains. For IgA and IgM, the Fc monomer may include the J chain. For IgG, the Fc portion comprises immunoglobulin domains CH2 and CH3 and the hinge between the first two domains and CH2. Although the boundaries of the Fc portion may vary an example for a human IgG heavy chain Fc portion comprising a functional hinge, CH2 and CH3 domain can be defined e.g. to comprise residues D231 (of the hinge domain– corresponding to D234 in Table 4 below) to P476, respectively L476 (for IgG4) of the carboxyl-terminus of the CH3 domain, wherein the numbering is according to Kabat. The two Fc portion or Fc monomer, which are fused to each other via a peptide linker define the third domain of the binding construct of the invention, which may also be defined as scFc domain.
In one embodiment of the invention it is envisaged that a scFc domain as disclosed herein, respectively the Fc monomers fused to each other are comprised only in the third domain of the binding construct.
In some embodiments an IgG hinge region can be identified by analogy using the Kabat numbering as set forth in Table 4. In line with the above, it is envisaged that for a hinge domain/region of the present invention the minimal requirement comprises the amino acid residues corresponding to the IgG1 sequence stretch of D231 D234 to P243 according to the Kabat numbering. It is likewise envisaged that a hinge domain/region of the present invention comprises or consists of the IgG1 hinge sequence DKTHTCPPCP (SEQ ID NO: 4) (corresponding to the stretch D234 to P243 as shown in Table 4 below – variations of said sequence are also envisaged provided that the hinge region still promotes dimerization). In a preferred embodiment of the invention the glycosylation site at Kabat position 314 of the CH2 domains in the third domain of the binding construct is removed by a N314X substitution, wherein X is any amino acid excluding Q. Said substitution is preferably a N314G substitution. In a more preferred embodiment, said CH2 domain additionally comprises the following substitutions (position according to Kabat) V321C and R309C (these substitutions introduce the intra domain cysteine disulfide bridge at Kabat positions 309 and 321).

Table 4: Kabat numbering of the amino acid residues of the hinge region

IMGT numbering for the hinge	IgG_1 amino acid translation	Kabat numbering
1	(E)	226
2	P	227

3	K	228
4	S	232
5	C	233
6	D	234
7	K	235
8	T	236
9	H	237
10	T	238
11	C	239
12	P	240
13	P	241
14	C	242
15	P	243

In further embodiments of the present invention, the hinge domain/region comprises or consists of the IgG2 subtype hinge sequence ERKCCVECPPCP (SEQ ID NO: 5), the IgG3 subtype hinge sequence ELKTPLDTTHTCPRCP (SEQ ID NO: 6) or ELKTPLGDTTHTCPRCP (SEQ ID NO: 7), and/or the IgG4 subtype hinge sequence ESKYGPPCPSCP (SEQ ID NO: 8). The IgG1 subtype hinge sequence may be the following one EPKSCDKTHTCPPCP (SEQ ID NO: 9). These core hinge regions are thus also envisaged in the context of the present invention.

The location and sequence of the IgG CH2 and IgG CH3 domain can be identified by analogy using the Kabat numbering as set forth in Table 5:

Table 5: Kabat numbering of the amino acid residues of the IgG CH2 and CH3 region

IgG subtype	CH2 aa translation	CH2 Kabat numbering	CH3 aa translation	CH3 Kabat numbering
IgG_1	APE... ...KAK	244... ...360	GQP.....PGK	361... ...478
IgG_2	APP... ...KTK	244... ...360	GQP.....PGK	361... ...478
IgG_3	APE... ...KTK	244... ...360	GQP.....PGK	361... ...478
IgG_4	APE... ...KAK	244... ...360	GQP.....LGK	361... ...478

In one embodiment of the invention, the emphasized bold amino acid residues in the CH3 domain of the first or both Fc monomers are deleted.

In a classical full-length antibody or immunoglobulin, each light (L) chain is linked to a heavy (H) chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more

disulfide bonds depending on the H chain isotype. The heavy chain constant (CH) domain most proximal to VH is usually designated as CH1. The constant ("C") domains are not directly involved in antigen binding, but exhibit various effector functions, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement activation (complement dependent cytotoxicity, CDC). The Fc region of an antibody is the "tail" region of a classical antibody that interacts with cell surface receptors called Fc receptors and some proteins of the complement system. In IgG, IgA and IgD antibody isotypes, the Fc region is composed of two identical protein fragments, derived from the second and third constant domains (CH2 and CH3) of the antibody's two heavy chains. IgM and IgE Fc regions contain three heavy chain constant domains (CH2, CH3 and CH4) in each polypeptide chain. The Fc regions also contains part of the so-called "hinge" region held together by one or more disulfides and noncovalent interactions. The Fc region of a naturally occurring IgG bears a highly conserved N-glycosylation site. Glycosylation of the Fc fragment is essential for Fc receptor-mediated activity.

In some embodiments of the invention the CH2 domain of one or preferably each (both) polypeptide monomers of the third domain comprises an intra domain cysteine disulfide bridge. As known in the art the term "cysteine disulfide bridge" refers to a functional group with the general structure R–S–S–R. The linkage is also called an SS-bond or a disulfide bridge or a cysteine clamp and is derived by the coupling of two thiol groups of cysteine residues. In certain embodiments, the cysteines forming the cysteine disulfide bridge in the mature binding construct are introduced into the amino acid sequence of the CH2 domain corresponding to 309 and 321 (Kabat numbering). In other embodiments, the cysteine clamps are introduced in other domains of the binding constructs. See also, e.g. US 2016/0193295.

In one embodiment of the invention a glycosylation site in Kabat position 314 of the CH2 domain is removed. It is preferred that this removal of the glycosylation site is achieved by a N314X substitution, wherein X is any amino acid excluding Q. Said substitution is preferably a N314G . In a more preferred embodiment, said CH2 domain additionally comprises the following substitutions (position according to Kabat) V321C and R309C (these substitutions introduce the intra domain cysteine disulfide bridge at Kabat positions 309 and 321).

Covalent modifications of the binding constructs are also included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the binding construct are introduced into the molecule by reacting specific amino acid residues of the binding construct with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives.

Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0. Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides ($R'—N=C=N--R'$), where R and R' are optionally different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking the binding constructs of the present invention to a water-insoluble support matrix or surface for use in a variety of methods. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming

crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates as described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, 1983, pp. 79-86), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the binding constructs included within the scope of this invention comprises altering the glycosylation pattern of the protein. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the binding construct is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the amino acid sequence of a binding construct is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the binding construct is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to

(a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330, and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306.

Removal of carbohydrate moieties present on the starting binding construct may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al., 1987, Arch. Biochem. Biophys. 259:52 and by Edge et al., 1981, Anal. Biochem. 118:131. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., 1987, Meth. Enzymol. 138:350. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., 1982, J. Biol. Chem. 257:3105. Tunicamycin blocks the formation of protein-N-glycoside linkages.

Other modifications of the binding construct are also contemplated herein. For example, another type of covalent modification of the binding construct comprises linking the binding construct to various non-proteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. In addition, as is known in the art, amino acid substitutions may be made in various positions within the binding construct, e.g. in order to facilitate the addition of polymers such as PEG.

In some embodiments, the covalent modification of the binding constructs of the invention comprises the addition of one or more labels. The labelling group may be coupled to the binding construct via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and can be used in performing the present invention. The term "label" or "labelling group" refers to any detectable label. In general, labels fall into a variety of classes, depending on the assay in which they are to be detected – the following examples include, but are not limited to:

isotopic labels, which may be radioactive or heavy isotopes, such as radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{89}Zr, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I)

magnetic labels (e.g., magnetic particles)

redox active moieties

optical dyes (including, but not limited to, chromophores, phosphors and fluorophores) such as fluorescent groups (e.g., FITC, rhodamine, lanthanide phosphors), chemiluminescent groups, and fluorophores which can be either "small molecule" fluors or proteinaceous fluors

enzymatic groups (e.g. horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase)

biotinylated groups

predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sides for secondary antibodies, metal binding domains, epitope tags, etc.)

By "fluorescent label" is meant any molecule that may be detected via its inherent fluorescent properties. Suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue‡, Texas Red, IAEDANS, EDANS, BODIPY FL, LC Red 640, Cy 5, Cy 5.5, LC Red 705, Oregon green, the Alexa-Fluor dyes (Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660, Alexa Fluor 680), Cascade Blue, Cascade Yellow and R-phycoerythrin (PE) (Molecular Probes, Eugene, OR), FITC, Rhodamine, and Texas Red (Pierce, Rockford, IL), Cy5, Cy5.5, Cy7 (Amersham Life Science, Pittsburgh, PA). Suitable optical dyes, including fluorophores, are described in Molecular Probes Handbook by Richard P. Haugland.

Suitable proteinaceous fluorescent labels also include, but are not limited to, green fluorescent protein, including a Renilla, Ptilosarcus, or Aequorea species of GFP (Chalfie et al., 1994, Science 263:802-805), EGFP (Clontech Laboratories, Inc., Genbank Accession Number U55762), blue fluorescent protein (BFP, Quantum Biotechnologies, Inc. 1801 de Maisonneuve Blvd. West, 8th Floor, Montreal, Quebec, Canada H3H 1J9; Stauber, 1998, Biotechniques 24:462-471; Heim et al., 1996, Curr. Biol. 6:178-182), enhanced yellow fluorescent protein (EYFP, Clontech Laboratories, Inc.), luciferase (Ichiki et al., 1993, J. Immunol. 150:5408-5417), β galactosidase (Nolan et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:2603-2607) and Renilla (WO92/15673, WO95/07463, WO98/14605, WO98/26277, WO99/49019, U.S. Patent Nos. 5,292,658; 5,418,155; 5,683,888; 5,741,668; 5,777,079; 5,804,387; 5,874,304; 5,876,995; 5,925,558).

Leucine zipper domains are peptides that promote oligomerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., 1988, Science 240:1759), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble oligomeric proteins are described in PCT application WO 94/10308, and the leucine zipper derived from lung surfactant protein D (SPD) described in Hoppe et al., 1994, FEBS Letters 344:191. The use of a modified leucine

zipper that allows for stable trimerization of a heterologous protein fused thereto is described in Fanslow et al., 1994, Semin. Immunol. 6:267-78.

The binding construct of the invention may also comprise additional domains, which are e.g. helpful in the isolation of the molecule or relate to an adapted pharmacokinetic profile of the molecule. Domains helpful for the isolation of a binding construct may be selected from peptide motives or secondarily introduced moieties, which can be captured in an isolation method, e.g. an isolation column. Non-limiting embodiments of such additional domains comprise peptide motives known as Myc-tag, HAT-tag, HA-tag, TAP-tag, GST-tag, chitin binding domain (CBD-tag), maltose binding protein (MBP-tag), Flag-tag, Strep-tag and variants thereof (e.g. StrepII-tag) and His-tag. All herein disclosed binding constructs may comprise a His-tag domain, which is generally known as a repeat of consecutive His residues in the amino acid sequence of a molecule, preferably of five, and more preferably of six His residues (hexa-histidine). The His-tag may be located e.g. at the N- or C-terminus of the binding construct, preferably it is located at the C-terminus. Most preferably, a hexa-histidine tag (HHHHHH) (SEQ ID NO: 30) is linked via peptide bond to the C-terminus of the binding construct according to the invention. Additionally, a conjugate system of PLGA-PEG-PLGA may be combined with a poly-histidine tag for sustained release application and improved pharmacokinetic profile.

Diagnostic Methods

The binding constructs that are provided herein are useful for detecting MAGEB2 in biological samples and can be used for diagnostic purposes to detect, diagnose, or monitor diseases and/or conditions associated with MAGEB2.

The disclosed binding constructs provide a means for the detection of the presence of MAGEB2 in a sample using, for example, classical immunohistological methods known to those of skill in the art (e.g., Tijssen, 1993, Practice and Theory of Enzyme Immunoassays, Vol 15 (Eds R. H. Burdon and P. H. van Knippenberg, Elsevier, Amsterdam); Zola, 1987, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc.); Jalkanen et al., 1985, J. Cell. Biol. 101:976-985; Jalkanen et al., 1987, J. Cell Biol. 105:3087-3096). The detection of MAGEB2 can be performed *in vivo* or *in vitro*, but is preferably done *in vitro* on a sample that comprises cells obtained from a patient. The methods used to detect MAGEB2 expression in human tissue, e.g., tumor tissue, can be qualitative, semi-quantitative, or quantitative. For example, art-recognized antibody-based methods for detecting protein levels in biological samples include, but are not limited to, enzyme-linked immunosorbent assay (ELISA), radioimmunoassays, electrochemiluninescence (ECL) assays, surface plasmon resonance, western blot, immunoprecipitation, fluorescence-activated cell sorting (FACS), immunofluorescence, immunohistochemistry, and the like. Methods for detecting MAGEB2 expression in a biological sample may include control samples (negative and positive controls). For example, a negative control sample

may be a sample containing no MAGEB2 protein, and a positive controls sample is a sample containing MAGEB2 protein. Comparing the results with the negative and positive controls can confirm the presence or absence of MAGEB2 in the biological sample.

In a specific embodiment, the binding construct can be used in an immunohistochemistry (IHC) assay. These IHC assays can be performed on modern, higher throughput platforms that in some instances are automated. For example, the Roche-Ventana BenchMark ULTRA system.

IHC is a commonly used assay that involves the use of binding constructs such as antibodies to specifically bind to a target antigen in a tissue sample. This allows the assay user to ascertain whether the target antigen is or is not present in the tissue sample.

Immunohistochemical methods are well-known in the art and are described in, e.g., Immunohistochemical Staining Methods 6th Edition 2013, Taylor C R, Rudbeck L, eds., Dako North America, available as an Education Guide at www.dako.com; "Immunohistochemical Staining Method guide"). Immunohistochemical staining may be conducted on fixed tissue sections or on frozen tissue sections. When using fixed tissue sections, e.g., formalin fixed paraffin embedded (FFPE) tissue sections, the procedure can generally use the following exemplary steps: obtaining a tumor tissue sample (e.g., by biopsy), fixation of the tumor sample; embedding (e.g., in paraffin); sectioning and mounting; antigen retrieval; incubation with a primary antibody (e.g., a MAGEB2 antibody described herein), detection (e.g., after amplification of the antigen/antibody complex signal), and interpretation by a skilled practitioner (e.g., using an art-recognized scoring system).

Suitable, non-limiting fixatives include, for example, paraformaldehyde, glutaraldehyde, formaldehyde, acetic acid, acetone, osmium tetroxide, chromic acid, mercuric chloride, picric acid, alcohols (e.g., methanol, ethanol), Gendre's fluid, Rossman's fluid, B5 fixative, Bouin's fluid, Carnoy's fixative, and methacarn. In one embodiment, the tumor sample is fixed in formaldehyde (e.g., 10% neutral buffered formalin, which corresponds to 4% formaldehyde in a buffered solution). Once fixed, the tumor sample can be serially dehydrated using alcohol or xylene, embedded in paraffin, and cut with a microtome to generate tumor tissue sections (e.g., with a thickness of about 4-5 μm), which can then be mounted onto microscope slides (e.g., adhesive-coated glass slides). Exemplary embedding materials include paraffin, celloidin, OCT™ compound, agar, plastics, or acrylics. Another exemplary material, and as described in the Examples herein, is Histogel containing agarose/glycerine (ThermoFisher Scientific).

Provided herein are methods for detecting MAGEB2 expression in a biological sample, e.g., a human tumor tissue sample, by contacting the sample with an anti-MAGEB2 antigen binding construct, such as an anti-MAGEB2 antibody comprising the heavy and light chain variable region CDR sequences set forth herein, or the heavy and light chain variable region sequences set forth herein, wherein the

antigen binding construct specifically binds to human MAGEB2, and detecting the binding of the antibody to MAGEB2 in the sample, e.g., by immunohistochemistry.

Accordingly, the invention provides a method of diagnosing a subject with a tumor, comprising: a) determining the level of MAGEB2 in a sample obtained from the subject; and b) diagnosing the subject as having a MAGEB2 positive tumor when the level of MAGEB2 is increased relative to a control.

Provided herein is a method of identifying a subject having a MAGEB2 positive tumor comprising: a) determining the level of MAGEB2 in a sample obtained from the subject; and b) identifying the subject as having a MAGEB2 positive tumor when the level of MAGEB2 is increased relative to a control.

Provided herein is a method of identifying a subject as needing an anti-MAGEB2 therapeutic comprising: a) determining the level of MAGEB2 in a sample obtained from the subject; and b) identifying the subject as needing the anti-MAGEB2 therapeutic when the level of MAGEB2 is increased relative to a control.

Provided herein is a method of determining treatment for a subject with a MAGEB2 positive tumor comprising: determining the level of MAGEB2 in a sample obtained from the subject; and determining the treatment as comprising an anti-MAGEB2 therapeutic when the level of MAGEB2 is increased, relative to a control.

In certain embodiments, exemplary biological samples include blood samples, serum samples, cells, surgically-resected tissue, and biopsied tissue (e.g. cancer tissue) obtained from the cancer patient. Biological samples for use in the methods described herein can be fresh, frozen, or fixed. Biological samples, e.g., tumor samples, can be obtained from the patient using routine methods, such as, but not limited to, biopsy, surgical resection, or aspiration.

MAGEB2 protein expression in tissue, e.g., tumor, samples can be detected using direct or indirect methods. For example, in assays where the binding construct is an antibody, the primary antibody may comprise a detectable moiety, such as the enzyme horseradish peroxidase (HRP) or a fluorescent label (e.g., FITC, TRITC), as described herein. In certain embodiments, the primary antibody does not itself comprise a detectable moiety, but is instead detected by binding of a secondary antibody to it, for indirect immunohistochemistry. Accordingly, in certain embodiments, the secondary antibody comprises a detectable label, e.g., an enzymatic, chromogenic, or fluorescent label. In certain embodiments, the primary antibody is a chimeric antibody comprising human variable region sequences and a non-human Fc region. A secondary antibody can be used to recognize the non-human Fc region of the primary antibody in order to reduce background staining.

In other embodiments, MAGEB2 protein expression can be detected using the avidin-biotin complex method (ABC method). In these embodiments, for example when the binding construct is an antibody, the secondary antibody is biotinylated and can serve as a bridge between the primary antibody and biotin-avidin-peroxidase complex. Other suitable non-limiting methods for immunohistochemistry

include those described in Chapter 6 of the Immunohistochemical Staining Method guide, e.g., methods described in Chilosi et al., Biotech Histochem 1994; 69:235; Sabattini et al., J Clin Pathol 1998; 51:506-11; and Gross et al., JBC 1959; 234:1622.

General methods for preparing and staining frozen tissue sections are well known in the art, and are described in, e.g., the Immunohistochemical Staining Method guide, Chapter 3 of Immunohistochemistry and Methods (Buchwalow and Bocker, Springer-Verlag Berlin Heidelberg 2010), and Chapter 21 of Theory and Practice of Histological Techniques (Bancroft and Gamble, 6.sup.th Edition, Elsevier Ltd., 2008). In some embodiments, slides stained with, e.g., the anti-MAGEB2 antibodies described herein can be further counterstained with, e.g., hematoxylin and/or eosin, using methods well known in the art.

MAGEB2 protein expression detected by immunohistochemistry can be digitally scanned and analyzed (e.g., as described herein in the Examples), or otherwise evaluated and scored using art-recognized scoring methods. Non-limiting examples of scoring methods are described below.

For example, in certain embodiments, MAGEB2 expression can be evaluated using the H-score (histochemical score) system, which is widely used in the art and is useful given its dynamic range and use of weighted percentiles. The H-score is a semiquantitative scoring system based on the formula: 3x percentage of strongly staining cells (3+ staining) + 2x percentage of moderately staining (2+ staining) cells +1x percentage of weakly staining (1+ staining) cells +0x percentage of non-stained (0 staining) cells, giving a score range of 0 to 300. See, e.g., McCarty et al., Cancer Res 1986; 46:4244-8; Bosman et al., J Clin Pathol 1992; 45:120-4; Dieset et al., Analyt Quant Cytol Histol 1996; 18:351-4. Control tissue can include, e.g., matching non-tumor tissue from the same subject. Accordingly, in certain embodiments, the number of cells staining positive for MAGEB2 using the binding constructs herein (e.g., antibodies), and the intensity of staining, can be used to determine an H-score.

In other embodiments, MAGEB2 expression can be evaluated using the Allred scoring system (Allred et al., Mod Pathol 1998; 11:155-68; Harvey et al., J Clin Oncol 1999; 17:1474-91). This scoring system involves adding proportion and intensity scores to obtain a total score. Accordingly, in some embodiments, the proportion score is obtained based on the estimated proportion of tumor cells that are positive for MAGEB2 (0: none, 1: < 1/100, 2: 1/100 to 1/10, 3: 1/10 to 1/3; 4: 1/3 to 2/3; and 5: >2/3), and the intensity score is obtained based on the average intensity of MAGEB2 expression in positive tumor cells (0: none, 1: weak, 2: intermediate, 3: strong). Allred scores range from 0 to 8, with a score ranging from 3 to 8 considered positive (i.e., positive detection). Accordingly, in certain embodiments, tumors with an Allred score of 3 to 8, for example, an Allred score of 3, 4, 5, 6, 7, or 8, for MAGEB2 staining are considered MAGEB2-positive tumors.

In other embodiments, and as discussed herein above, the scoring system can be automated, e.g., computerized, and quantified by image analysis. Automated methods are well known in the art. For

example, an average threshold measure (ATM) score, which obtains an average of 255 staining intensity levels, can be calculated as described in, e.g., Choudhury et al., J Histochem Cytochem 2010; 58:95-107, Rizzardi et al., Diagnostic Pathology 2012; 7:42-52. Another automated scoring system is AQUA® (automated quantitative analysis), which is performed using, e.g., tissue microarrays (TMAs), on a continuous scale. AQUA® is a hybrid of standard immunohistochemistry and flow cytometry that provides an objective numeric score ranging from 1-255, and involves antigen retrieval, use of primary and secondary antibodies, and multiplexed fluorescent detection. Optimal cutoff points can be determined as described in Camp et al. Clin Cancer Res 2004; 10:7252-9. The AQUA® scoring system is described in detail in Camp et al., Nat Med 2002; 8:1323-7; Camp et al., Cancer Res 2003; 63:1445-8; Ghosh et al., Hum Pathol 2008; 39:1835-43; Bose et al., BMC Cancer 2012; 12:332; Mascaux et al., Clin Cancer Res 2011; 17:7796-807. Other suitable automated immunohistochemistry platforms include commercially available platforms, such as the Leica BOND RX staining platform. The platform detects target protein expression in tumor tissue on the basis of staining intensity on the following scale: minimal, <1 cells per 20x objective field; mild, 1 - 10 cells per 20x objective field; moderate, 10 - 50 cells per 20x objective field, marked, 50 - 200 cells per 20x objective; and intense, >200 cells per 20x objective field.

In some embodiments, a tumor is considered MAGEB2-positive and likely to benefit from a therapeutic that targets MAGEB2 if a sample of the tumor has more MAGEB2-positive cells than a matching normal tissue from the same cancer patient.

In certain embodiments, the cancer patient is likely to or predicted to benefit from a therapeutic that targets MAGEB2 if the number of MAGEB2-positive cells in the tumor sample exceeds a threshold level. A threshold level may be the lowest level of MAGEB2 as determined with a given detection system, e.g., immunohistochemistry, as further described herein, such as in the Examples. In certain embodiments, the cancer patient is likely to or predicted to benefit from a therapeutic that targets MAGEB2 if the number of MAGEB2-positive cells in the tumor sample exceeds a threshold number or proportion using an art-recognized scoring system described supra. Accordingly, in some embodiments, if the number or proportion of MAGEB2-positive cells exceeds a certain threshold number or proportion using a scoring system described herein, then the sample can be defined as being "MAGEB2-positive."

In certain embodiments, a tumor sample having at least 1%, for example, at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 90%, or at least 95% of cells in the tumor expressing MAGEB2 is designated as being "MAGEB2-positive" and

indicates that the cancer patient is likely to or predicted to respond to a therapeutic that targets MAGEB2.

In some embodiments, an H score can be calculated for the tumor sample. Accordingly, in certain embodiments, a tumor sample with an H score of at least 5, such as at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, or at least 290 is designated as being "MAGEB2-positive" and indicates that the cancer patient is likely to or predicted to respond to a therapeutic that targets MAGEB2.

In some embodiments, an Allred score can be calculated for the tumor sample. Accordingly, in certain embodiments, a tumor sample with an Allred score of at least 3, such as at least 4, at least 5, at least 6, at least 7, or 8 is designated as being "MAGEB2-positive" and indicates that the cancer patient is likely to or predicted to respond to a therapeutic that targets MAGEB2.

In some embodiments, an AQUA score can be calculated. Accordingly, in certain embodiments, a tumor sample with an AQUA score of at least 5, such as at least 10, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, or at least 250 is designated as being "MAGEB2-positive" and indicates that the cancer patient is likely to or predicted to respond to a therapeutic that targets MAGEB2.

In certain embodiments, a combination of one of the scoring systems above with another detection modality, such as FISH, is used to increase the accuracy of determining whether a tumor is MAGEB2-positive or MAGEB2-negative.

In certain embodiments, the patient has a cancer selected from the group consisting of squamous cell carcinoma, small-cell lung cancer, non-small cell lung cancer, squamous non-small cell lung cancer (NSCLC), non squamous NSCLC, glioma, gastrointestinal cancer, renal cancer (e.g. clear cell carcinoma), ovarian cancer, liver cancer, colorectal cancer, endometrial cancer, kidney cancer (e.g., renal cell carcinoma (RCC)), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), thyroid cancer, neuroblastoma, pancreatic cancer, glioblastoma (glioblastoma multiforme), cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer (or carcinoma), gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, melanoma (e.g., metastatic malignant melanoma, such as cutaneous or intraocular malignant melanoma), bone cancer, skin cancer, uterine cancer, cancer of the anal region, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the ureter, carcinoma of the

renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain cancer, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally-induced cancers including those induced by asbestos, virus-related cancers or cancers of viral origin (e.g., human papilloma virus (HPV-related or -originating tumors)), and hematologic malignancies derived from either of the two major blood cell lineages, i.e., the myeloid cell line (which produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells) or lymphoid cell line (which produces B, T, NK and plasma cells), such as all types of leukemias, lymphomas, and myelomas, e.g., acute, chronic, lymphocytic and/or myelogenous leukemias, such as acute leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML), undifferentiated AML (M0), myeloblastic leukemia (M1), myeloblastic leukemia (M2; with cell maturation), promyelocytic leukemia (M3 or M3 variant [M3V]), myelomonocytic leukemia (M4 or M4 variant with eosinophilia [M4E]), monocytic leukemia (M5), erythroleukemia (M6), megakaryoblastic leukemia (M7), isolated granulocytic sarcoma, and chloroma; lymphomas, such as Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), B cell hematologic malignancy, e.g., B-cell lymphomas, T-cell lymphomas, lymphoplasmacytoid lymphoma, monocytoid B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, anaplastic (e.g., Ki 1+) large-cell lymphoma, adult T-cell lymphoma/leukemia, mantle cell lymphoma, angio immunoblastic T-cell lymphoma, angiocentric lymphoma, intestinal T-cell lymphoma, primary mediastinal B-cell lymphoma, precursor T-lymphoblastic lymphoma, T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL), peripheral T-cell lymphoma, lymphoblastic lymphoma, post-transplantation lymphoproliferative disorder, true histiocytic lymphoma, primary central nervous system lymphoma, primary effusion lymphoma, B cell lymphoma, lymphoblastic lymphoma (LBL), hematopoietic tumors of lymphoid lineage, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, Burkitt's lymphoma, follicular lymphoma, diffuse histiocytic lymphoma (DHL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, cutaneous T-cell lymphoma (CTLC) (also called mycosis fungoides or Sezary syndrome), and lymphoplasmacytoid lymphoma (LPL) with Waldenstrom's macroglobulinemia; myelomas, such as IgG myeloma, light chain myeloma, nonsecretory myeloma, smoldering myeloma (also called indolent myeloma), solitary plasmocytoma, and multiple myelomas, chronic lymphocytic leukemia (CLL), hairy cell lymphoma; hematopoietic tumors of myeloid lineage, tumors of mesenchymal origin, including fibrosarcoma and rhabdomyoscarcoma; seminoma, teratocarcinoma, tumors of the central and peripheral nervous, including astrocytoma, schwannomas; tumors of mesenchymal origin, including fibrosarcoma, rhabdomyoscaroma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not

limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL) preferably of the T-cell type; a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angiocentric (nasal) T-cell lymphoma; cancer of the head or neck, renal cancer, rectal cancer, cancer of the thyroid gland; acute myeloid lymphoma, as well as any combinations of said cancers. In certain embodiments, the cancer is a metastatic cancer, refractory cancer, or recurrent cancer.

Treatment of Patients

Once a cancer patient has been identified as being likely to benefit from treatment with a therapeutic that targets MAGEB2 expressing tumors (i.e., has a MAGEB2-positive tumor), the patient can be treated with an anti-MAGEB2 therapeutic (e.g., an anti-MAGEB2 immunotherapeutic such as those found in U.S. Patent Application Nos. 63/027,148 filed on May 19, 2020 and entitled "MAGEB2 Binding Constructs" and 63/128,773 filed on December 21, 2020 and entitled "MAGEB2 Binding Constructs." Accordingly, provided herein is a method for treating a tumor in a subject, said method comprising: determining the subject as responsive to treatment with an anti-MAGEB2 therapeutic by obtaining a sample from the subject, wherein the sample comprises a cell from the tumor, measuring the level of MAGEB2 in the sample, and determining the subject as responsive to treatment with an anti-MAGEB2 therapeutic, and administering to the subject an effective amount of the anti-MAGEB2 therapeutic. Provided herein are methods of treating a cancer patient comprising: (a) determining whether a tumor of a cancer patient is MAGEB2-positive using the methods described herein, e.g., contacting a tumor sample from the patient with binding construct described herein, wherein the antibody or antigen-binding fragment thereof specifically binds to human MAGEB2, detecting the binding of the antibody to MAGEB2 in the sample, and determining the level of MAGEB2 protein expression in the sample, wherein a level of MAGEB2 protein above a threshold level in tumor cells indicates that the tumor is MAGEB2-positive, and (b) if the tumor is determined to be MAGEB2-positive, then administering a therapeutically effective amount of an agent that targets cells that express MAGEB2 and kills them. Provided herein is a method of treating a tumor in a subject, wherein the subject's tumor has been tested for MAGEB2 levels and the subject's tumor is MAGEB2 positive, comprising administering an anti-MAGEB2 therapeutic to the subject.

Provided herein is a method of determining efficacy of treatment with an anti-MAGEB2 therapeutic in a subject comprising: determining the level of MAGEB2 in a sample obtained from the subject before treatment with an anti-MAGEB2 therapeutic and after treatment with an anti-MAGEB2 therapeutic;

and determining the treatment as effective when the level of MAGEB2 positive tumor cells is decreased after treatment with the anti-MAGEB2 therapeutic.

Provided herein is a method of monitoring treatment with an anti-MAGEB2 therapeutic in a subject comprising: determining the level of MAGEB2 in a sample obtained from the subject at a first timepoint; determining the level of MAGEB2 in a sample obtained from the subject at a second timepoint; and optionally continuing treatment with an anti-MAGEB2 therapeutic when the level of MAGEB2 is decreased at the second timepoint relative to the level at the first timepoint.

Provided herein is a method of identifying a subject as responsive to treatment with an anti-MAGEB2 therapeutic comprising: determining the level of MAGEB2 in a sample obtained from the subject; identifying the subject as responsive to treatment with an anti-MAGEB2 therapeutic when the level of MAGEB2 decreases upon treatment with an anti-MAGEB2 therapeutic.

In a specific embodiment of the above embodiments, the subject or patient is treated with a therapeutically-effective amount of any of the molecules disclosed in U.S. Patent Application Nos. 63/027,148 filed on May 19, 2020 and entitled "MAGEB2 Binding Constructs" and 63/128,773 filed on December 21, 2020 and entitled "MAGEB2 Binding Constructs," the contents of which are herein incorporated by reference.

In a further specific embodiment of the above embodiments, various reference levels of MAGEB2 in a patient sample can be compared to each other. For example, a first reference level taken at a first time point can be compared to a second level taken at a second time point. Further, e.g., MAGEB2 levels can be compared prior to drug treatment and after drug treatment. In this example, it can be envisaged that successful treatment with a drug will yield a lower measured MAGEB2 level after treatment as compared to the reference MAGEB2 level measured before treatment. The first and the second tissue sample can be obtained, e.g., within 3-7 days, 1 week to 3 weeks, or 1 month to 3 months from each other, or even a longer duration.

In certain embodiments, a method for monitoring a MAGEB2-expressing tumor in a cancer patient comprises: (a) detecting MAGEB2 protein expression in a tissue (e.g., tumor) sample at a first time point by using an anti-MAGEB2 antibody, or antigen-binding portion thereof, (b) determining the level of MAGEB2 protein expression at the first time point, (c) detecting MAGEB2 protein expression in a tissue (e.g., tumor, which may be the same tumor as the one that was biopsied at the first time point) sample at a second time point using the same antibody used in step (a), and (d) determining the level of MAGEB2 protein expression in the tumor from the second time point.

Provided herein are methods for monitoring the efficacy of anti-MAGEB2 immunotherapy (e.g., one of the molecules provided in U.S. Patent Application Nos. 63/027,148 filed on May 19, 2020 and entitled "MAGEB2 Binding Constructs" and 63/128,773 filed on December 21, 2020 and entitled "MAGEB2 Binding Constructs") in a patient having a MAGEB2-positive tumor comprising: (a) detecting MAGEB2

protein expression in the MAGEB2-positive tumor at a first time point before or after initiating anti-MAGEB2 immunotherapy by using an anti- MAGEB2 antibody, (b) determining the level of MAGEB2 protein expression in the tumor from the first time point, (c) detecting MAGEB2 protein expression in the MAGEB2-positive tumor at a second time point after initiating anti- MAGEB2 immunotherapy using the same antibody from step (a), (d) determining the level of MAGEB2 protein expression in the tumor from the second time point; (e) comparing the levels of MAGEB2 protein expression determined at the first and second time points, wherein a higher level at the first time point relative to the second time point may be indicative of effective anti- MAGEB2 immunotherapy, a lower score at the first time point relative to the second time point is indicative of ineffective anti- MAGEB2 immunotherapy, and an unchanged score at the first time point relative to the second time point may be indicative of anti-MAGEB2 immunotherapy being stabilizing. MAGEB2 expression may be determined in the tumor, tumor tissue, or tumor cells.

In certain embodiments, the status of a MAGEB2-expressing tumor can be monitored repeatedly after initial diagnosis (i.e., after making a determination that the tumor is MAGEB2-positive), such as one month after initial diagnosis, two months after initial diagnosis, three months after initial diagnosis, four months after initial diagnosis, five months after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc. In other embodiments, the efficacy of anti- MAGEB2 immunotherapy against a MAGEB2-expressing tumor can be monitored repeatedly after initiating anti-MAGEB2 immunotherapy, such as one month after initiation of therapy, two months after initiation of therapy, three months after initiation of therapy, four months after initiation of therapy, five months after initiation of therapy, six months after initiation of therapy, one year after initiation of therapy, etc.

Sequence Modifications of MAGEB2 Binding Constructs

The term "amino acid" or "amino acid residue" typically refers to an amino acid having its art recognized definition such as an amino acid selected from the group consisting of: alanine (Ala or A); arginine (Arg or R); asparagine (Asn or N); aspartic acid (Asp or D); cysteine (Cys or C); glutamine (Gln or Q); glutamic acid (Glu or E); glycine (Gly or G); histidine (His or H); isoleucine (Ile or I): leucine (Leu or L); lysine (Lys or K); methionine (Met or M); phenylalanine (Phe or F); proline (Pro or P); serine (Ser or S); threonine (Thr or T); tryptophan (Trp or W); tyrosine (Tyr or Y); and valine (Val or V), although

modified, synthetic, or rare amino acids may be used as desired. There are basically four different classes of amino acids determined by different side chains:

(1) non-polar and neutral (uncharged): Ala, Gly, Ile, Leu, Met, Phe, Pro, Val

(2) polar and neutral (uncharged): Asn, Cys (being only slightly polar), Gln, Ser, Thr, Trp (being only slightly polar), Tyr

(3) acidic and polar (negatively charged): Asp and Glu

(4) basic and polar (positively charged): Arg, His, Lys

Hydrophobic amino acids can be divided according to whether they have aliphatic or aromatic side chains. Phe and Trp (being very hydrophobic), Tyr and His (being less hydrophobic) are classified as aromatic amino acids. Strictly speaking, aliphatic means that the side chain contains only hydrogen and carbon atoms. By this strict definition, the amino acids with aliphatic side chains are alanine, isoleucine, leucine (also norleucine), proline and valine. Alanine's side chain, being very short, means that it is not particularly hydrophobic, and proline has an unusual geometry that gives it special roles in proteins. It is often convenient to consider methionine in the same category as isoleucine, leucine and valine, although it also contains a sulphur atom. The unifying theme is that these amino acids contain largely non-reactive and flexible side chains. The amino acids alanine, cysteine, glycine, proline, serine and threonine are often grouped together for the reason that they are all small in size. Gly and Pro may influence chain orientation.

Amino acid modifications include, for example, deletions of residues from, insertions of residues into, and/or substitutions of residues within the amino acid sequences of the binding constructs. Any combination of deletion, insertion, and/or substitution is made to arrive at a final binding construct, provided that the final construct possesses the desired characteristics, e.g. the biological activity of the unmodified parental molecule (such as binding to MAGEB2). The amino acid changes may also alter post-translational processes of the binding constructs, such as changing the number or position of glycosylation sites.

For example, 1, 2, 3, 4, 5, or 6 amino acids may be inserted, deleted and/or substituted in each of the CDRs (of course, dependent on their respective length), while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be inserted, deleted and/or substituted in each of the FRs. Amino acid sequence insertions also include N-terminal and/or C-terminal additions of amino acids ranging in length from e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues to polypeptides containing more than 10, e.g. one hundred or more residues, as well as intra-sequence insertions of single or multiple amino acid residues. An insertional variant of the binding construct of the invention includes the fusion of a polypeptide which increases or extends the serum half-life of the binding construct to the N-

terminus or to the C-terminus of the binding construct. It is also conceivable that such insertion occurs within the binding construct, e.g. between the first and the second domain.

The sites of greatest interest for amino acid modifications, in particular for amino acid substitutions, include the the hypervariable regions, in particular the individual CDRs of the heavy and/or light chain, but FR alterations in the heavy and/or light chain are also contemplated. The substitutions can be conservative substitutions as described herein. Preferably, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids may be substituted in a CDR, while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be substituted in the framework regions (FRs), depending on the length of the CDR or FR, respectively. For example, if a CDR sequence encompasses 6 amino acids, it is envisaged that one, two or three of these amino acids are substituted. Similarly, if a CDR sequence encompasses 15 amino acids it is envisaged that one, two, three, four, five or six of these amino acids are substituted.

A useful method for the identification of certain residues or regions within the binding constructs that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" and is described e.g. in Cunningham B.C. and Wells J.A. (Science. 1989 Jun 2;244(4908):1081-5). Here, a residue or group of residues within the binding construct is/are identified (e.g. charged residues such as Arg, His, Lys, Asp, and Glu) and replaced by a neutral or non-polar amino acid (most preferably alanine or polyalanine) to affect the interaction of the respective amino acid(s) with the epitope of the target protein. Alanine scanning is a technique used to determine the contribution of a specific residue to the stability or function of given protein. Alanine is used because of its non-bulky, chemically inert, methyl functional group that nevertheless mimics the secondary structure preferences that many of the other amino acids possess. Sometimes bulky amino acids such as valine or leucine can be used in cases where conservation of the size of mutated residues is needed. This technique can also be useful to determine whether the side chain of a specific residue plays a significant role in bioactivity. Alanine scanning is usually accomplished by site-directed mutagenesis or randomly by creating a PCR library. Furthermore, computational methods to estimate thermodynamic parameters based on a theoretical alanine substitutions have been developed. The data can be tested by IR, NMR Spectroscopy, mathematical methods, bioassays, etc.

Those amino acid locations demonstrating functional sensitivity to the substitutions (as determined e.g. by alanine scanning) can then be refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site or region for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se needs not to be predetermined. For example, to analyze or optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at a target codon or region, and the expressed binding construct variants are screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in the DNA having a known sequence are well known,

for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants is done e.g. using assays of antigen (e.g. MAGEB2) binding activity and/or of cytotoxic activity.

Generally, if amino acids are substituted in one or more or all of the CDRs of the heavy and/or light chain, it is envisaged that the then-obtained "substituted" sequence is at least 60% or 65%, more preferably 70% or 75%, even more preferably 80% or 85%, and particularly preferably 90% or 95% identical / homologous to the "original" or "parental" CDR sequence. This means that the degree of identity / homology between the original and the substituted sequence depends on the length of the CDR. For example, a CDR having 5 amino acids in total and comprising one amino acid substitution is 80% identical to the "original" or "parental" CDR sequence, while a CDR having 10 amino acids in total and comprising one amino acid substitution is 90% identical to the "original" or "parental" CDR sequence. Accordingly, the substituted CDRs of the binding construct of the invention may have different degrees of identity to their original sequences, e.g., CDRL1 may have 80%, while CDRL3 may have 90% of homology. The same considerations apply to the framework regions and to the entire VH and VL regions.

A "variant CDR" is a CDR with a specific sequence homology, similarity, or identity to the parent CDR of the invention, and shares biological function with the parent CDR, including, but not limited to, at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the specificity and/or activity of the parent CDR. Generally, the amino acid homology, similarity, or identity between individual variant CDRs is at least 60% to the parent sequences depicted herein, and more typically with increasing homologies, similarities or identities of at least 65% or 70%, preferably at least 75% or 80%, more preferably at least 85%, 90%, 91%, 92%, 93%, 94%, and most preferably 95%, 96%, 97%, 98%, 99%, and almost 100%. The same applies to "variant VH" and "variant VL". According to one embodiment, the sequence variations within a "variant VH" and/or a "variant VL" do not extend to the CDRs. The present invention is hence directed to a binding construct as defined herein, comprising VH and VL sequences having a certain sequence homology (see above) to the specific sequences as defined herein (the "parental" VH and VL), wherein the CDR sequences are 100% identical to the specific CDR sequences as defined herein (the "parental" CDRs).

Preferred substitutions (or replacements) are conservative substitutions. However, any substitution (including non-conservative substitutions or one or more from the "exemplary substitutions" listed in Table 6, below) is envisaged, as long as the binding construct retains its capacity to bind to MAGEB2, and/or provided its CDRs, FRs, VH and/or VL sequences have a degree of identity to the original or

parental sequence of at least 60% or 65%, more preferably at least 70% or 75%, even more preferably at least 80% or 85%, and particularly preferably at least 90% or 95%.

A conservative replacement (also called a conservative mutation or a conservative substitution) is an amino acid replacement that changes a given amino acid to a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity, size). Conservative replacements in proteins often have a smaller effect on protein function than non-conservative replacements. Conservative substitutions are shown in Table 6. Exemplary conservative substitutions are shown as "exemplary substitutions". If such substitutions result in a change in biological activity, then more substantial changes, as further described herein in reference to amino acid classes, may be introduced and the products screened for a desired characteristic.

Table 6: Amino acid substitutions (aa = amino acid)

Original aa	**Conservative substitutions**	**Exemplary Substitutions**
Ala (A)	Small aa	Gly, Ser, Thr
Arg (R)	Polar aa, in particular Lys	Lys, Gln, Asn
Asn (N)	Polar aa, in particular Asp	Asp, Gln, His, Lys, Arg
Asp (D)	Glu or other polar aa, in particular Asn	Glu, Asn
Cys (C)	Small aa	Ser, Ala
Gln (Q)	Polar aa, in particular Glu	Glu, Asn
Glu (E)	Asp or other polar aa, in particular Gln	Asp, Gln
Gly (G)	Small aa, such as Ala	Ala
His (H)		Asn, Gln, Arg, Lys, Tyr
Ile (I)	Hydrophobic, in particular aliphatic aa	Ala, Val, Met, Leu, Phe
Leu (L)	Hydrophobic, in particular aliphatic aa	Norleucine, Ile, Ala, Val, Met
Lys (K)	Polar aa, in particular Arg	Arg, Gln, Asn
Met (M)	Hydrophobic, in particular aliphatic aa	Leu, Ala, Ile, Val, Phe
Phe (F)	Aromatic or hydrophobic aa, in particular Tyr	Tyr, Trp, Leu, Val, Ile, Ala
Pro (P)	Small aa	Ala
Ser (S)	Polar or small aa, in particular Thr	Thr
Thr (T)	Polar aa, in particular Ser	Ser
Trp (W)	Aromatic aa	Tyr, Phe
Tyr (Y)	Aromatic aa, in particular Phe	Phe, Trp, Thr, Ser
Val (V)	Hydrophobic, in particular aliphatic aa	Leu, Ile, Ala, Met, Phe

Substantial modifications in the biological properties of the binding construct of the present invention are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Non-conservative substitutions will usually entail exchanging a member of one of the above defined amino acid classes (such as polar, neutral, acidic, basic, aliphatic, aromatic, small…) for another class. Any cysteine residue not involved in maintaining the proper conformation of the binding construct may be substituted, generally with serine, to improve the oxidative stability of the binding construct.

In addition to the above described substitutions, other substitutions within the CDRs that contribute to binding can be made. For example, the consensus sequences set forth in Table 25 herein provide such substitutions. In certain embodiments, deletions as guided by the provided consensus sequences may also be made. With the guidance provided herein by the consensus sequences set forth in Table 25, various amino acid substitutions, and functional equivalents thereof, or deletions, can be readily made by one of ordinary skill in the art.

Sequence identity, homology and/or similarity of amino acid sequences is determined by using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith and Waterman, 1981, Adv. Appl. Math. 2:482, the sequence identity alignment algorithm of Needleman and Wunsch (J Mol Biol. 1970 Mar;48(3):443-53), the search for similarity method of Pearson and Lipman (Proc Natl Acad Sci USA. 1988 Apr;85(8):2444-8), computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.), the Best Fit sequence program described by Devereux et al. (Nucleic Acids Res.1984 Jan 11;12(1 Pt 1):387-95), preferably using the default settings, or by inspection. It is envisaged that percent identity is calculated by FastDB based upon the following parameters: mismatch penalty of 1; gap penalty of 1; gap size penalty of 0.33; and joining penalty of 30. See also "Current Methods in Sequence Comparison and Analysis," Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp 127-149 (1988), Alan R. Liss, Inc.

An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng and Doolittle (J Mol Evol.1987;25(4):351-60); the method is similar to that

described by Higgins and Sharp (Comput Appl Biosci. 1989 Apr;5(2):151-3). Useful PILEUP parameters include a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps. Another example of a useful algorithm is the BLAST algorithm, described in: Altschul et al. (J Mol Biol. 1990 Oct 5;215(3):403-10.); Altschul et al., (Nucleic Acids Res. 1997 Sep 1;25(17):3389-402); and Karlin and Altschul (Proc Natl Acad Sci U S A. 1993 Jun 15;90(12):5873-7). A particularly useful BLAST program is the WU-Blast-2 program which was obtained from Altschul et al., (Methods Enzymol. 1996; 266:460-80). WU-Blast-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T)=II. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

An additional useful algorithm is gapped BLAST as reported by Altschul et al. (Nucleic Acids Res. 1997 Sep 1;25(17):3389-402). Gapped BLAST uses BLOSUM-62 substitution scores; threshold T parameter set to 9; the two-hit method to trigger ungapped extensions, charges gap lengths of k a cost of 10+k; Xu set to 16, and Xg set to 40 for database search stage and to 67 for the output stage of the algorithms. Gapped alignments are triggered by a score corresponding to about 22 bits.

Nucleotides Encoding the Binding constructs

The invention provides a polynucleotide / nucleic acid molecule encoding a binding construct of the invention. Nucleic acid molecules are biopolymers composed of nucleotides. A polynucleotide is a biopolymer composed of 13 or more nucleotide monomers covalently bonded in a chain. DNA (such as cDNA) and RNA (such as mRNA) are examples of polynucleotides / nucleic acid molecules with distinct biological function. Nucleotides are organic molecules that serve as the monomers or subunits of nucleic acid molecules like DNA or RNA. The nucleic acid molecule or polynucleotide of the present invention can be double stranded or single stranded, linear or circular. It is envisaged that the nucleic acid molecule or polynucleotide is comprised in a vector. It is furthermore envisaged that such vector is comprised in a host cell. Said host cell is, e.g. after transformation or transfection with the vector or the polynucleotide / nucleic acid molecule of the invention, capable of expressing the binding construct. For this purpose, the polynucleotide or nucleic acid molecule is operatively linked with control sequences.

The genetic code is the set of rules by which information encoded within genetic material (nucleic acids) is translated into proteins. Biological decoding in living cells is accomplished by the ribosome which links amino acids in an order specified by mRNA, using tRNA molecules to carry amino acids and to read the mRNA three nucleotides at a time. The code defines how sequences of these nucleotide

triplets, called codons, specify which amino acid will be added next during protein synthesis. With some exceptions, a three-nucleotide codon in a nucleic acid sequence specifies a single amino acid. Because the vast majority of genes are encoded with exactly the same code, this particular code is often referred to as the canonical or standard genetic code.

Degeneracy of codons is the redundancy of the genetic code, exhibited as the multiplicity of three-base pair codon combinations that specify an amino acid. Degeneracy results because there are more codons than encodable amino acids. The codons encoding one amino acid may differ in any of their three positions; however, more often than not, this difference is in the second or third position. For instance, codons GAA and GAG both specify glutamic acid and exhibit redundancy; but, neither specifies any other amino acid and thus demonstrate no ambiguity. The genetic codes of different organisms can be biased towards using one of the several codons that encode the same amino acid over the others – that is, a greater frequency of one will be found than expected by chance. For example, leucine is specified by six distinct codons, some of which are rarely used. Codon usage tables detailing genomic codon usage frequencies for most organisms are available. Recombinant gene technologies commonly take advantage of this effect by implementing a technique termed codon optimization, in which those codons are used to design a polynucleotide which are preferred by the respective host cell (such as a cell of human hamster origin, an Escherichia coli cell, or a Saccharomyces cerevisiae cell), e.g. in order to increase protein expression. It is hence envisaged that the polynucleotides / nucleic acid molecules of the present disclosure are codon optimized. Nevertheless, the polynucleotide / nucleic acid molecule encoding a binding construct of the invention may be designed using any codon that encodes the desired amino acid.

In line herewith, the term "percent (%) nucleic acid sequence identity / homology / similarity" with respect to the nucleic acid sequence encoding the binding constructs identified herein is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the coding sequence of the binding construct. One method to align two sequences and thereby dertermine their homology uses the BLASTN module of WU-Blast2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively. Generally, the nucleic acid sequence homology, similarity, or identity between the nucleotide sequences encoding individual variant CDRs and the nucleotide sequences depicted herein are at least 60%, and more typically with increasing homologies, similarities or identities of at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and almost 100%. Again, the same applies to nucleic acid sequence encoding the "variant VH" and/or "variant VL".

In one embodiment, the percentage of identity to human germline of the binding constructs according to the invention, or of the first and second domain (binding domains) of these binding constructs, is

≥ 70% or ≥ 75%, more preferably ≥ 80% or ≥ 85%, even more preferably ≥ 90%, and most preferably ≥ 91%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95% or even ≥ 96%. Identity to human antibody germline gene products is thought to be an important feature to reduce the risk of therapeutic proteins to elicit an immune response against the drug in the patient during treatment. Hwang W.Y. and Foote J. (Methods. 2005 May;36(1):3-10) demonstrate that the reduction of non-human portions of drug binding constructs leads to a decrease of risk of inducing anti-drug antibodies in the patients during treatment. By comparing an exhaustive number of clinically evaluated antibody drugs and the respective immunogenicity data, the trend is shown that humanization of the variable regions of antibodies / binding constructs makes the protein less immunogenic (average 5.1 % of patients) than antibodies / binding constructs carrying unaltered non-human variable regions (average 23.59 % of patients). A higher degree of identity to human sequences is hence desirable for protein therapeutics based on variable regions and in the form of binding constructs. For the purpose of determining the germline identity, the V-regions of VL can be aligned with the amino acid sequences of human germline V segments and J segments (http://www2.mrc-lmb.cam.ac.uk/vbase/) using Vector NTI software and the amino acid sequence calculated by dividing the identical amino acid residues by the total number of amino acid residues of the VL in percent. The same can be done for the VH segments (http://www2.mrc-lmb.cam.ac.uk/vbase/) with the exception that the VH CDR3 may be excluded due to its high diversity and a lack of existing human germline VH CDR3 alignment partners. Recombinant techniques can then be used to increase sequence identity to human antibody germline genes.

According to one embodiment, the polynucleotide / nucleic acid molecule of the present invention encoding the binding construct of the invention is in the form of one single molecule or in the form of two or more separate molecules. If the binding construct of the present invention is a single chain binding construct, the polynucleotide / nucleic acid molecule encoding such construct will most likely also be in the form of one single molecule.

The same applies for the vector comprising a polynucleotide / nucleic acid molecule of the present invention. If the binding construct of the present invention is a single chain binding construct, one vector may comprise the polynucleotide which encodes the binding construct in one single location (as one single open reading frame, ORF). One vector may also comprise two or more polynucleotides / nucleic acid molecules at separate locations (with individual ORFs), each one of them encoding a different component of the binding construct of the invention. It is envisaged that the vector comprising the polynucleotide / nucleic acid molecule of the present invention is in the form of one single vector or two or more separate vectors. In one embodiment, and for the purpose of expressing the binding construct in a host cell, the host cell of the invention should comprise the polynucleotide / nucleic acid molecule encoding the binding construct or the vector comprising such polynucleotide / nucleic acid molecule in their entirety, meaning that all components of the binding construct –

whether encoded as one single molecule or in separate molecules / locations – will assemble after translation and form together the biologically active binding construct of the invention.

The invention also provides a vector comprising a polynucleotide / nucleic acid molecule of the invention. A vector is a nucleic acid molecule used as a vehicle to transfer (foreign) genetic material into a cell, usually for the purpose of replication and/or expression. The term "vector" encompasses – but is not restricted to – plasmids, viruses, cosmids, and artificial chromosomes. Some vectors are designed specifically for cloning (cloning vectors), others for protein expression (expression vectors). So-called transcription vectors are mainly used to amplify their insert. The manipulation of DNA is normally conducted on E. coli vectors, which contain elements necessary for their maintenance in E. coli. However, vectors may also have elements that allow them to be maintained in another organism such as yeast, plant or mammalian cells, and these vectors are called shuttle vectors. Insertion of a vector into the target or host cell is usually called transformation for bacterial cells and transfection for eukaryotic cells, while insertion of a viral vector is often called transduction.

In general, engineered vectors comprise an origin of replication, a multicloning site and a selectable marker. The vector itself is generally a nucleotide sequence, commonly a DNA sequence, that comprises an insert (transgene) and a larger sequence that serves as the "backbone" of the vector. While the genetic code determines the polypeptide sequence for a given coding region, other genomic regions can influence when and where these polypeptides are produced. Modern vectors may therefore encompass additional features besides the transgene insert and a backbone: promoter, genetic marker, antibiotic resistance, reporter gene, targeting sequence, protein purification tag. Vectors called expression vectors (expression constructs) specifically are for the expression of the transgene in the target cell, and generally have control sequences.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, a Kozak sequence and enhancers.

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the nucleotide sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction

sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

"Transfection" is the process of deliberately introducing nucleic acid molecules or polynucleotides (including vectors) into target cells. The term is mostly used for non-viral methods in eukaryotic cells. Transduction is often used to describe virus-mediated transfer of nucleic acid molecules or polynucleotides. Transfection of animal cells typically involves opening transient pores or "holes" in the cell membrane, to allow the uptake of material. Transfection can be carried out using biological particles (such as viral transfection, also called viral transduction), chemical-based methods (such as using calcium phosphate, lipofection, Fugene, cationic polymers, nanoparticles) or physical treatment (such as electroporation, microinjection, gene gun, cell squeezing, magnetofection, hydrostatic pressure, impalefection, sonication, optical transfection, heat shock).

The term "transformation" is used to describe non-viral transfer of nucleic acid molecules or polynucleotides (including vectors) into bacteria, and also into non-animal eukaryotic cells, including plant cells. Transformation is hence the genetic alteration of a bacterial or non-animal eukaryotic cell resulting from the direct uptake through the cell membrane(s) from its surroundings and subsequent incorporation of exogenous genetic material (nucleic acid molecules). Transformation can be effected by artificial means. For transformation to happen, cells or bacteria must be in a state of competence, which might occur as a time-limited response to environmental conditions such as starvation and cell density, and can also be artificially induced.

Moreover, the invention provides a host cell transformed or transfected with the polynucleotide / nucleic acid molecule of the invention or with the vector of the invention.

As used herein, the terms "host cell" or "recipient cell" are intended to include any individual cell or cell culture that can be or has been recipient of vectors, exogenous nucleic acid molecules and/or polynucleotides encoding the binding construct of the present invention; and/or recipients of the binding construct itself. The introduction of the respective material into the cell is carried out by way of transformation, transfection and the like (vide supra). The term "host cell" is also intended to include progeny or potential progeny of a single cell. Because certain modifications may occur in succeeding generations due to either natural, accidental, or deliberate mutation or due to environmental influences, such progeny may not, in fact, be completely identical (in morphology or in genomic or total DNA complement) to the parent cell, but is still included within the scope of the term as used herein. Suitable host cells include prokaryotic or eukaryotic cells, and also include -- but are not limited to -- bacteria (such as E. coli), yeast cells, fungi cells, plant cells, and animal cells such as insect cells and mammalian cells, e.g., hamster, murine, rat, macaque or human.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for the binding construct of the invention. Saccharomyces cerevisiae, or common

baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as Schizosaccharomyces pombe, Kluyveromyces hosts such as K. lactis, K. fragilis (ATCC 12424), K. bulgaricus (ATCC 16045), K. wickeramii (ATCC 24178), K. waltii (ATCC 56500), K. drosophilarum (ATCC 36906), K. thermotolerans, and K. marxianus; yarrowia (EP 402 226); Pichia pastoris (EP 183 070); Candida; Trichoderma reesia (EP 244 234); Neurospora crassa; Schwanniomyces such as Schwanniomyces occidentalis; and filamentous fungi such as Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as A. nidulans and A. niger.

Suitable host cells for the expression of a glycosylated binding construct are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodoptera frugiperda (caterpillar), Aedes aegypti (mosquito), Aedes albopictus (mosquito), Drosophila melanogaster (fruit fly), and Bombyx mori have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of Autographa californica NPV and the Bm-5 strain of Bombyx mori NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of Spodoptera frugiperda cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, Arabidopsis and tobacco can also be used as hosts. Cloning and expression vectors useful in the production of proteins in plant cell culture are known to those of skill in the art. See e.g. Hiatt et al., Nature (1989) 342: 76-78, Owen et al. (1992) Bio/Technology 10: 790-794, Artsaenko et al. (1995) The Plant J 8: 745-750, and Fecker et al. (1996) Plant Mol Biol 32: 979-986.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (cell culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (such as COS-7, ATCC CRL 1651); human embryonic kidney line (such as 293 or 293 cells subcloned for growth in suspension culture, Graham et al. , J. Gen Virol. 36 : 59 (1977)); baby hamster kidney cells (such as BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (such as CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77: 4216 (1980)); mouse sertoli cells (such as TM4, Mather, Biol. Reprod. 23: 243-251 (1980)); monkey kidney cells (such as CVI ATCC CCL 70); African green monkey kidney cells (such as VERO-76, ATCC CRL1587); human cervical carcinoma cells (such as HELA, ATCC CCL 2); canine kidney cells (such as MDCK, ATCC CCL 34); buffalo rat liver cells (such as BRL 3A, ATCC CRL 1442); human lung cells (such as W138, ATCC CCL 75); human liver cells (such as Hep G2,1413 8065); mouse mammary tumor (such as MMT 060562, ATCC CCL-51); TRI cells

(Mather et al., Annals N. Y Acad. Sci. (1982) 383: 44-68); MRC 5 cells; FS4 cells; and a human hepatoma line (such as Hep G2).

Production of Binding constructs

In a further embodiment, the invention provides a process for producing a binding construct of the invention, said process comprising culturing a host cell of the invention under conditions allowing the expression of the binding construct of the invention and recovering the produced binding construct from the culture.

As used herein, the term "culturing" refers to the in vitro maintenance, differentiation, growth, proliferation and/or propagation of cells under suitable conditions in a medium. Cells are grown and maintained in a cell growth medium at an appropriate temperature and gas mixture. Culture conditions vary widely for each cell type. Typical growth conditions are a temperature of about 37°C, a CO2 concentration of about 5% and a humidity of about 95%. Recipes for growth media can vary e.g. in pH, concentration of the carbon source (such as glucose), nature and concentration of growth factors, and the presence of other nutrients (such as amino acids or vitamins). The growth factors used to supplement media are often derived from the serum of animal blood, such as fetal bovine serum (FBS), bovine calf serum (FCS), equine serum, and porcine serum. Cells can be grown either in suspension or as adherent cultures. There are also cell lines that have been modified to be able to survive in suspension cultures so they can be grown to a higher density than adherent conditions would allow.

The term "expression" includes any step involved in the production of a binding construct of the invention including, but not limited to, transcription, post-transcriptional modification, translation, folding, post-translational modification, targeting to specific subcellular or extracellular locations, and secretion. The term "recovering" refers to a series of processes intended to isolate the binding construct from the cell culture. The "recovering" or "purification" process may separate the protein and non-protein parts of the cell culture, and finally separate the desired binding construct from all other polypeptides and proteins. Separation steps usually exploit differences in protein size, physico-chemical properties, binding affinity and biological activity. Preparative purifications aim to produce a relatively large quantity of purified proteins for subsequent use, while analytical purification produces a relatively small amount of a protein for a variety of research or analytical purposes.

When using recombinant techniques, the binding construct can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the binding construct is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. The binding construct of the invention may e.g. be produced in bacteria such as E. coli. After expression, the construct is isolated from the bacterial cell

paste in a soluble fraction and can be purified e.g. via affinity chromatography and/or size exclusion. Final purification can be carried out in a manner similar to the process for purifying a binding construct expressed in mammalian cells and secreted into the medium. Carter et al. (Biotechnology (NY) 1992 Feb;10(2):163-7) describe a procedure for isolating antibodies which are secreted to the periplasmic space of E. coli.

Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an ultrafiltration unit.

The binding construct of the invention prepared from the host cells can be recovered or purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography. Other techniques for protein purification such as fractionation on an ion-exchange column, mixed mode ion exchange, HIC, ethanol precipitation, size exclusion chromatography, reverse phase HPLC, chromatography on silica, chromatography on heparin sepharose, chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), immunoaffinity (such as Protein A/G/L) chromatography, chromato-focusing, SDS-PAGE, ultracentrifugation, and ammonium sulfate precipitation are also available depending on the binding construct to be recovered.

A protease inhibitor may be included in any of the foregoing steps to inhibit proteolysis, and antibiotics may be included to prevent the growth of contaminants.

Formulations

Moreover, the invention provides a composition or formulation comprising a binding construct of the invention or a binding construct produced according to the process of the invention.

Compositions of the invention include, but are not limited to, liquid, frozen, and lyophilized compositions.

The compositions may comprise a pharmaceutically acceptable carrier. In general, as used herein, "pharmaceutically acceptable carrier" means any and all aqueous and non-aqueous solutions, sterile solutions, solvents, buffers, e.g. phosphate buffered saline (PBS) solutions, water, suspensions, emulsions, such as oil/water emulsions, various types of wetting agents, liposomes, dispersion media and coatings, which are compatible with pharmaceutical administration, in particular with parenteral administration. The use of such media and agents in pharmaceutical compositions is well known in the

art, and the compositions comprising such carriers can be formulated by well-known conventional methods.

Certain embodiments provide compositions comprising the binding construct of the invention and further one or more excipients such as those illustratively described in this section and elsewhere herein. Excipients can be used in the invention for a wide variety of purposes, such as adjusting physical, chemical, or biological properties of formulations, such as adjustment of viscosity, and or processes of the invention to improve effectiveness and/or to stabilize such formulations and processes against degradation and spoilage e.g. due to stresses that occur during manufacturing, shipping, storage, pre-use preparation, administration, and thereafter. Excipients should in general be used in their lowest effective concentrations.

In certain embodiments, the composition may contain formulation materials for the purpose of modifying, maintaining or preserving certain characteristics of the composition such as the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration (see, Remington's Pharmaceutical Sciences, 18" Edition, 1990, Mack Publishing Company). In such embodiments, suitable formulation materials may include, but are not limited to, e.g., amino acids, antimicrobials such as antibacterial and antifungal agents, antioxidants, buffers, buffer systems and buffering agents which are used to maintain the composition at physiological pH or at a slightly lower pH, typically within a pH range of from about 5 to about 8 or 9, non-aqueous solvents, vegetable oils, and injectable organic esters, aqueous carriers including water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media, biodegradable polymers such as polyesters, bulking agents, chelating agents, isotonic and absorption delaying agents, complexing agents, fillers, carbohydrates, (low molecular weight) proteins, polypeptides or proteinaceous carriers, preferably of human origin, coloring and flavouring agents, sulfur containing reducing agents, diluting agents, emulsifying agents, hydrophilic polymers, salt-forming counter-ions, preservatives, metal complexes, solvents and co-solvents, sugars and sugar

alcohols, suspending agents, surfactants or wetting agents, stability enhancing agents, tonicity enhancing agents, parenteral delivery vehicles, or intravenous delivery vehicles.

Different constituents of the composition can have different effects, for example, and amino acid can act as a buffer, a stabilizer and/or an antioxidant; mannitol can act as a bulking agent and/or a tonicity enhancing agent; sodium chloride can act as delivery vehicle and/or tonicity enhancing agent; etc.

Salts may be used in accordance with certain embodiments of the invention, e.g. in order to adjust the ionic strength and/or the isotonicity of a composition or formulation and/or to improve the solubility and/or physical stability of a binding construct or other ingredient of a composition in accordance with the invention. Ions can stabilize the native state of proteins by binding to charged residues on the protein's surface and by shielding charged and polar groups in the protein and reducing the strength of their electrostatic interactions, attractive, and repulsive interactions. Ions also can stabilize the denatured state of a protein by binding to, in particular, the denatured peptide linkages (--CONH) of the protein. Furthermore, ionic interaction with charged and polar groups in a protein also can reduce intermolecular electrostatic interactions and, thereby, prevent or reduce protein aggregation and insolubility.

Ionic species differ significantly in their effects on proteins. A number of categorical rankings of ions and their effects on proteins have been developed that can be used in formulating pharmaceutical compositions in accordance with the invention. One example is the Hofmeister series, which ranks ionic and polar non-ionic solutes by their effect on the conformational stability of proteins in solution. Stabilizing solutes are referred to as "kosmotropic". Destabilizing solutes are referred to as "chaotropic". Kosmotropes are commonly used at high concentrations to precipitate proteins from solution ("salting-out"). Chaotropes are commonly used to denature and/or to solubilize proteins ("salting-in"). The relative effectiveness of ions to "salt-in" and "salt-out" defines their position in the Hofmeister series.

Free amino acids can be used in formulations or compositions comprising the binding construct of the invention in accordance with various embodiments of the invention as bulking agents, stabilizers, and antioxidants, as well as for other standard uses. Certain amino acids can be used for stabilizing proteins in a formulation, others are useful during lyophilization to ensure correct cake structure and properties of the active ingredient. Some amino acids may be useful to inhibit protein aggregation in both liquid and lyophilized formulations, and others are useful as antioxidants.

Polyols are kosmotropic and are useful as stabilizing agents in both liquid and lyophilized formulations to protect proteins from physical and chemical degradation processes. Polyols are also useful for adjusting the tonicity of formulations and for protecting against freeze-thaw stresses during transport

or the preparation of bulks during the manufacturing process. Polyols can also serve as cryoprotectants in the context of the present invention.

Certain embodiments of the formulation or composition comprising the binding construct of the invention can comprise surfactants. Proteins may be susceptible to adsorption on surfaces and to denaturation and resulting aggregation at air-liquid, solid-liquid, and liquid-liquid interfaces. These deleterious interactions generally scale inversely with protein concentration and are typically exacerbated by physical agitation, such as that generated during the shipping and handling of a product. Surfactants are routinely used to prevent, minimize, or reduce surface adsorption. Surfactants also are commonly used to control protein conformational stability. The use of surfactants in this regard is protein specific, since one specific surfactant will typically stabilize some proteins and destabilize others.

Certain embodiments of the formulation or composition comprising the binding construct of the invention can comprise one or more antioxidants. To some extent deleterious oxidation of proteins can be prevented in pharmaceutical formulations by maintaining proper levels of ambient oxygen and temperature and by avoiding exposure to light. Antioxidant excipients can also be used to prevent oxidative degradation of proteins. It is envisaged that antioxidants for use in therapeutic protein formulations in accordance with the present invention can be water-soluble and maintain their activity throughout the shelf life of the product (the compositon comprising the binding construct). Antioxidants can also damage proteins and should hence – among other things – be selected in a way to eliminate or sufficiently reduce the possibility of antioxidants damaging the binding construct or other proteins in the formulation.

Formulations in accordance with the invention may include metal ions that are protein co-factors and that are necessary to form protein coordination complexes, such as zinc necessary to form certain insulin suspensions. Metal ions also can inhibit some processes that degrade proteins. However, metal ions also catalyze physical and chemical processes that degrade proteins. Magnesium ions (10-120 mM) can be used to inhibit isomerization of aspartic acid to isoaspartic acid. Ca+2 ions (up to 100 mM) can increase the stability of human deoxyribonuclease. Mg+2, Mn+2, and Zn+2, however, can destabilize rhDNase. Similarly, Ca+2 and Sr+2 can stabilize Factor VIII, it can be destabilized by Mg+2, Mn+2 and Zn+2, Cu+2 and Fe+2, and its aggregation can be increased by Al+3 ions.

Certain embodiments of the formulation or composition comprising the binding construct of the invention can comprise one or more preservatives. Preservatives are necessary for example when developing multi-dose parenteral formulations that involve more than one extraction from the same container. Their primary function is to inhibit microbial growth and ensure product sterility throughout the shelf-life or term of use of the drug product. Although preservatives have a long history of use with small-molecule parenterals, the development of protein formulations that include preservatives can

be challenging. Preservatives very often have a destabilizing effect (aggregation) on proteins, and this has become a major factor in limiting their use in multi-dose protein formulations. Several aspects need to be considered during the formulation and development of preserved dosage forms. The effective preservative concentration in the product must be optimized. This requires testing a given preservative in the dosage form with concentration ranges that confer anti-microbial effectiveness without compromising protein stability.

Development of liquid formulations containing preservatives are more challenging than lyophilized formulations. Freeze-dried products can be lyophilized without the preservative and reconstituted with a preservative containing diluent at the time of use. This shortens the time during which a preservative is in contact with the binding construct, significantly minimizing the associated stability risks. With liquid formulations, preservative effectiveness and stability should be maintained over the entire product shelf-life. An important point to note is that preservative effectiveness should be demonstrated in the final formulation containing the active drug and all excipient components. Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, crystal, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration.

Kits

In further embodiments, the invention provides a kit comprising a binding construct that binds to MAGEB2 and is useful for detection of MAGEB2 in a tumor sample, wherein the binding construct comprises any of the binding constructs disclosed herein. In one embodiment, the binding construct that is useful for detection of MAGEB2 is a monoclonal antibody that binds to MAGEB2. In further embodiments, the kit further comprises a therapeutic that is effective for treating cancer that expresses MAGEB2. In certain embodiments, the kit can optionally provide a package insert comprising instructions for detecting MAGEB2 and administration of a cancer therapeutic.

In further embodiments, the kit further comprises binding constructs (e.g., antibodies) that are useful to detect different biomarkers other than MAGEB2, e.g., further comprising a MAGEA4 or a MAGEA8 binding construct.

As used herein, the singular forms "a", "an", and "the" include plural references unless the context clearly indicates otherwise. Thus, for example, reference to "a reagent" includes one or more of such different reagents and reference to "the method" includes reference to equivalent steps and methods

known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention.

The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

The term "about" or "approximately" as used herein means within ±20%, preferably within ±15%, more preferably within ±10%, and most preferably within ±5% of a given value or range. It also includes the concrete value, e.g., "about 50" includes the value "50".

Throughout this specification and the claims, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term "comprising" can be substituted with the term "containing" or "including" or sometimes when used herein with the term "having".

When used herein "consisting of" excludes any element, step, or ingredient not specified in the claim element. When used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim.

In each instance herein, any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms.

It should be understood that the above description and the below examples provide exemplary arrangements, but the present invention is not limited to the particular methodologies, techniques, protocols, material, reagents, substances, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims. Aspects of the invention are provided in the independent claims. Some optional features of the invention are provided in the dependent claims.

All publications and patents cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. To

the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

A better understanding of the present invention and of its advantages will be obtained from the following examples, offered for illustrative purposes only. The examples are not intended and should not be construed as to limit the scope of the present invention in any way.

EXAMPLES

Example 1: MAGEB2 Immunogen Design

Protein sequences of 35 *H. sapiens* MAGE family members were downloaded from UniProtKB (www.uniprot.org): MAGEA1 (P43355), MAGEA2 (P43356), MAGEA3 (P43357), MAGEA4 (P43358), MAGEA5 (P43359), MAGEA6 (P43360), MAGEA8 (P43361), MAGEA9 (P43362), MAGEA10 (P43363), MAGEA11 (P43364), MAGEA12 (P43365), MAGEB1 (P43366), MAGEB2 (O15479), MAGEB3 (O15479), MAGEB4 (O15481), MAGEB5 (Q98Z81), MAGEB6 (Q8N7X4), MAGEB10 (Q96LZ2), MAGEB16 (A2A368), MAGEB17 (A8MXT2), MAGEB18 (Q96M61), MAGEC1 (O60732), MAGEC2 (Q9UBF10), MAGEC3 (Q8TD91), MAGED1 (Q9Y5V3), MAGED2 (Q9UNF1), MAGED4 (Q96JG8), MAGEE1 (Q9HCI5), MAGEE2 (Q8TD90), MAGEH1 (Q9H213), MAGEF1 (Q9HAY2), MAGEL2 (Q9UJ55), NSE3 (Q96MG7), TROP (Q12816), and NECD (Q99608). Clades were determined using the Neighbor-Joining Tree Builder algorithm in Geneious v10.2.

Shannon entropy, a quantitative metric describing the diversity of amino acids observed at a given position in an alignment, was used to select MAGEB2 peptide immunogens having low amino acid conservation across the entire MAGE family and MAGE-B clade (B1, B2, B3, B4, B5, B17). MAGE family or MAGE-B clade members were aligned using MUSCLE (R. C. Edgar. Nucleic Acids Res. 2004; 32(5): 1792-1797). The Shannon entropy was calculated at each position in the alignment using $\mathrm{H_S}(j) = -\sum_i \mathrm{f}_{ij} \log_2 \mathrm{f}_{ij}$, where HS(*j*) is the Shannon entropy at position *j* and f*ij* is the frequency of AA *i* at position *j*. Shannon entropy was mapped onto the structure of the MAGE homology domain (MHD) of MAGEA4 (PDB ID 2wa0) or a Rosetta homology model of the MHD of MAGEB2 using a custom script that replaces the PDB b-factor of each atom with the normalized Shannon entropy value for its corresponding residue and then visualizing in PyMOL v1.8 (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC) using color by b-factor.

A custom script was used to deterministically rank regions of length n (n = 5-40 amino acids) in the alignment from highest to lowest average Shannon entropy (lowest to highest conservation) using a sliding window approach. For the entire MAGE family, regions of length 10-30 with lowest conservation across the family cluster to three distinct areas: two areas N-terminal to the MHD and

one area within the helical N-terminus of the MHD. Analysis of the MAGE-B clade revealed a clade-specific area of low conservation located on a loop at the interface of two globular lobes of the MHD. MAGEB2 peptide immunogens were selected from the following three identified regions of low conservation: the region N-terminal to the MHD, the helical N-terminus of the MHD, and the MDH interface loop region. The peptide ranges within these regions were refined based on average Shannon entropy score, visual inspection of sequence alignments, consideration of structural features such as solvent accessibility and conservation of secondary structure as an isolated peptide as predicted by PSIPRED (Jones DT. (1999) Protein secondary structure prediction based on position-specific scoring matrices. J. Mol. Biol. 292: 195-202), and whether a sequence blast against the non-reductant h. sapiens proteome revealed hits with high homology in non-MAGE proteins. Regions of MAGEB2 selected as peptides immunogens were MAGEB2 a.a.43-76: SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV (N-terminal region peptide); MAGEB2 a.a.95-125: SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK (MHD N-terminal helix peptide); and MAGEB2 a.a.185-200: DLTDEESLLSSWDFPR (MHD middle loop peptide).

Peptides from MAGE homologs with >50% pairwise identity to the MAGEB2 immunogens were selected for experimental counter-screening. Additional MAGEB2 homolog peptides were selected for counter-screening. For MAGEB2 a.a.43-76 (N-terminal region peptide), selected counter screening peptides were derived from MAGE- B1, B4, B17, A4, A1, A5, A12, A6, A3, A2, A11, and A8. For MAGE B2 a.a.95-125 (MHD N-terminal helix peptide), selected counter screening peptides were derived from MAGE- B4, B5, B17, C3, A11, A8, A9, A3, A10, F1, A4, A5, A12, C2, A2, E1, and C1. For MAGEB2 a.a.185-200 (MHD middle loop peptide), a single counter screening peptide derived from MAGEB1 was selected.

Example 2: Generation and Selection of Antibodies

Immunizations

Rabbits were immunized according to standard protocols, and splenocytes were isolated and frozen from immune animals.

Preparation of Peptides for Antigen-Specific Sorting

27 biotin-labelled MAGE counter-screening peptides (Table 7) were pooled together in equal molar concentration to create a working cocktail. The cocktail was complexed to neutravidin (Fisher: PI31000) at a molar ratio of 4:1 (neutravidin:peptide) in 2% FBS FACS buffer (calcium and magnesium free PBS with 2 % v/v FBS). The mixture was left to incubate at 4 °C for 15 minutes. Each of the 3 biotin-labelled MAGEB2 target peptides (N-Terminal Region Peptide, Middle Loop Region Peptide, N-

Terminal Helix Peptide, see Table 8) was complexed to a different fluorescently-tagged streptavidin using the same 4:1 (streptavidin:peptide) molar ratio in 2% FBS FACS buffer and then left to incubate at 4 °C for 15 minutes. MAGEB2 N-Terminal Peptide was complexed to BV421 streptavidin (BD: 563259), MAGEB2 Middle Loop Region Peptide was complexed to BV605 streptavidin (BD: 563855), and MAGEB2 N-Terminal Helix Peptide was complexed to Alexa Fluor 647 streptavidin (Jackson: 016-600-084).

Unbound streptavidin or neutravidin was quenched by adding excess amount of D-biotin (Fisher: BP232-1) at molar ratio of 600:1 (D-biotin:streptavidin or neutravidin), the peptides were then left to incubate for another 15 minutes at 4 °C. The 3 MAGEB2 target peptides were pooled together just before use.

Table 7: 27 MAGE counter-screening peptides.

MAGE Counter-Screening Peptides		
Item	Peptide Name	Peptide Sequence
1	A1_CTermHelix	{Biotin-NH} SYVKVLEYVIKVSARVRFFFPSLREAA {COOH}
2	A1_NTerminalPeptide	{Biotin-NH} SSPLVLGTLEEVPTAGSTDPPQSPQGASAFPTTINFT {COOH}
3	A2_NTermHelix	{Biotin-NH} PDLESEFQAAISRKMVELVHFLLLK {COOH}
4	A2_NTerminalPeptide	{Biotin-NH} SSTLVEVTLGEVPAADSPSPPHSPQGASSFSTTINYT {COOH}
5	A3_NTermHelix	{Biotin-NH} STFPDLESEFQAALSRKVAELVHFLLLK {COOH}
6	A3_NTerminalPeptide	{Biotin-NH} SSTLVEVTLGEVPAAESPDPPQSPQGASSLPTTMNY {COOH}
7	A5_NTermHelix	{Biotin-NH} STSPDPESVFRAALSKKVADLIHFLLLK {COOH}
8	A5_NTerminalPeptide	{Biotin-NH} SSPLVPGTLGEVPAAGSPGPLKSPQGASAIPTAIDFT {COOH}
9	A9_CTermHelix (C19S)	{Biotin-NH} SYEKVINYLVMLNAREPISYPSLYEEV {COOH}
10	A9_NTermHelix	{Biotin-NH} SSSVDPAQLEFMFQEALKLKVAELVHFLLHK {COOH}
11	A10_NTermHelix	{Biotin-NH} STLQVLPDSESLPRSEIDEKVTDLVQFLLFK {COOH}
12	A11_NTermHelix	{Biotin-NH} STSPDLIDPESFSQDILHDKIIDLVHLLLRK {COOH}
13	A11_NTerminalPeptide	{Biotin-NH} SSTLNVGTLEELPAAESPSPPQSPQEESFSPTAMDAI {COOH}
14	A12_NTermHelix	{Biotin-NH} STFPDLETSFQVALSRKMAELVHFLLLK {COOH}
15	A12_NTerminalPeptide	{Biotin-NH} SSTLVEVTLREVPAAESPSPPHSPQGASTLPTTINYT {COOH}
16	B1_MiddleLoopRegion	{Biotin-NH} NLTNDGNLSNDWDFPR {COOH}
17	B1_NTerminalPeptide	{Biotin-NH} SSPVLGDTPTSSPAAGIPQKPQGAPPTTTAAAAV {COOH}
18	B4_NTermHelix	{Biotin-NH} SSSQASTSTERSLKDSLTRKTKMLVQFLLYK {COOH}
19	B4_NTerminalPeptide	{Biotin-NH} SSVLRDTASSSLAFGIPQEPQREPPTTSAAAA {COOH}

20	B5_NtermHelix (C12S)	{Biotin-NH} SSEVSPSTESSSSNFINIKVGLLEQFLLYK {COOH}
21	B17_NTermHelix	{Biotin-NH} NSFHGPSSSESTGRDLLNTKTGELVQFLLNK {COOH}
22	B17_NTerminalPeptide (C5S)	{Biotin-NH} SSPASQSPPQSFPNAGIPQESQRASYPSSPASAV {COOH}
23	C1_NTermHelix	{Biotin-NH} TDSESLIESEPLFTYTLDEKVDELARFLLLK {COOH}
24	C2_NTermHelix (C3S)	{Biotin-NH} GTSQGLPDSESSFTYTLDEKVAELVEFLLLK {COOH}
25	C3_NTermHelix	{Biotin-NH} HALPESESLPRYALDEKVAELVQFLLLK {COOH}
26	E1_NTermHelix	{Biotin-NH} NTSRVAITLKPQDPMEQNVAELLQFLLVK {COOH}
27	F1_NTermHelix	{Biotin-NH} ALAAKALARRRAYRRLNRTVAELVQFLLVK {COOH}

Table 8: 3 MAGEB2 Target Peptides

MAGEB2 Target Peptides		
Item	Peptide Name	Peptide Sequence
1	B2_MiddleLoopRegion	{Biotin-NH} DLTDEESLLSSWDFPR {COOH}
2	B2_NTermHelix	{Biotin-NH} SSSQASTSTKSPSEDPLTRKSGSLVQFLLYK {COOH}
3	B2_NTerminalPeptide	{Biotin-NH} SSVSGGAASSSPAAGIPQEPQRAPTTAAAAAAGV {COOH}

Preparation of Rabbit Splenocytes for Antigen-Specific Sorting

Rabbit splenocytes were thawed into 10% ICM (Immune Cell Culture Medium) and dead cells were removed using the Miltenyi Biotec MACS Dead Cell Removal Kit (Miltenyi: 130-090-101). The cells were then washed in 10 mL of cold 2% FBS FACS buffer. After a 4-minute centrifugation at 400G, the supernatant was removed using a serological pipette and the cocktail of neutravidin-complexed MAGE counter-screening peptides was added. The cells were left to incubate at 4 °C for 30 minutes, at which point the 3 streptavidin-complexed MAGEB2 target peptides along with 5 µg of FITC-conjugated goat anti-rabbit IgG Fc antibody (Serotec: STAR121F) were added to stain IgG-expressing, antigen-specific cells. The final concentrations of the peptides in the 1 mL reaction volume were: 120 nM for the cocktail of counter-screening peptides, and 16.5 nM for each target peptide. The cells were incubated for another 30 minutes at 4 °C, then washed in 10 mL of cold 2% FBS FACS buffer and centrifuged again. After removing the supernatant, the cells were resuspended in 2 mL of 10% ICM and then put

through a 40 µm cell strainer to remove any clumps. 10 µL of 7-AAD viability staining solution (Fisher: 00-6993-50) was added to the cells 10 minutes prior to sorting.

Sorting and Culturing of Antigen-Specific Cells

Cells were sorted on a BD FACSAria III equipped with 405 nm laser (detection: 450/50 nm, 610/20 nm), 488 nm laser (detection: 530/30 nm, 695/40 nm), and 640 nm laser (detection: 730/45 nm) using a 100 micron nozzle at 20 Psi sheath pressure. Dead cells were excluded by gating on population not fluorescent in 7-AAD, and MAGEB2 target peptide binders were sorted by drawing 3 gates using the following selection criteria: FITC+/BV421+, FITC+/BV605+, FITC+/Alexa Fluor 647+ (see Figure 1). An initial sort was performed using Yield precision mode to enrich target populations from ~0.1 % to ~50%, this was followed by a second round of sorting using Purity precision mode to single cell sort MAGEB2 target peptide binders into 384-well tissue culture plates pre-filled with 10% ICM supplemented with 4% rabbit T-cell supernatant, 2 µL/mL of anti-rabbit IgG microbeads (Miltenyi: 130-048-602), and gamma-ray-irradiated EL4 cells at a density of 437,500 cells/mL. The plates were stored in tissue culture incubator (37 °C, 5 % CO2), and outgrowth was assessed 7 days later by visual scoring using an inverted microscope. After 8 days, supernatants were collected from the tissue culture plates and the cells were lysed in Buffer RLT (Qiagen: 79216) and frozen at -80 °C.

Initial Detection of MAGEB2 Antibodies

B-cell culture supernatants were screened for the presence of positive secreting antibodies using a rabbit IgG capture ELISA. Goat anti rabbit IgG (Fc) (Jackson, Cat. No. 111-005-046) was coated in 1 x phosphate buffered saline (PBS) overnight at 4 °C onto Corning 3702 384 well polystyrene plates. Plates were washed 4 cycles using a Titertek plate washer and blocked with 1xPBS/1%milk. Then the plates were aspirated and B-cell culture supernatants were diluted at 1:5 final in 1xPBS/1%milk and incubated for 1 hour at room temperature. Plates were washed 4 cycles and then horseradish peroxidase (HRP) conjugated goat anti human IgG (Fc) (Jackson, Cat.No. 111-035-046) made up in 1xPBS/1%milk was added and incubated for 1 hour at room temperature. Plates were washed 4 cycles, then 1 step TMB (Neogen cat. No. 319177) was added for 30 minutes at room temperature and subsequently quenched with 1N hydrochloric acid. Plates were read on the Multiscan Ascent plate reader at OD 450nm and a total of 129 rabbit antibodies were positive with the breakdown of hits per region represented in Table 9 below.

Table 9:

Binding region peptide	Total # Positive
MAGEB2 middle loop region peptide	50
MAGEB2 N-term helix peptide	35
MAGEB2 N-term peptide	45

Identification of MAGEB2 Specific Antibodies

MAGEB2 antibodies were tested for binding using biotinylated MAGEB2 peptides coated on xMAP LumAvidin Microspheres or Luminex beads (Luminex Cat No. L100-LXXX-01). These avidin coupled microparticles are color coded into different spectrally distinct regions, with unique fluorescent dye combinations allowing for non-covalent binding of biotinylated proteins. To validate the rabbit IgG capture ELISA results in the first bead-based FACS assay the panel of 129 rabbit antibodies was tested against the same 3 peptides that the rabbits were immunized with: MAGEB2 middle loop region peptide, MAGEB2 N-terminal helix peptide and MAGEB2 N-terminal region peptide. The three biotinylated peptides were coated using three different LumAvidin beads in FACS buffer [1xPBS+2% fetal bovine serum (Hyclone Cat No. SH30396.03)] for 30 minutes at room temperature in the dark. The beads were then washed twice in FACS buffer by centrifugation for 2 min at 3500 RPM. The coated beads were resuspended and pooled together (3-plex) and added to the B-cell culture supernatants at a 1:2 final dilution onto Corning 3897 96 well V-bottom polystyrene plates and incubated for 1 hour at room temperature in the dark. Another 2 washes in FACS buffer were done before the addition of Alexa 488 conjugated goat anti rabbit IgG (Fc) (Jackson, Cat No. 111-545-046) made up in FACS buffer was added and incubated for 15 minutes at room temperature in the dark. A final wash was done before plates were read on the iQue FACS machine with Intellicyte autosampler to obtain flow cytometry measurements according to the manufacturer's recommendations; data analysis was done on Intellicyt ForeCyt® Enterprise Client Edition 6.2 (R3). A total of 82 wells were identified as MAGEB2 positive with the breakdown of hits per region as presented in Table 10 below.

Table 10.

Binding region	Total # Positive
MAGEB2 middle loop region peptide	21
MAGEB2 N-term helix peptide	24
MAGEB2 N-term region peptide	37

In the second bead-based FACS assay the panel of 129 rabbit antibodies was tested against 9 other peptides of similar sequence to determine MAGEB2 specificity: MAGEB1 middle loop region peptide, MAGEB4 N-term helix peptide, MAGEB5 N-term helix peptide, MAGE17 N-term helix peptide, MAGEF1 N-term helix peptide, MAGEE1 N-term helix peptide; MAGEB1 N-term peptide peptide, MAGEB4 N-

term region peptide and MAGEB17 N-term region peptide. The nine biotinylated peptides were coated using nine different LumAvidin beads in FACS buffer [1xPBS+2% fetal bovine serum (FBS)] for 30 minutes at room temperature in the dark. The beads were then washed twice in FACS buffer by centrifugation for 2 min at 3500 RPM. The coated beads were resuspended and pooled together (9-plex) and added to the rabbit B-cell supernatants at a 1:2 final dilution onto Corning 3897 96 well V-bottom polystyrene plates and incubated for 1 hour at room temperature in the dark. Another 2 washes in FACS buffer were done before the addition of Alexa 488 conjugated goat anti rabbit IgG (Fc) (Jackson, Cat No. 111-545-046) made up in FACS buffer was added and incubated for 15 minutes at room temperature in the dark. A final wash was done before plates were read on the iQue FACS machine with Intellicyte autosampler to obtain flow cytometry measurements according to the manufacturer's recommendations; data analysis was done on Intellicyt ForeCyt® Enterprise Client Edition 6.2 (R3). A total of 43 wells were identified as MAGEB2 specific with the breakdown of hits per region as represented in Table 11 below. All 43 wells underwent heavy chain and light chain sequencing.

Table 11.

Binding region	Total # Positive
MAGEB2 middle loop region peptide	12
MAGEB2 N-term helix peptide	19
MAGEB2 N-term region peptide	12

Identification of MAGEB2 Highest Affinity Antibodies

To determine which of the 43 MAGEB2 specific antibodies to take forward to cloning, a limited antigen (LA) kinetics FACS assay done on LumAvidin beads using full length MAGEB2 protein, to rank the panel and select the highest affinity antibodies.

huMAGEB2(1-319) in pET23 was transformed into BL21(DE)-star pLysS strain (Invitrogen). Single colonies were used to inoculate 2mL Luria Broth supplemented with carbenicillin (50 µg/mL) and chloramphenicol (34 µg/mL) and grown shaking at 37°C for several hours until turbid. Two hundred microliters of culture were used to inoculate 400 mL of Luria Broth (Teknova) supplemented with selection antibiotics and antifoam 204 (Sigma). These cultures were grown shaking at 24 °C overnight. Turbid cultures were used to seed 1-liter Terrific Broth (Teknova) supplemented with 1% (w/v) glucose, 2mM $MgSO_4$, 17 µg/L carbenicillin, 12 µg/L chloramphenicol and antifoam 204 (in 4L baffled shake flasks) to an OD_{600} = 0.2. These cultures were grown shaking at 37°C until an OD_{600} = 1.0, then were shifted to 16°C and continued to grow to OD_{600} = 2.0. At this point, protein expression was induced,

with 100 µM IPTG, for 16 hrs. The cells were pelleted by centrifugation and stored at -80°C until processing.

The MAGEB2 pellets were resuspended in Lysis Buffer (100mM TRIS pH 8.0, 500 mM NaCl, 15 mM bME, 5mM Benzamidine, 0.01mg/mL lysozyme, 0.05 mg/mL Dnase1, 5%(v/v) glycerol, and Halt Protease Inhibitor Tablets (Pierce)) using a Polytron PT2100 homogenizer. The homogenized cells were lysed using Microfluidizer M-110Y (Mircofluidics) in three passes. The lysate was clarified by centrifugation at 13,000 rpm in a Beckman Centrifuge in a JLA-16.250 rotor. The clarified lysate was filtered through 0.22 µm filter before being applied to HisTrap Excel column (Cytiva). The resin was washed with 25mM TRIS pH 8.5, 0.5M NaCl, 10mM BME, 5% glycerol, 10 mM imidazole and MAGEB2 was eluted with 25mM TRIS pH 8.5, 0.5M NaCl, 10mM BME, 5% glycerol, 500 mM imidazole.

The affinity captured MAGEB2 was dialyzed against 20mM TRIS pH 8.5, 0.5M NaCl, 5%(v/v) glycerol, 1 mM TCEP and diluted 25-fold into 20 mM TRIS pH 8.5, 5% (v/v) glycerol, 1 mM TCEP before ion-exchange chromatography using Q-sepharose HP (Cytiva). Bound MAGEB2 was eluted of the column using a 20mM to 500 mM NaCl gradient over 20 CV. Peak fractions were analyzed by SDS-PAGE and pooled based on the absence of low-molecular weight bands. The n-terminal 6xHis tag was removed from MAGEB2 using caspase 3 protease at a 1:100 (w/w) ratio, after the addition of 1 mM EDTA and 0.1% CHAPS. The digest proceeded overnight at 4°C. The digest was dialyzed against 20 mM TRIS pH 8.5, 0.25M NaCl, 1 mM TCEP to remove CHAPS and EDTA before ion-exchange chromatography. Dialyzed MAGEB2 was diluted 10-fold into 20 mM TRIS pH 8.5, 1mM TCEP and applied to Q-sepharose HP resin (Cytiva). Bound MAGEB2 was eluted of the column using a 20mM to 500 mM NaCl gradient over 20 CV. Peak fractions were analyzed by SDS-PAGE and pooled based on the absence of high-molecular weight bands. This pool was biotinylated using recombinant BirA with 50 mM Bicine pH 8.3, 10 mM Mg-acetate, 10 mM ATP and 50µM d-biotin. The reaction proceeded overnight at 4°C and 5 µg of protein was analyzed by Mass spec (ask Dylan for the about instrument and methods) to monitor biotin addition to MAGEB2.

The biotinylation reaction was dialyzed against 20 mM TRIS pH 8.5, 150 mM NaCl, 1 mM TCEP to remove excess ATP and biotin. After dialysis the sample was diluted 10-fold, in 20 mm TRIS pH 8.5, 1mM TCEP, and applied to Q-sepharose HP (Cytiva). Bound MAGEB2 was eluted off the column using a 20mM to 500 mM NaCl gradient over 20 CV. Peak fractions were analyzed by SDS-PAGE and pooled based on the presence of a 37 Kd band. The pooled fractions were concentrated using 10Kd MWCO spin filters (Sartorius). The concentrated MAGEB2 was dialyzed against 30 mM HEPES pH 8.2, 0.15M NaCl, 1 mM TCEP, filtered through 0.22 µm Posidyne syringe filter (Pall) and stored at -80°C.

During purification, mass spec analysis was used to confirm the identity of intact MAGEB2, caspase-3 cleaved MAGEB2, and biotinylated MAGEB2. The homogeneity of purification intermediates and final protein lot was measured by sizing HPLC analysis on Agilent 1200 LC system using a YARA 3 µm SEC

2000 column (Phenomenex). Protein concentration was determined by A280 absorbance using Spectramax spectrophotometer (Molecular Devices).

Biotinylated MAGEB2 full length protein was titrated 1:2 across 6 different limited antigen concentrations and then coated onto six different LumAvidin beads in FACS buffer [1xPBS+2% fetal bovine serum (Hyclone Cat No. SH30396.03)] for 30 minutes at room temperature in the dark. The beads were then washed twice in FACS buffer by centrifugation for 2 min at 3500 RPM. The coated beads were resuspended and pooled together (6-plex) and added to the rabbit B-cell supernatants at a 1:2 final dilution onto Corning 3897 96 well V-bottom polystyrene plates and incubated overnight at room temperature in the dark. The next day 2 washes were done in FACS buffer before the addition of Alexa 488 conjugated goat anti rabbit IgG (Fc) (Jackson, Cat No. 111-545-046) made up in FACS buffer was added and incubated for 15 minutes at room temperature in the dark. A final wash was done before plates were read on the iQue FACS machine with intellicyte autosampler to obtain flow cytometry measurements according to the manufacturer's recommendations; data analysis was done on Intellicyt ForeCyt® Enterprise Client Edition 6.2 (R3). The LA data was aligned with the sequence data and a total of 24 highest affinity, sequence diverse antibodies were identified, with the breakdown of hits per region as shown in Table 12 below and in Figure 2 herein.

Table 12.

Binding region	Total # Positive
MAGEB2 middle loop region peptide	6
MAGEB2 N-term helix peptide	16
MAGEB2 N-term region peptide	2

Heavy and Light Chain Sequencing of Antibodies

Messenger RNA (mRNA) was purified from wells containing the antibody-producing cells using the mRNA catcher plus kit (Invitrogen). Purified RNA was used to amplify the antibody heavy and light chain variable region (V) genes using cDNA synthesis via reverse transcription, followed by a polymerase chain reaction (RT-PCR). The rabbit antibody gamma heavy chain was obtained using the Qiagen One Step Reverse Transcriptase PCR kit (Qiagen). This method was used to generate the first strand cDNA from the RNA template and then to amplify the variable region of the gamma heavy chain using multiplex PCR. The 5' gamma chain-specific primer annealed to the signal sequence of the antibody heavy chain, while the 3' primer annealed to a region of the gamma constant domain. The rabbit kappa light chain was obtained using the One Step Reverse Transcriptase PCR kit (Qiagen). This method was used to generate the first strand cDNA from the RNA template and then to amplify the variable region of the kappa light chain using multiplex PCR. The 5' kappa light chain-specific primer

annealed to the signal sequence of the antibody light chain while the 3' primer annealed to a region of the kappa constant domain.

The PCR product was sequenced and amino acid sequences were deduced from the corresponding nucleic acid sequences bioinformatically. Two additional, independent RT-PCR amplification and sequencing cycles were completed for each sample in order to confirm that any mutations observed were not a consequence of the PCR. The derived amino acid sequences were then analyzed to determine the germline sequence origin of the antibodies and to identify deviations from the germline sequence. A comparison of each of the heavy and light chain sequences to their original germline sequences are indicated in sequence Table 4 herein. The amino acid sequences were used to group the clones by similarity.

The variable region kappa light chain DNA sequences for the selected antibodies were made as a synthetic genes. These synthetic genes were cloned with the rabbit kappa constant region in to a pTT5 based expression vector. The variable region heavy chain DNA sequences for the selected antibodies were made as synthetic genes. These synthetic genes were cloned with a rabbit IgG constant region in to a pTT5 based expression vector. After cloning, these antibodies were expressed in HEK2936E cells transfected with PEI.

Example 3: Immunohistochemical Assays

Objectives

To assess the specificity of twenty-four rabbit anti-human MAGEB2 antibodies to human MAGEB2, and potential cross reactivity for other MAGE-B and MAGE-A family members by immunohistochemical (IHC)staining.

Overview Summary

Twenty-four rabbit-anti human MAGEB2 supernatants were assessed for binding to human MAGEB2 on transfected human MAGEB2+ control cells by IHC. After initial screening assays, small batch purified antibody aliquots from four MAGEB2 clones (4G17, 1J15, 1C3, 1I14) with desired attributes were assessed on transfected human MAGE-A+ and MAGE-B+ family member control cells, normal human testes tissues, normal human tissue TMAs, and thirteen human cancer cell lines with known

endogenous MAGEB2 RNA expression level by IHC. MAGEB2 antibody clones 1H4 and 1C3 displayed properties that were desired for IHC.

Materials and Methods

Control Cells and tissues

Negative control cells included parental Chinese hamster ovary (CHO) cells, CHO cells stably transfected with mCherry Vector Control (minus Myc/DDK tags), CHO cells stably transfected with mCherry-human DCAF4L2 (plus Myc/DDK tags). The cross-reactivity control cells included CHO cells transfected with mCherry Vector Control (plus Myc/DDK tags) with one of the human MAGE-B or MAGE-A family members (MAGEA1, MAGEA2, MAGEA3, MAGEA4, MAGEA5, MAGEA6, MAGEA8, MAGEA9, MAGEA11, MAGEA12, MAGEB1, MAGEB3, MAGEB4, MAGEB5, MAGEB6, MAGEB10, MAGEB16, MAGEB17, MAGEB18). Positive control cell lines included CHO cells transfected with mCherry Vector (plus Myc/DDK tags) with human MAGE-B2 or CHO cells transfected with mCherry Vector (open reading frame (ORF); minus Myc/DDK tags) with human MAGEB2, and thirteen human cancer cell lines with known endogenous RNA expression level of MAGEB2 (U266B1, T98G, KMM-1, U2OS, CFPAC-1, SCaBER, UACC-257, CFPAC-1, VMRC-LCD, NCI-H1703, UM-UC-3, NCI-H1395, NCIH82).

Cell pellets were fixed in 10% neutral buffered formalin, suspended in Histogel™ containing agarose/glycerine (cat # HG-4000-12, Thermo Fisher Scientific, Waltham, MA), and processed to paraffin blocks. Two FFPE normal human testes tissues, and one FFPE normal human liver tissue were used as positive and negative controls, respectively, obtained from Human Tissue Science Center (HTSC).

Table 13. Cells and Tissues

Species	HTSC Inventory Code (Block ID)	Descriptions
Hamster	694001	Amgen in house TMA contain CHO, CHO- mCherry, CHO- mCherry- human DCAF4L2+, CHO- MAGE-A1+, MAGE-A2+, MAGE-A3+, MAGE-A4+ (variant 2), MAGE-A5+, MAGE-A6+, and MAGE-A8+ cell line
Hamster	697782	Amgen in house TMA contain CHO, CHO- mCherry, CHO- mCherry- human DCAF4L2+, CHO- MAGE-A4+ (variant 4), MAGE-A9+, MAGE-A10+, MAGE-

		A11+ (variant 1), MAGE-A11+ (variant 2), MAGE-A12+ cell lines
Hamster	697548	Amgen in house TMA contain CHO, CHO- mCherry, CHO- mCherry- human DCAF4L2+, CHO- MAGE-B1+ (variant 1), MAGE-B1+ (variant 3), MAGE-B2+ (ORF = minus Myc/DDK tags), MAGE-B2 MAGE-B3+, MAGE-B5+ (variant 1) cell lines
Hamster	697639	Amgen in house TMA contain CHO, CHO- mCherry, CHO- mCherry- human DCAF4L2+, CHO- MAGE-B5+ (variant 2), MAGE-B6+, MAGE-B10+, MAGE-B16+, MAGE-17+, MAGE-18+cell lines
Hamster	694686	Amgen in house TMA contain CHO, CHO- mCherry, CHO- mCherry- human DCAF4L2+, CHO- MAGE-B5+ (variant 2), MAGE-B6+, MAGE-B10+, MAGE-B16+, MAGE-17+, MAGE-18+cell lines
Human	627446	Amgen in house TMA contain U266B1, KMM-1, NCI-H1703, SCaBER, T98G, UACC-257 and CFPAC-1 cell
Human	685358	Amgen in house TMA contain NCI-H1703, NCI-H1395, VMRC-LCD, SCaBER cell
Human	685359	Amgen in house TMA contain U2OS, UM-UC-3, NCI-H82, CFPAC-1 cell
Human	14749	Normal Testis
Human	8497	Normal Testis
Human	356137	Normal Liver

Tissues Microarray

FFPE normal human TMA slides (normal human multiple organ tissue microarray) were obtained from US BioMax (FDA999x, Crabbs Branch Way, Derwood, MD; https://www.biomax.us/FDA999x).

Antibodies

Twenty-four human MAGEB2 clone supernatants (monoclonal rabbit IgG1 antibody) were assessed for binding to human MAGEB2 and potential cross reactivity for other MAGE-A and MAGE-B family members on control cells by IHC staining. Four of the twenty-four human MAGEB2 clones (4G17, 1J15, 1C3, 1I14) were the most promising candidates for further assessment of MAGEB2 specificity and sensitivity by IHC staining. Small batch purified antibody aliquots from the four MAGEB2 clones (4G17, 1J15, 1C3, 1I14) were assessed on MAGE-A and MAGE-B family cross-reactivity control cells, control testes tissues, and normal human tissue TMAs by IHC. Rabbit Monoclonal IgG1 isotype control (EPR25A) was purchased from Abcam (cat # ab172730, Lot # GR3235749-11, Cambridge, United Kingdom; https://www.abcam.com/rabbit-igg-monoclonal-epr25a-isotype-control-ab172730.html).

Immunohistochemistry Assay

FFPE samples were sectioned at 4 µm and mounted on positive charged glass slides. After mounting, paraffin sections were air-dried at room temperature. FFPE TMA samples were received as freshly sectioned and mounted on glass slides. The fully automated IHC assay was performed on a Discovery Ultra Stainer (Ventana Medical Systems, Tucson, AZ) using Ventana Anti-Rabbit HQ (cat # 760-4815), Ventana Anti-HQ HRP (cat # 760-4820), and Ventana Antibody Diluent with Casein (cat # 760-219) (Ventana Medical Systems, Tucson, AZ). The slides were baked on the Discovery Ultra Stainer at 60°C for 8 minutes, de-paraffinized with Ventana Discovery wash (cat # 950-510) for 3 cycles (8 minutes each) at 69°C, and underwent cell conditioning (target retrieval) at 95°C for 16 minutes (for cell arrays) or 32 minutes (tissues or TMAs) in CC1 buffer (cat # 950-500). Following this, slides were incubated with Background Sniper (cat# BS966M, Biocare Medical, Pacheco, CA) in a Ventana Option Prep kit (cat # 771-751) for 28 minutes, incubated with CM inhibitor (a part of the Ventana DAB kit cat # 760-159) for 12 minutes, and incubated for 1h at RT with MAGE-B2 antibody diluted with Ventana diluent with Casein in a Ventana Prep kit (cat # 770-001) (working concentration at 0.1 µg/ml for cell pellet, or 1-5 ug/ml for tissues) or with a matched concentration of isotype control Rabbit IgG1 antibody. Following this, slides were incubated with the Ventana Anti-Rabbit HQ (cat # 760-4815) for 28 minutes at RT, Ventana Anti-HQ HRP (cat # 760-4820) for 28 minutes at RT, and signals detected with Ventana DAB chromogen kit (cat # 760-159) for 5 minutes at RT. Slides were counterstained with Ventana Hematoxylin II (cat # 5266726001) for 8 minutes and Ventana Bluing reagent (cat # 526676900) for 4

minutes. Slides were coverslipped and digitized using an Aperio AT2 scanner (Leica Biosystems Inc, Buffalo Grove, IL).

Table 14. Assay steps.

Steps on Ventana Ultra	Vendor / Catalog	Time
Baking	Ventana / NA	60°C, 8 minutes
Deparaffinization	Ventana / 950-510	69°C, 3 cycles (8 minutes each)
Antigen Retrieval	Ventana / 950-500	CC1, 95°C, 16 minutes (for FFPE cell pellets) or 32 minutes (for FFPE tissues)
Background Sniper	Biocare Medical / BS966M	RT, 28 minutes
Peroxidase inhibitor (CM)	Ventana / 760-159	RT, 12 minutes
In house MAGEB2 antibody at 0.1 (cells) or 1-5 ug/ml (tissues) in Ventana diluent with Casein	Ventana / 760-219	RT, 60 minutes
Anti-Rabbit HQ	Ventana / 760-4815	RT, 28 minutes
Anti-HQ HRP	Ventana / 760-4820	RT, 28 minutes
DAB+ Chromogen	Ventana / 760-159	RT, 5 minutes
Hematoxylin II	Ventana / 5266726001	RT, 8 minutes
Bluing reagent	Ventana / 526676900	RT,4 minutes

Results

Twenty-four rabbit-anti human MAGEB2 antibody supernatants were assessed for binding to MAGEB2 and potential cross reactivity for other MAGE-A and MAGE-B family members by IHC. Five of twenty-four human MAGEB2 antibody supernatants (5H20, 1J13, 1J14, 714, 7J6) did not detect MAGEB2 by IHC on human MAGEB2+ transfected cells. Nineteen of the twenty-four antibody supernatants detected MAGEB2 by IHC on human MAGEB2+ transfected cells and were assessed on other human MAGE-A+ and MAGE-B+ family member cross-reactivity control cells. Four of nineteen supernatants (4G17, 1J15, 1C3, 1I14) showed the strongest IHC signal on human MAGEB2+ transfected control cells, and the lowest/ or no cross reactivity on other MAGE-A and MAGE-B family members and selected as candidates for further assessment.

Note that the twenty-four MAGEB2 antibody supernatants were not purified antibodies. A false positive cross reactivity result with one of the discarded clones may be due to non-specific staining by the supernatants. Small batch purified antibody aliquots from four MAGEB2 clones (4G17, 1J15, 1C3, 1I14) were re-tested on transfected human MAGEA+ and MAGE-B+ family member control cells, and

tested on control testes tissues, normal tissue TMAs, and thirteen human cancer cell lines with known endogenous RNA expression level of MAGEB2 by IHC.

There was intense membranous/ cytoplasmic staining in the transfected human MAGEB2+ cells with the four anti-MAGEB2 antibodies (4G17, 1J15, 1C3, 1I14) (Figure 3), no cross reactivity on other human MAGE-A+ and MAGE-B+ family member cross reactivity control cells (Figure 4), (Table 15). There was intense MAGEB2 IHC staining in testis, characterized by cytoplasmic to membrane staining of spermatogonia cells. Nuclear staining in a subset of testis germ cells was observed with 4G17, 1J15, and 1C3 clones, and immunostaining in Leydig cells was observed with 1J15, and 4G17 clones. Leydig staining is likely non-specific (Figure 5). Nuclear staining was considered likely specific because it was observed only in testis tissues, and nuclear localization is thought to play a role in enhancement of E2F transcriptional activity, cell proliferation, and resistance to ribotoxin stress (Peche et al 2015).

The four anti-MAGEB2 antibodies (4G17, 1J15, 1C3, 1I14) were assessed on normal tissue TMA. MAGEB2 specific immunostaining was observed only in testis cores. Immunostaining observed with 4G17, 1J15, and 1C3 clones in other tissues such as liver, adrenal gland, and intestines were presumed nonspecific. The MAGEB2 antibody (1I14) showed the lowest non-specific/ background immunostaining when compared to Immunoglobulin G (IgG) isotype control (Tables 16, 17, 18, 19). Antibody clone 1I14 displays properties that indicate it will be an excellent IHC reagent for detecting MAGEB2 (Figure 6). Furthermore, down titration of MAGEB2 (1C3) antibody completely mitigated the nonspecific background immunostaining that was observed in liver tissues, while still retaining MAGEB2 specific immunostaining in testis (Figure 7).

Anti-MAGEB2 antibodies 4G17, 1J15, 1C3, 1I14 detected varying levels of MAGEB2 expression in the thirteen human cancer cell lines. MAGEB2 IHC expression frequency was high (~90 %) in U266B1 cells, moderate (~70%) in T98G, KMM-1, U2OS cells, and undetectable in CFPAC-1, UACC-257, CFPAC-1, VMRC-LCD, NCI-H1703, UM-UC-3, NCI-H1395, NCI-H82 cell lines. MAGEB2 IHC expression frequency in SCaBER cells were inconsistent with the four MAGEB2 clones (4G17, 1J15, 1C3, 1I14) and maybe due to different binding intensity of each antibody or unoptimized IHC assay conditions (Table 20).

Conclusions

Twenty-four rabbit anti-human MAGEB2 antibodies were assessed for specificity to MAGEB2, and potential cross reactivity for other MAGE family members by IHC staining on FFPE tissues. Nineteen of the twenty-four detected MAGEB2 by IHC on control MAGEB2+ cells. Four of the nineteen antibodies (4G17, 1J15, 1C3, 1I14) were tested thoroughly and showed no cross reactivity to other MAGE-A or MAGE-B family members, strong IHC signal on control testis tissues, and detected the MAGEB2 IHC signal in thirteen human cancer cell lines with known endogenous RNA expression level of MAGEB2. Three of the four MAGEB2 antibodies (4G17, 1J15, 1C3) showed some non-specific background IHC

staining on liver and adrenal gland in normal human tissue TMA. Data is summarized in Tables 15 - 20 herein.

SEQUENCES

Human MAGEB2 Amino Acid Sequence (SEQ ID NO: 1):

>sp|O15479|MAGB2_HUMAN Melanoma-associated antigen B2 OS=Homo sapiens OX=9606 GN=MAGEB2 PE=1 SV=3

MPRGQKSKLRAREKRRKARDETRGLNVPQVTEAEEEEAPCCSSSVSGGAASSSPAAGIPQ
EPQRAPTTAAAAAAGVSSTKSKKGAKSHQGEKNASSSQASTSTKSPSEDPLTRKSGSLVQ
FLLYKYKIKKSVTKGEMLKIVGKRFREHFPEILKKASEGLSVVFGLELNKVNPNGHTYTF
IDKVDLTDEESLLSSWDFPRRKLLMPLLGVIFLNGNSATEEEIWEFLNMLGVYDGEEHSV
FGEPWKLITKDLVQEKYLEYKQVPSSDPPRFQFLWGPRAYAETSKMKVLEFLAKVNGTTP
CAFPTHYEEALKDEEKAGV

Full amino acid sequences for three exemplary monoclonal antibodies light chains and heavy chains follow:

>1C3_LC (SEQ ID NO: 555)
MDMRVPAQLLGLLLLWLRGARCDIVMTQTPSSVEAAVGGTVTIKCQASQNISSYLAWYQQKPGQPPKLLIYRASTLA
SGVPSRFKGSGSGTQFTLTISDLECADAATYYCQSYDDSRSSNFFYAFGGGTEVVVKGDPVAPTVLLFPPSSDEVATGT
VTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQSFSR
KNC

>1C3_HC (SEQ ID NO: 556)
MDMRVPAQLLGLLLLWLRGARCQSLEESGGGLVQPEGSLTLTCTAFGVTLTNYYICWVRQAPGKGLEWVGCIDNAN
GRTYYASWAKGRFTISKTSSTTGTLQMTSLTAADTATYFCARSLATPLWGPGTLVTVSSGQPKAPSVFPLAPCCGDTP
SSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPS
TCSKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIR
VVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVE
WEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK

>1I14_LC (SEQ ID NO: 557)
MDMRVPAQLLGLLLLWLRGARCDVVMTQTPASVEATVGGTVTIKCQSSQSVYDNNALAWYQQNAGQRPRLLIYG
ASTLASGVPSRFSASGSGTEFTLTISDLECADAATYYCQCTYYVSSYQNDFGGGTEVVVKGDPVAPTVLLFPPSSDEVA
TGTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQ
SFSRKNC

>1I14_HC (SEQ ID NO: 558)
MDMRVPAQLLGLLLLWLRGARCQSVEESGGRLVTPGTPLTLTCTISGFSLSSYAMSWVRQAPGKGLEWIGSIGGGGS
AVYASWAKGRFTISKTSTTVDLRITSPTTEDTAMYFCGRGFYSIDLWGPGTLVTVSSGQPKAPSVFPLAPCCGDTPSST
VTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPATNTKVDKTVAPSTC

SKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTARPPLREQQFNSTIRVV
STLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTCMINGFYPSDISVEWE
KNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRSPGK

>1H17_LC (SEQ ID NO: 559)
MDMRVPAQLLGLLLLWLRGARCAAVLTQTPSPVSAAVGGTVSASCQSSKSVYNKNWLSWFQQKPGQPPKLLIYGA
STLASGVPSRFKGSGSGTQFTLTISDVQCDDAATYYCAGGYSSSSDTFAFGGGTEVVVKGDPVAPTVLLFPPSSDEVAT
GTVTIVCVANKYFPDVTVTWEVDGTTQTTGIENSKTPQNSADCTYNLSSTLTLTSTQYNSHKEYTCKVTQGTTSVVQS
FSRKNC

>1H17_HC (SEQ ID NO: 560)
MDMRVPAQLLGLLLLWLRGARCQEQLVESGGGLVKPGASLTLTCKASGFSFSSGQLMCWVRQAPGKGLEWIACIGS
GSNAISTFYASWAQGRFTISKSSSTTVTLQLTSLTAADTATYFCARVGSDDYGDSDVFDPWGPGTLVTVSSGQPKAPS
VFPLAPCCGDTPSSTVTLGCLVKGYLPEPVTVTWNSGTLTNGVRTFPSVRQSSGLYSLSSVVSVTSSSQPVTCNVAHPA
TNTKVDKTVAPSTCSKPTCPPPELLGGPSVFIFPPKPKDTLMISRTPEVTCVVVDVSQDDPEVQFTWYINNEQVRTAR
PPLREQQFNSTIRVVSTLPIAHQDWLRGKEFKCKVHNKALPAPIEKTISKARGQPLEPKVYTMGPPREELSSRSVSLTC
MINGFYPSDISVEWEKNGKAEDNYKTTPAVLDSDGSYFLYSKLSVPTSEWQRGDVFTCSVMHEALHNHYTQKSISRS
PGK

Additional exemplary sequences of the present invention are provided in Tables 21 – 25 herein.

Table 15. MAGEB2 Immunohistochemistry Staining in MAGE-A/B control cells

Antibody ID	HTSC Inventory Code (Block ID)	CHO cells	MAGE-B2 cells	Other MAGE-B family members	Other MAGE-A family members
4G17	694001, 697782, 697548, 697639, 694686	negative	positive	negative	negative
1J15	694001, 697782, 697548, 697639, 694686	negative	positive	negative	negative
1C3	694001, 697782, 697548, 697639, 694686	negative	positive	negative	negative
1i14	694001, 697782, 697548, 697639, 694686	negative	positive	negative	negative
IgG	694001, 697782, 697548, 697639, 694686	negative	negative	negative	negative

Table 16. MAGE-B2 (1i14) Immunohistochemistry Staining in Normal Human Tissue TMA (FDA999x)

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localiza-tion	Comment
A1	1	Cerebrum	Nct05N012	X			X					
A2	2	Cerebrum	Nct05N016	X			X					
A3	3	Cerebrum	Nct08N046	X			X					Very weak non-specific staining in the vessels
A4	4	Cerebellum	Ncb05N008	X			X					
A5	5	Cerebellum	Ncb15N146	X			X					Very weak non-specific staining in the vessels
A6	6	Cerebellum	Ncb05N011	X			X					Very weak non-specific staining in the vessels
A7	7	Adrenal gland	Eag06N019	X		Non-specific particulate staining in cortex (Zona fasiculata and zona reticularis)	X					
A8	8	Adrenal gland	Eag06N010	X		Non-specific particulate staining in cortex (Zona fasiculata and zona reticularis)	X					Non-specific particulate staining in cortex (Zona fasiculata and zona reticularis) as observed in isotype
A9	9	Adrenal gland	Eag15N145	X		Non-specific particulate staining in cortex (Zona fasiculata and zona reticularis)	X					
A10	10	Ovary	Fov07N034	X			X					
A11	11	Ovary	Fov13N015	X			X					

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
A12	12	Ovary	Fov13N023	X			X					
B1	13	Pancreas	Dpa15N145	TI		Tissue autolyzed	TI					Tissue autolyzed
B2	14	Pancreas	Dpa06N023	X			X					
B3	15	Pancreas	Dpa11N012	X			X					
B4	16	Lymph node	lly06N015	X			X					
B5	17	Lymph node	lly08N047	X		Non-specific staining in exfoliated cells	X					Non-specific staining in exfoliated cells as observed in isotype
B6	18	Lymph node	lly08N014	X			X					
B7	19	Hypophysis	Ept14N001	X			X					
B8	20	Hypophysis	Ept13N019	X			X					
B9	21	Hypophysis	Ept13N020	X			X					
B10	22	Testis	Mtt09N048	X			X	X	Spermatogonia cells	3	Cytoplasmic/ membrane	
B11	23	Testis	Mtt08N001	X			X	X	Spermatogonia cells	3	Cytoplasmic/ membrane	
B12	24	Testis	Mtt08N009	X			X	X	Spermatogonia cells	3	Cytoplasmic/ membrane	

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
C1	25	Thyroid gland	Etg07N002	X		Non-specific staining in colloid						Non-specific staining as observed in isotype
C2	26	Thyroid gland	Etg08N047	X		Non-specific staining in colloid						Non-specific staining as observed in isotype
C3	27	Thyroid gland	Etg06N003	X		Non-specific staining in colloid						Non-specific staining as observed in isotype
C4	28	Breast	Fmg08N034	X			X					
C5	29	Breast	Fmg50N034	X			X					
C6	30	Breast	Fmg07N040	X			X					
C7	31	Spleen	Isp15N145	X			X					
C8	32	Spleen	Isp15N146	X			X					
C9	33	Spleen	Isp11N001	X			X					
C10	34	Tonsil	Rts13N011	TI		Only adipose tissue present	X					
C11	35	Tonsil	Rts50N001	X			X					
C12	36	Tonsil	Rts08N042	X		Minimal lymphoid tissue present	X					
D1	37	Thymus gland	Ith06N024	X			X					
D2	38	Thymus gland	Ith06N002	X			X					
D3	39	Thymus gland	Ith15N145	X			X					
D4	40	Bone marrow	Ibm06N024	X		Non-specific staining in exfoliated cells	X					Non-specific staining as observed in isotype

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localiza-tion	Comment
D5	41	Bone marrow	Ibm11N015	X		Non-specific staining in exfoliated cells	X					Non-specific staining as observed in isotype
D6	42	Bone marrow	Ibm12N001	X		Non-specific staining in exfoliated cells	X					Non-specific staining as observed in isotype
D7	43	Lung	Rln06N004	X			X					
D8	44	Lung	Rln06N027	X			X					
D9	45	Lung	Rln06N023	X			X					
D10	46	Heart	Cht11N009	X			X					
D11	47	Heart	Cht05N005	X			X					
D12	48	Heart	Cht11N001	X			X					
E1	49	Esophagus	Des06N027	X			X					Intense non-specific staining in superficial epithelium
E2	50	Esophagus	Des17N004	X		Minimal epithelial tissue	X					
E3	51	Esophagus	Des15N117	X			X					
E4	52	Stomach	Dst07N016	X		Hazy non-specific staining in gastric gland cells	X					Non-specific staining as observed in isotype
E5	53	Stomach	Dst15N003	X		Hazy non-specific staining in gastric gland cells	X					Non-specific staining as observed in isotype

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localiza-tion	Comment
E6	54	Stomach	Dst06N017	X		Hazy non-specific staining in gastric gland cells	X					
E7	55	Small intestine	Din07N016	X		Weak non-specific mucosal staining	X					Non-specific cytoplasmic staining in epithelial cells as observed in isotype
E8	56	Small intestine	Din08N014	X			X					
E9	57	Small intestine	Din08N007	X			X					
E10	58	Colon	Dco15N083	X			X					
E11	59	Colon	Dco06N022	X			X					
E12	60	Colon	Dco15N082	TI		No mucosal tissue	X					
F1	61	Liver	Dlv05N015		X		X					
F2	62	Liver	Dlv15N146	X		Non-specific particulate staining in hepatocytes	X					
F3	63	Liver	Dlv15N145	X			X					
F4	64	Salivary gland	Doc11N007	X			X					
F5	65	Salivary gland	Doc06N023	X			X					
F6	66	Salivary gland	Doc06N022	X			X					
F7	67	Kidney	Ukn08N047	X			X					
F8	68	Kidney	Ukn08N035	X			X					
F9	69	Kidney	Ukn06N004	X			X					

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localiza-tion	Comment
F10	70	Prostate	Mpr08N004	X			X					
F11	71	Prostate	Mpr08N005	X			X					
F12	72	Prostate	Mpr11N013	X			X					
G1	73	Uterus	Fur17N003	X			X					
G2	74	Uterus	Fur50N029	X			X					
G3	75	Uterus	Fur07N040	X			X					
G4	76	Cervix	Fdu50N020	X			X					
G5	77	Cervix	Fdu17N003	X			X					
G6	78	Cervix	Fdu50N019	X			X					
G7	79	Bladder	Ubd08N007	X			X					
G8	80	Bladder	Ubd08N001	X			X					
G9	81	Bladder	Ubd08N009	X			X					
G10	82	Skeletal muscle	Srm15N008	X			X					
G11	83	Skeletal muscle	Srm15N009	X			X					
G12	84	Skeletal muscle	Srm05N014	X			X					
H1	85	Skin	Kin15N007	X			X					
H2	86	Skin	Kin07N025	X			X					
H3	87	Skin	Kin15N009	X			X					
H4	88	Nerve	Scf10N003	X			X					
H5	89	Nerve	Scf08N010	X			X					
H6	90	Nerve	Scf11N005	X			X					
H7	91	Artery	Sbv07N029	X			X					
H8	92	Pericardium	Apr15N145	X			X					
H9	93	Pericardium	Apr15N146	X			X					
H10	94	Eye	Vey130002	TI		Only pigmented	TI					Only

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localiza-tion	Comment
						retina present						pigmented retina present
H11	95	Eye	Vey140001	TI		Only pigmented retina present	TI					Only pigmented retina present
H12	96	Eye	Vey070007	X		Intense pigmentation	X					Intense pigmentation
I1	97	Larynx	Rla13N011	X			X					
I2	98	Larynx	Rla13N020	X			X					
I3	99	Larynx	Rla07N010	X			X					

MAGE-B2 specific IHC staining with MAGE-B2 (1114) was observed only in testis tissues characterized by cytoplasmic to membrane staining of spermatogonia cells. Staining in other tissues were either observed in isotype or deemed non-specific.

Table 17. MAGE-B2 (IJ15) Immunohistochemistry Staining in Normal Human Tissue TMA (FDA999x)

Tissue identification				Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
A1	1	Cerebrum	Nct03N002	X					
A2	2	Cerebrum	Nct03N005	X					Non-specific staining in blood vessels
A3	3	Cerebrum	Nct04N001	X					Non-specific staining in blood vessels
A4	4	Cerebrum	Nct11N001	X					
A5	5	Cerebrum	Nct07N015	X					Non-specific staining in blood vessels
A6	6	Cerebrum	Nct05N006	X					Non-specific staining in blood vessels
A7	7	Cerebellum	Ncb04N001	X					
A8	8	Cerebellum	Ncb05N006	X					Non-specific staining in blood vessels
A9	9	Cerebellum	Ncb05N011	X					
B1	10	Adrenal gland	Eag07N001		X	Epithelial cells	3+	Cytoplasmic/ membrane	Intense staining in cortex and likely non-specific
B2	11	Adrenal gland	Eag06N010		X	Epithelial cells	3+	Cytoplasmic/ membrane	Intense staining in cortex and likely non-specific
B3	12	Adrenal gland	Eag06N009		X	Epithelial cells	3+	Cytoplasmic/ membrane	Intense staining in cortex and likely non-specific
B4	13	Ovary	Fov110136	X					
B5	14	Ovary	Fov160119	X					

Tissue identification				Target IHC					
B6	15	Ovary	Fdu100022	X					
B7	16	Pancreas	Dpa05N010		X	Epithelial cells	2+	Cytoplasmic/ membrane	Weak to mild staining in acinar epithelium and likely non-specific
B8	17	Pancreas	Dpa03N009	X					Non-specific staining in blood vessels
B9	18	Pancreas	Dpa08N040		X	Epithelial cells	1+	Cytoplasmic/ membrane	Very weak staining in acinar epithelium and likely non-specific
C1	19	Lymph node	Ily08N047	X					Non-specific staining in exfoliated cells and some blood vessels
C2	20	Lymph node	Ily11N004	X					Non-specific staining in exfoliated cells and some blood vessels
C3	21	Lymph node	Ily07N015	X					
C4	22	Hypophysis	Ept17N003	X					Non-specific staining in blood vessels
C5	23	Hypophysis	Ept17N003	X					Non-specific staining in blood vessels
C6	24	Hypophysis	Ept17N003	X					Non-specific staining in blood vessels
C7	25	Testis	Mtt07N016		X	Spermato-gonia cells; Leydig cells	3+	Cytoplasmic/ membrane; nuclear staining in some	Leydig cells staining is likely non-specific
C8	26	Testis	Mtt11N004		X	Spermato-gonia cells; Leydig cells	2+	Cytoplasmic/ membrane; nuclear staining in some	Leydig cells staining is likely non-specific
C9	27	Testis	Mtt07N026		X	Spermato-gonia cells; Leydig cells	3+	Cytoplasmic/ membrane; nuclear staining in some	Leydig cells staining is likely non-specific
D1	28	Thyroid gland	Etg06N003	X					Non-specific staining in colloid and serum

Tissue identification				Target IHC					
D2	29	Thyroid gland	Etg06N010	X					Non-specific staining in colloid and serum
D3	30	Thyroid gland	Etg05N007	X					Non-specific staining in colloid and serum
D4	31	Breast	Fmg140091	X					
D5	32	Breast	Fmg07N034	X					
D6	33	Breast	Fmg12N003	X					
D7	34	Spleen	Isp06N023	X					Non-specific staining in exfoliated cells and some blood vessels
D8	35	Spleen	Isp05N009	X					Non-specific staining in exfoliated cells and some blood vessels
D9	36	Spleen	Isp07N028	X					Non-specific staining in exfoliated cells and some blood vessels
E1	37	Tonsil	Doc041188	X					
E2	38	Tonsil	Doc041188	X					
E3	39	Tonsil	Doc041188	X					
E4	40	Thymus gland	Ith06N002	X					Non-specific staining in endothelial cells
E5	41	Thymus gland	Ith06N024	X					Non-specific staining in endothelial cells
E6	42	Thymus gland	Ith06N025	X					Non-specific staining in endothelial cells
E7	43	Bone marrow	Ibm07N029	X					Non-specific staining in exfoliated cells
E8	44	Bone marrow	Ibm07N023	X					Non-specific staining in exfoliated cells

Tissue identification				Target IHC					
E9	45	Bone marrow	Ibm07N026	X					Non-specific staining in exfoliated cells
F1	46	Lung	Rln05N023	X					Non-specific staining in alveolar septum, exfoliated cells
F2	47	Lung	Rln05N010	X					Non-specific staining in alveolar septum, exfoliated cells
F3	48	Lung	Rln08N014	X					Non-specific staining in alveolar septum, exfoliated cells
F4	49	Heart	Cht05N005	X					Non-specific staining in serum and blood vessels
F5	50	Heart	Cht03N009	X					Non-specific staining in serum and blood vessels
F6	51	Heart	Cht11N005	X					Non-specific staining in serum and blood vessels
F7	52	Esophagus	Des06N003		X		3+	Cytoplasmic/ membrane	Staining in superficial epithelium, blood vessels and connective tissues are likely non-specific
F8	53	Esophagus	Des15N145		X		2+	Cytoplasmic/ membrane	Staining in superficial epithelium, blood vessels and connective tissues are likely non-specific
F9	54	Esophagus	Des06N009	X					Non-specific staining n blood vessels and connective tissue
G1	55	Stomach	Dst08N033		X		2+	Cytoplasmic/ membrane	Non-specific staining in glandular epithelial cells
G2	56	Stomach	Dst08N011		X	Epithelium	3+	Cytoplasmic/ membrane	Non-specific staining in glandular epithelial cells
G3	57	Stomach	Dst17N005	X					

Tissue identification				Target IHC					
G4	58	Small intestine	Din07N016	X					Non-specific staining in blood vessels; Weak hazy cytoplasmic staining in mucosal epithelium
G5	59	Small intestine	Din09N051	X					Non-specific staining in blood vessels; Weak hazy cytoplasmic staining in mucosal epithelium
G6	60	Small intestine	Din06N022	X					Non-specific staining in blood vessels; Weak hazy cytoplasmic staining in mucosal epithelium
G7	61	Colon	Dco15N142	X					
G8	62	Colon	Dco15N103	X					
G9	63	Colon	Dco15N115	X					
H1	64	Liver	Dlv05N015	X	X	Hepatocytes	2+	Cytoplasmic/ membrane	Mild to intense staining and likely non-specific
H2	65	Liver	Dlv15N145	X	X	Hepatocytes	2+	Cytoplasmic/ membrane	Mild staining and likely non-specific
H3	66	Liver	Dlv03N005	X	X	Hepatocytes	1+	Cytoplasmic/ membrane	Very weak staining and likely non-specific
H4	67	Salivary gland	Doc120045	X					Non-specific staining in blood vessels and serum
H5	68	Salivary gland	Doc05N009	X					Non-specific staining in blood vessels and serum
H6	69	Salivary gland	Doc06N019	X					Non-specific staining in blood vessels and serum
H7	70	Kidney	Ukn06N006	X					Non-specific staining in vasculature serum proteins

Tissue identification				Target IHC					
H8	71	Kidney	Ukn05N003	X					Non-specific staining in vasculature serum proteins
H9	72	Kidney	Ukn06N023		X	Tubular epithelium	2+	C/M	Staining in vasculature serum proteins and tubular epithelium likely non-specific
I1	73	Prostate	Mpr07N029	TI					Tissue inadequate
I2	74	Prostate	Mpr11N008	X					Weak hazy non-specific staining in stroma and glandular epithelium
I3	75	Prostate	Mpr09N052	X					Weak hazy non-specific staining in stroma and glandular epithelium
I4	76	Uterus	Fur07N010	X					
I5	77	Uterus	Fur07N001	X					Non-specific staining in blood vessels
I6	78	Uterus	Fur150196	X					Non-specific nuclear staining in some stromal cells; hazy cytoplasmic staining in glandular epithelium that is likely non-specific
I7	79	Cervix	Fur170174	X					
I8	80	Cervix	Fdu060889	X					
I9	81	Cervix	Fdu170148	X					
J1	82	Skeletal muscle	Srm06N015	X					Very weak non-specific hazy staining
J2	83	Skeletal muscle	Srm05N005	X					Very weak non-specific hazy staining
J3	84	Skeletal muscle	Srm05N007	X					Very weak non-specific hazy staining

Tissue identification				Target IHC					
J4	85	Skin	Kin07N017	X					
J5	86	Skin	Kin06N025	X					
J6	87	Skin	Kin08N034	X					
J7	88	Nerve	Scf15N098	X					
J8	89	Nerve	Scf15N009	X					
J9	90	Nerve	Scf07N017	X					
K1	91	Pericardium	Apr07N027	TI					Tissue inadequate
K2	92	Diaphragm	Srm17N006	X					
K3	93	Pericardium	Apr07N026	X					
K4	94	Eye	Vey090007	X					Intense pigmentation
K5	95	Eye	Vey140007	TI					Tissue inadequate
K6	96	Eye	Vey090002	X					
K7	97	Larynx	Rla17N006	X					Non-specific staining in superficial epithelium
K8	98	Larynx	Rla17N005	X					
K9	99	Larynx	Rla06N023	TI					Tissue inadequate

MAGE-B2 IHC staining with MAGE-B2 (IJ15) is observed in testis tissues characterized by cytoplasmic to membrane staining of spermatogonia cells, nuclear staining in some and staining in Leydig cells. Leydig staining is likely non-specific. Staining in other tissues were either observed in isotype or deemed non-specific.

Table 18. MAGE-B2 (IC3) Immunohistochemistry Staining in Normal Human Tissue TMA (FDA999x)

No.	Organ	Tissue ID	IHC isotype Control			Target IHC					
			Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Local-ization	Comment
1	Cerebrum	Nct05N012	X			X					
2	Cerebrum	Nct05N016	X			X					Scattered glial cell staining that is likely non-specific due to high antibody concentration
3	Cerebrum	Nct08N046	X			X					Scattered glial cell staining that is likely non-specific due to high antibody concentration
4	Cerebellum	Ncb05N008	X			X					
5	Cerebellum	Ncb15N146	X			X					
6	Cerebellum	Ncb05N011	X			X					
7	Adrenal gland	Eag06N019	X		Non-specific particulate staining in cortex (Zona fasciculata and zona reticularis)		X	Epithelial cells	3+	Cytoplasmic/ membrane	Intense staining in cortex and likely non-specific
8	Adrenal gland	Eag06N010	X		Non-specific particulate staining in cortex (Zona fasciculata and zona reticularis)		X	Epithelial cells	3+	Cytoplasmic/ membrane	Intense staining in cortex and likely non-specific

No.	Organ	Tissue ID	IHC Isotype Control			Target IHC					
			Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Local-ization	Comment
9	Adrenal gland	Eag15N145	X		Non-specific particulate staining in cortex (Zona fasciculata and zona reticularis)		X	Epithelial cells	3+	Cytoplasmic/ membrane	Intense staining in cortex and likely non- specific
10	Ovary	Fov07N034	X			X					
11	Ovary	Fov13N015	X			X					
12	Ovary	Fov13N023	X			X					
13	Pancreas	Dpa15N145	TI		Tissue autolyzed	TI					Severe autolysis and non-specific staining
14	Pancreas	Dpa06N023	X				X	Epithelial cells	3+	C/M	Intense staining and likely non-specific
15	Pancreas	Dpa11N012	X				X	Epithelial cells	1+	C/M	Very weak staining and likely non-specific
16	Lymph node	lly06N015	X			X					
17	Lymph node	lly08N047	X		Non-specific staining in exfoliated cells	X					Non-specific staining in exfoliated cells
18	Lymph node	lly08N014	X			X					
19	Hypophysis	Ept14N001	X			X					
20	Hypophysis	Ept13N019	X			X					Non-specific nuclear staining in glandular cells
21	Hypophysis	Ept13N020	X			X					Non-specific nuclear staining in glandular cells

No.	Organ	Tissue ID	IHC Isotype Control			Target IHC					Comment
			Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	
22	Testis	Mtt09N048	X				X	Spermatogonia cells; spermatids; Leydig cells	3+	C/M in Leydig; N in others	Intense nuclear staining suggests non-specific staining with this antibody clone at this high concentration
23	Testis	Mtt08N001	X				X	Spermatogonia cells; spermatids; Leydig cells	3+	C/M in Leydig; N in others	Intense nuclear staining suggests non-specific staining with this antibody clone at this high concentration
24	Testis	Mtt08N009	X				X	Spermatogonia cells; spermatids; Leydig cells	3+	C/M in Leydig; N in others	ntense nuclear staining suggests non-specific staining with this antibody clone at this high concentration
25	Thyroid gland	Etg07N002	X		Hazy non-specific staining in colloid						Non-specific staining in colloid and serum
26	Thyroid land	Etg08N047	X		Hazy non-specific staining in colloid						Non-specific staining in colloid and serum
27	Thyroid gland	Etg06N003	X		Hazy non-specific staining in colloid						Non-specific staining in colloid and serum
28	Breast	Fmg08N034	X			X					
29	Breast	Fmg50N034	X			X					
30	Breast	Fmg07N040	X			X					
31	Spleen	Isp15N145	X			X					Hazy non-specific staining in red pulp
32	Spleen	Isp15N146	X			X					Hazy non-specific staining in red pulp
33	Spleen	Isp11N001	X			X					

			IHC Isotype Control			Target IHC					
No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Local-ization	Comment
34	Tonsil	Rts13N011	TI		Only adipose tissue present	TI					Only adipose tissue present
35	Tonsil	Rts50N001	X			X					Few nuclear staining in superficial epithelium
36	Tonsil	Rts08N042	X		Minimal lymphoid tissue present	X					Minimal lymphoid tissue present
37	Thymus gland	Ith06N024	X			X					
38	Thymus gland	Ith06N002	X			X					
39	Thymus gland	Ith15N145	X			X					
40	Bone marrow	Ibm06N024	X		Non-specific staining in exfoliated cells	X					Non-specific staining in exfoliated cells
41	Bone marrow	Ibm11N015	X		Non-specific staining in exfoliated cells	X					Non-specific staining in exfoliated cells
42	Bone marrow	Ibm12N001	X		Non-specific staining in exfoliated cells	X					Non-specific staining in exfoliated cells
43	Lung	Rln06N004	X			X					Non-specific staining in exfoliated cells and alveolar macrophages
44	Lung	Rln06N027	X			X					
45	Lung	Rln06N023	X			X					Non-specific staining in exfoliated cells and alveolar macrophages
46	Heart	Cht11N009	X			X					Non-specific hazy staining in cardiomyocytes

No.	Organ	Tissue ID	IHC Isotype Control			Target IHC					
			Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Local-ization	Comment
47	Heart	Cht05N005	X			X					Non-specific hazy staining in cardiomyocytes
48	Heart	Cht11N001	X			X					
49	Esophagus	Des06N027	X			X					Non-specific cytoplasmic staining in squamous epithelial
50	Esophagus	Des17N004	X		Minimal epithelial tissue	X					Minimal epithelial tissue
51	Esophagus	Des15N117	X			X					
52	Stomach	Dst07N016	X		Hazy non-specific staining in gastric gland cells	X					Intense non-specific staining in gastric gland cells
53	Stomach	Dst15N003	X		Hazy non-specific staining in gastric gland cells	X					Weak non-specific staining in gastric gland cells
54	Stomach	Dst06N017	X		Hazy non-specific staining in gastric gland cells	X					Intense non-specific staining in gastric gland cells
55	Small intestine	Din07N016	X		Weak non-specific mucosal staining	X					Non-specific cytoplasmic staining in epithelial cells as observed in isotype
56	Small intestine	Din08N014	X			X					
57	Small intestine	Din08N007	X			X					
58	Colon	Dco15N083	X			X					
59	Colon	Dco06N022	X			X					

			IHC Isotype Control			Target IHC					
No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
60	Colon	Dco15N082	TI		No mucosal tissue	TI					No mucosal tissue
61	Liver	Div05N015	X				X	Hepatocytes	3+	C/M	Very intense staining and likely non-specific
62	Liver	Div15N146	X		Non-specific particulate staining in hepatocytes		X	Hepatocytes	3+	C/M	Very intense staining and likely non-specific
63	Liver	Div15N145	X				X	Hepatocytes	3+	C/M	Very intense staining and likely non-specific
64	Salivary gland	Doc11N007	X								
65	Salivary gland	Doc06N023	X			X					
66	Salivary gland	Doc06N022	X			X					
67	Kidney	Ukn08N047	X			X					
68	Kidney	Ukn08N035	X			X					
69	Kidney	Ukn06N004	X			X					
70	Prostate	Mpr08N004	X			X					
71	Prostate	Mpr08N005	X			X					

			IHC Isotype Control			Target IHC					
No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Local-ization	Comment
72	Prostate	Mpr11N013	X			X					
73	Uterus	Fur17N003	X			X					
74	Uterus	Fur50N029	X			X					on-specific nuclear staining in glandular epithelial cells and stromal cells
75	Uterus	Fur07N040	X			X					
76	Cervix	Fdu50N020	X			X					Non-specific nuclear staining in epithelial and stromal cells
77	Cervix	Fdu17N003	X			X					
78	Cervix	Fdu50N019	X			X					
79	Bladder	Ubd08N007	X			X					Non-specific nuclear staining in epithelial and stromal cells
80	Bladder	Ubd08N001	X			X					Non-specific nuclear staining in epithelial and stromal cells
81	Bladder	Ubd08N009	X			X					
82	Skeletal muscle	Srm15N008	X			X					
83	Skeletal muscle	Srm15N009	X			X					
84	Skeletal muscle	Srm05N014	X			X					Hazy non-specific staining in cytoplasm
85	Skin	Kin15N007	X			X					

			IHC Isotype Control			Target IHC					
No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Local-ization	Comment
86	Skin	Kin07N025	X			X					
87	Skin	Kin15N009	X			X					
88	Nerve	Scf10N003	X			X					
89	Nerve	Scf08N010	X			X					
90	Nerve	Scf11N005	X			X					
91	Artery	Sbv07N029	X			X					
92	Pericardium	Apr15N145	X			X					
93	Pericardium	Apr15N146	X			X					
94	Eye	Vey130002	TI		Only pigmented retina present	TI					Only pigmented retina present
95	Eye	Vey140001	TI		Only pigmented retina present	TI					Only pigmented retina present
96	Eye	Vey070007	X		Intense pigmentation	X					Intense pigmentation
97	Larynx	Rla13N011	X			X					
98	Larynx	Rla13N020	X			X					Non-specific nuclear staining in epithelial and stromal cells
99	Larynx	Rla07N010	X			X					Non-specific nuclear staining in epithelial and stromal cells

MAGE-B2 IHC staining with MAGE-B2 (IC3) is observed in testes tissues characterized by cytoplasmic to membrane staining of spermatogonia cells, and nuclear staining in some cells. There are MAGE-B2 IHC staining in other tissues such as liver, adrenal gland, intestines that are most likely non-specific.

Table 19. MAGE-B2 (4G17) Immunohistochemistry Staining in Normal Human Tissue TMA (FDA999x)

Tissue identification				IHC Isotype Control			Target IHC				
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
A1	1	Cerebrum	Nct05N012	X			X					
A2	2	Cerebrum	Nct05N016	X			X					Non-specific staining in few glial cells
A3	3	Cerebrum	Nct08N046	X			X					Non-specific staining in few capillaries
A4	4	Cerebellum	Ncb05N008	X			X					
A5	5	Cerebellum	Ncb15N146	X			X					Non-specific staining in few capillaries
A6	6	Cerebellum	Ncb05N011	X			X					Non-specific staining in few capillaries
A7	7	Adrenal gland	Eag06N019	X		Non-specific particulate staining in cortex (Zona fasciculata and zona reticularis)	X					Non-specific staining as observed in isotype but with increased intensity

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
A8	8	Adrenal gland	Eag06N010	X		Non-specific particulate staining in cortex (Zona fasiculata and zona reticularis)	X					Non-specific staining as observed in isotype but with increased intensity
A9	9	Adrenal gland	Eag15N145	X		Non-specific particulate staining in cortex (Zona fasiculata and zona reticularis)	X					Non-specific staining as observed in isotype but with increased intensity
A10	10	Ovary	Fov07N034	X			X					Non-specific particulate staining in cortex (Zona fasiculata and zona reticularis) as observed in isotype
A11	11	Ovary	Fov13N015	X			X					
A12	12	Ovary	Fov13N023	X			X					
B1	13	Pancreas	Dpa15N145	TI		Tissue autolyzed	TI					Tissue autolyzed
B2	14	Pancreas	Dpa06N023	X				X	Epithelial cells	1+	Cytoplasmic/ membrane	Weak to mild staining and likely non-specific
B3	15	Pancreas	Dpa11N012	X			X					
B4	16	Lymph node	lly06N015	X			X					

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
B5	17	Lymph node	lly08N047	X		Non-specific staining in exfoliated cells	X					Non-specific staining as in isotype, but with increased intensity
B6	18	Lymph node	lly08N014	X			X					
B7	19	Hypophysis	Ept14N001	X			X					
B8	20	Hypophysis	Ept13N019	X				X	Epithelial cells	2+	Cytoplasmic/ membrane	Predominantly in loosely adhered cells and likely non-specific
B9	21	Hypophysis	Ept13N020	X				X	Epithelial cells	2+	Cytoplasmic/ membrane	Predominantly in loosely adhered cells and likely non-specific
B10	22	Testis	Mtt09N048	X				X	Spermato-gonia cells	3	Cytoplasmic/ membrane, Nuclear	There is nuclear staining in some spermatids and hazy cytoplasmic staining in some Leydig cells. Both are likely non-specific
B11	23	Testis	Mtt08N001	X				X	Spermato-gonia cells	3	Cytoplasmic/ membrane, Nuclear	There is nuclear staining in some spermatids and hazy cytoplasmic staining in some Leydig cells. Both are likely non-specific
B12	24	Testis	Mtt08N009	X				X	Spermato-gonia cells	3	C/M; N	There is nuclear staining in some spermatids and hazy cytoplasmic staining in some Leydig cells. Both are likely non-specific

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
C1	25	Thyroid gland	Etg07N002	X		Hazy non-specific staining in colloid						Non-specific staining as observed in isotype but with increased intensity
C2	26	Thyroid gland	Etg08N047	X		Hazy non-specific staining in colloid						Non-specific staining as observed in isotype but with increased intensity
C3	27	Thyroid gland	Etg06N003	X		Hazy non-specific staining in colloid						Non-specific staining as observed in isotype but with increased intensity
C4	28	Breast	Fmg08N034	X			X					
C5	29	Breast	Fmg50N034	X			X					
C6	30	Breast	Fmg07N040	X			X					
C7	31	Spleen	Isp15N145	X			X					Weak non-specific staining in exfoliated cells in red pulp
C8	32	Spleen	Isp15N146	X			X					Weak non-specific staining in exfoliated cells in red pulp
C9	33	Spleen	Isp11N001	X			X					Weak non-specific staining in exfoliated cells in red pulp

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
C10	34	Tonsil	Rts13N011	TI		Only adipose tissue present	TI					Only adipose tissue present
C11	35	Tonsil	Rts50N001	X			X					Non-specific nuclear staining in some cells
C12	36	Tonsil	Rts08N042	X		Minimal lymphoid tissue present	X					
D1	37	Thymus gland	Ith06N024	X			X					
D2	38	Thymus gland	Ith06N002	X			X					
D3	39	Thymus gland	Ith15N145	X			X					
D4	40	Bone marrow	Ibm06N024	X		Non-specific staining in exfoliated cells	X					Non-specific staining as observed in isotype but with increased intensity
D5	41	Bone marrow	Ibm11N015	X		Non-specific staining in exfoliated cells	X					Non-specific staining as observed in isotype but with increased intensity
D6	42	Bone marrow	Ibm12N001	X		Non-specific staining in exfoliated cells	X					Non-specific staining as observed in isotype but with increased intensity
D7	43	Lung	Rln06N004	X			X					Non-specific staining in some degenerating alveolar macrophages

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
D8	44	Lung	Rln06N027	X			X					
D9	45	Lung	Rln06N023	X			X					
D10	46	Heart	Cht11N009	X			X					
D11	47	Heart	Cht05N005	X			X					
D12	48	Heart	Cht11N001	X			X					
E1	49	Esophagus	Des06N027	X			X					
E2	50	Esophagus	Des17N004	X		Minimal epithelial tissue	X					
E3	51	Esophagus	Des15N117	X			X					
E4	52	Stomach	Dst07N016	X		Hazy non-specific staining in gastric gland cells	X					Non-specific staining as observed in isotype but with increased intensity
E5	53	Stomach	Dst15N003	X		Hazy non-specific staining in gastric gland cells	X					Non-specific staining as observed in isotype but with increased intensity
E6	54	Stomach	Dst06N017	X		Hazy non-specific staining in gastric gland cells	X					Non-specific staining as observed in isotype but with increased intensity
E7	55	Small intestine	Din07N016	X		Weak non-specific mucosal staining	X					Non-specific cytoplasmic staining in epithelial cells as observed in isotype

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
E8	56	Small intestine	Din08N014	X			X					
E9	57	Small intestine	Din08N007	X			X					
E10	58	Colon	Dco15N083	X			X					
E11	59	Colon	Dco06N022	X			X					
E12	60	Colon	Dco15N082	TI		No mucosal tissue	X					
F1	61	Liver	Dlv05N015	X			X					
F2	62	Liver	Dlv15N146	X		Non-specific particulate staining in hepatocytes		X	Hepatocytes	2+	Cytoplasmic/ membrane	Weak to mild staining in hepatocytes, likely non-specific
F3	63	Liver	Dlv15N145	X				X	Hepatocytes	2+	Cytoplasmic/ membrane	Weak to mild staining in hepatocytes, likely non-specific
F4	64	Salivary gland	Doc11N007	X				X	Hepatocytes	2+	Cytoplasmic/ membrane	Weak to mild staining in hepatocytes, likely non-specific
F5	65	Salivary gland	Doc06N023	X			X					
F6	66	Salivary gland	Doc06N022	X			X					

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
F7	67	Kidney	Ukn08N047	X			X					
F8	68	Kidney	Ukn08N035	X			X					
F9	69	Kidney	Ukn06N004	X			X					
F10	70	Prostate	Mpr08N004	X			X					
F11	71	Prostate	Mpr08N005	X			X					
F12	72	Prostate	Mpr11N013	X			X					
G1	73	Uterus	Fur17N003	X			X	X	Epithelial	1+	Cytoplasmic/ membrane	Granular cytoplasmic staining in few prostate gland epithelial cells, likely non-specific
G2	74	Uterus	Fur50N029	X			X					Non-specific nuclear staining in some stromal cells and epithelial cells
G3	75	Uterus	Fur07N040	X			X					
G4	76	Cervix	Fdu50N020	X			X					
G5	77	Cervix	Fdu17N003	X			X					
G6	78	Cervix	Fdu50N019	X			X					
G7	79	Bladder	Ubd08N007	X			X					Non-specific nuclear staining in some stromal cells and epithelial cells

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
G8	80	Bladder	Ubd08N001	X			X					
G9	81	Bladder	Ubd08N009	X			X					
G10	82	Skeletal muscle	Srm15N008	X			X					
G11	83	Skeletal muscle	Srm15N009	X			X					
G12	84	Skeletal muscle	Srm05N014	X			X					
H1	85	Skin	Kin15N007	X			X					
H2	86	Skin	Kin07N025	X			X					
H3	87	Skin	Kin15N009	X			X					
H4	88	Nerve	Scf10N003	X			X					
H5	89	Nerve	Scf08N010	X			X					
H6	90	Nerve	Scf11N005	X			X					
H7	91	Artery	Sbv07N029	X			X					
H8	92	Pericardium	Apr15N145	X			X					
H9	93	Pericardium	Apr15N146	X			X					

Tissue identification				IHC Isotype Control			Target IHC					
Position	No.	Organ	Tissue ID	Negative	Positive	Comment	Negative	Positive	Cell type	Intensity (0-3+)	Localization	Comment
H10	94	Eye	Vey130002	TI		Only pigmented retina present	TI					Only pigmented retina present
H11	95	Eye	Vey140001	TI		Only pigmented retina present	TI					Only pigmented retina present
H12	96	Eye	Vey070007	X		Intense pigmentation	X					Intense pigmentation
I1	97	Larynx	Rla13N011	X			X					
I2	98	Larynx	Rla13N020	X			X					
I3	99	Larynx	Rla07N010	X			X					

MAGE-B2 IHC staining with MAGE-B2 (4G17) was observed in testes, characterized by cytoplasmic to membrane staining of spermatogonia cells, nuclear staining in some and staining in Leydig cells. Leydig staining is likely non-specific. There was MAGE-B2 IHC staining in other tissues such as liver, adrenal gland, intestines that was most likely non-specific.

Table 20. Testing the 4 MAGE-B2 antibodies (4G17, 1J15, 1C3, 1i14) on thirteen human cancer cell lines with knownendogenous RNA expression level by IHC.

Cell line	Tron Database [RPKM]	RNASeq (exon specific FPKM)	CCLE (FPKM)	IHC (1J15)		IHC (1i14)		IHC(1C3)		IHC (4G17)	
	MAGEB2 expression	MAGEB2 expression	MAGEB2 expression	Intensity	Frequency %	Intensity	Frequency %	Intensity	Frequency %	Intensity	Frequency %
T98G	0.05	0	0.01	1	70	2	70	2	70	2	70
UACC-257	0.04	0.01	0.01	0	0	0/1	0	0/1	0	0	0
KMM-1	56.91	31.58	42.69	0/1	70	1	70	1	70	2	70
U266B1	102.32	103.73	89.35	1	90	3	90	2	90	2	80
CFPAC-1	0.45	0.18	0.40	0/1	0	0/1	0	0/1	0	0/1	0
SCaBER	0.01	0.01	0.02	2	50	0	0	2	70	2	70
VMRC-LCD	0			0	0	0	0	0	0	0	0
NCI-H1703	0	0	0.00	0/1	0	0/1	0	0/1	0	0	0
UM-UC-3			0.02	0	0	0		0/1		0	0
NCI-H1395			3.52	0	0	0	0	0	0	0	0
U2OS			45.42	1	50	2	70	2	60	2	60
NCI-H82		6.33	0.06	0	0	0	0	1	0	0	0

MAGEB2 IHC expression frequency with the four MAGE-B2 (4G17, 1J15, 1C3, 1i14) was high (~90 %) in U266B1 cells, and moderate (~70%) in T98G, KMM-1, U2OS cells; and undetectable in CFPAC-1, UACC-257, CFPAC-1, VMRC-LCD, NCI-H1703, UM-UC-3, NCI-H1395, NCI-H82 cell lines with the 4 MAGE-B2 clones (4G17, 1J15, 1C3, 1i14). MAGEB2 IHC expression frequencies in SCaBER cells were inconsistent with the four MAGE-B2 clones (4G17, 1J15, 1C3, 1i14).

Table 21

Standard IgG Antibody VL CDRs

Ab	Type	CDR1	CDR2	CDR3
7M5	NA	CAGGCCAGTCAGAGCATTAGGAAT GAATTATTT (SEQ ID NO: 5)	GCTGCATCCAAACTGGCCTCT (SEQ ID NO: 6)	CAATGCAGTTATGTTAGTAGTGGTACT TATGGAAATGTT (SEQ ID NO: 7)
	AA	QASQSIRNELF (SEQ ID NO: 7)	AASKLAS (SEQ ID NO: 8)	QCSYVSSSGTYGNV (SEQ ID NO: 9)
1J14	NA	CAGGCCAGTGAAAGCATTAGCAAC TACTTATCC (SEQ ID NO: 10)	TGGGCATCCACTCTGGCATCT (SEQ ID NO: 11)	CAACAGGGTTATAGTAGTAATGTTGAT AATCTT (SEQ ID NO: 12)
	AA	QASESISNYLS (SEQ ID NO: 13)	WASTLAS (SEQ ID NO: 14)	QQGYSSSNVDNL (SEQ ID NO: 15)
7F15	NA	CAGTCCAGTCAGAGTGTTTGGCATA ACGACTACTTTATCC (SEQ ID NO: 16)	GGTGCATCCACTTTGGCATCT (SEQ ID NO: 17)	GCAGGCGGTTATGGACGTAGTAGTGAAAA TGGT (SEQ ID NO: 18)
	AA	QSSQSVWHNDYLS (SEQ ID NO: 19)	GASTLAS (SEQ ID NO: 20)	AGGYGRSSENG (SEQ ID NO: 21)
5I9	NA	CAGTCCAGTAAGAGTGTTTATAATA ACAACTGGTTAGCC (SEQ ID NO: 22)	GATGCATCGACTCTAGATTCT (SEQ ID NO: 23)	GTAGGGCGGTTATAGTCGTAGTGATAAT GGT (SEQ ID NO: 24)
	AA	QSSKSVYNNNWLA (SEQ ID NO: 25)	DASTLDS (SEQ ID NO: 26)	VGGYSSRSDNG (SEQ ID NO: 27)
1J15	NA	CAGGCCAGTCAGAGTATTAGTAGTT ACTTATCC (SEQ ID NO: 28)	AGGGCATCCACTCTGGCATCT (SEQ ID NO: 29)	CAAAGCTATGATGATAGTAGTGATAATAAT TTTTTTTATGGT (SEQ ID NO: 30)
	AA	QASQSISSYLS (SEQ ID NO: 31)	RASTLAS (SEQ ID NO: 32)	QSYDDSSDNNFFYG (SEQ ID NO: 33)
4G17	NA	CAGGCCAGTCAGAACATTGATAGT TACTTAGCC (SEQ ID NO: 34)	AGGGCATCCACTCTGGCATCT (SEQ ID NO: 35)	CAAAGCTATGATGATAGTAGGAGTAGTAG TTTTTTTTATGGT (SEQ ID NO: 36)
	AA	QASQNIDSYLA (SEQ ID NO: 37)	RASTLAS (SEQ ID NO: 38)	QSYDDSRSSSFFYG (SEQ ID NO: 39)
	NA	CAGGCCAGTCAGAACATTAATAGT TACTTAGCC	AGGGCATCCACTCTGGCATCT	CAAAGCTATGATGATAGTAGGAGTATTAGT TTTTTTATGCT

4A15		(SEQ ID NO: 40)	(SEQ ID NO: 41)	(SEQ ID NO: 42)	
	AA	QASQNINSYLA	RASTLAS	QSYDDSRSISFFYA	
		(SEQ ID NO: 43)	(SEQ ID NO: 44)	(SEQ ID NO: 45)	
1J16	NA	CAGGCCAGTCAGAGCATTAGTAGC TACTTAGCC	GCTGCATCCACTCTGGCATCT	CAAAGCTATGATAGTAGGAGTAGTAG TTTTTTTTATGCT	
		(SEQ ID NO: 46)	(SEQ ID NO: 47)	(SEQ ID NO: 48)	
	AA	QASQSISSYLA	AASTLAS	QSYDDSRSSSFFYA	
		(SEQ ID NO: 49)	(SEQ ID NO: 50)	(SEQ ID NO: 51)	
1C18	NA	CAGGCCAGTCAGAGCATTAGCAGT TGGTTATCC	AGGGCAACCACTCTGGCATCT	CAAAGTTATGATAGTAGTAGTAGTAAT TTTTTTTATGCT	
		(SEQ ID NO: 52)	(SEQ ID NO: 53)	(SEQ ID NO: 54)	
	AA	QASQSISSWLS	RATTLAS	QSYDDSSSSNFFYA	
		(SEQ ID NO: 55)	(SEQ ID NO: 56)	(SEQ ID NO: 57)	
7H4	NA	CAGTCCAGTCAGAGTGTTTATAGTA ACAACCTCTTATCT	AAGGCATCCACTCTGGCATCT	CAAGGCTATTATAGTGGTGTTATTTATATG	
		(SEQ ID NO: 58)	(SEQ ID NO: 59)	(SEQ ID NO: 60)	
	AA	QSSQSVYSNNLLS	KASTLAS	QGYYSGVIYM	
		(SEQ ID NO: 61)	(SEQ ID NO: 62)	(SEQ ID NO: 63)	
1H24	NA	CAGGCCAGTCAGAGCATTAGTAGT TACTTATCC	AGGGCATCCACTCTGGCATCT	CAAAGCTATGATGATAGTAGTAGTAATAAT TTTTTTTATGGT	
		(SEQ ID NO: 64)	(SEQ ID NO: 65)	(SEQ ID NO: 66)	
	AA	QASQSISSYLS	RASTLAS	QSYDDSSSNNFFYG	
		(SEQ ID NO: 67)	(SEQ ID NO: 68)	(SEQ ID NO: 69)	
1C3	NA	CAGGCCAGTCAGAACATTAGTAGC TACTTAGCC	AGGGCATCCACTCTGGCATCT	CAAAGCTATGATGATAGTAGGAGTAGTAA TTTTTTTTATGCT	
		(SEQ ID NO: 70)	(SEQ ID NO: 71)	(SEQ ID NO: 72)	
	AA	QASQNISSYLA	RASTLAS	QSYDDSRSSNFFYA	
		(SEQ ID NO: 73)	(SEQ ID NO: 74)	(SEQ ID NO: 75)	
5H20	NA	CAGTCCAGTGAGAGCGTTTATAATC ACAACTGGTTAGGC	GATGCATCCACTCTGGCATCT	CAAGGCTATTATCAAACTAGTGTTTGGGCT	
		(SEQ ID NO: 76)	(SEQ ID NO: 77)	(SEQ ID NO: 78)	
	AA	QSSESVYNHNWLG	DASTLAS	QGYYQTSVWA	
		(SEQ ID NO: 79)	(SEQ ID NO: 80)	(SEQ ID NO: 81)	
1I14	NA	CAGTCCAGTCAGAGTGTTTATGATA ACAATGCTTTAGCC	GGTGCATCCACTCTGGCATCT	CAATGTACTTATTATGTTAGTAGTTATCAA AATGAT	
		(SEQ ID NO: 82)	(SEQ ID NO: 83)	(SEQ ID NO: 84)	

1H17	AA	QSSQSVYYDNNALA	GASTLAS	QCTYYVSSYQND	
		(SEQ ID NO: 85)	(SEQ ID NO: 86)	(SEQ ID NO: 87)	
	NA	CAGTCCAGTAAGAGTGTTTATAATA AGAACTGGTTATCC	GGTGCATCCACTCTGGCATCT	GCAGGCGGTTATAGTAGTGATACA TTTGCT	
		(SEQ ID NO: 88)	(SEQ ID NO: 89)	(SEQ ID NO: 90)	
	AA	QSSKSVYYNKNWLS	GASTLAS	AGGYSSSSDTFA	
		(SEQ ID NO: 91)	(SEQ ID NO: 92)	(SEQ ID NO: 93)	
7B20	NA	CAGTCCAGTCAGAGCGTTTATAGTA GCGACCTCTTATCC	AAGGCATCCACTCTGGCATCT	CAAGGCTACTATAGTGGTGTGGTTTATATT	
		(SEQ ID NO: 94)	(SEQ ID NO: 95)	(SEQ ID NO: 96)	
	AA	QSSQSVYSSDLLS	KASTLAS	QGYYSGVVYI	
		(SEQ ID NO: 97)	(SEQ ID NO: 98)	(SEQ ID NO: 99)	
7J6	NA	CAGTCCAGTCAGAGCGTTTATAGTA GCGACCTCTTATCC	AAGGCATCCACTCTGGCATCT	CAAGGCTACTATAGTGGTGTGGTTTATATT	
		(SEQ ID NO: 100)	(SEQ ID NO: 101)	(SEQ ID NO: 102)	
	AA	QSSQSVYSSDLLS	KASTLAS	QGYYSGVVYI	
		(SEQ ID NO: 103)	(SEQ ID NO: 104)	(SEQ ID NO: 105)	
1D2	NA	CAGGCCAGTCAGAGCATTAGTAGT TACTTATCT	AGGGCATCCCCTCTGGCATCT	CAAAGCTACGATGATAGTAGTAGTAATAAT TTTTTTTATGGT	
		(SEQ ID NO: 106)	(SEQ ID NO: 107)	(SEQ ID NO: 108)	
	AA	QASQSISSYLS	RASPLAS	QSYDDSSSNNFFYG	
		(SEQ ID NO: 109)	(SEQ ID NO: 110)	(SEQ ID NO: 111)	
4G8	NA	CAGTCCAGTAAGAGTGTTTATAAATA AGAACTGGTTATCC	GGTGCATCCACTCTGGCATCT	GCAGGCGGTTATAGTAGTAGTGATACG TTTGCT	
		(SEQ ID NO: 112)	(SEQ ID NO: 113)	(SEQ ID NO: 114)	
	AA	QSSKSVYNKNWLS	GASTLAS	AGGYSSSSDTFA	
		(SEQ ID NO: 115)	(SEQ ID NO: 116)	(SEQ ID NO: 117)	
7I4	NA	CAGTCCAGTCAGAGCCTTTATAATA GCGACCTCTTATCC	AAGGCATCCACTCTGGCATCT	CAAGGCTACTATAGTGGTGTGGTTTATATT	
		(SEQ ID NO: 118)	(SEQ ID NO: 119)	(SEQ ID NO: 120)	
	AA	QSSQSLYNSDLLS	KASTLAS	QGYYSGVVYI	
		(SEQ ID NO: 121)	(SEQ ID NO: 122)	(SEQ ID NO: 123)	
1I23	NA	CAGTCCAGTAAGAGTGTTTATAATA AGAACTGGTTATCC	GGTGCATCCACTCTGGCATCT	GCAGGCGGTTATAGTAGTAGTGATACA TTTGCT	
		(SEQ ID NO: 124)	(SEQ ID NO: 125)	(SEQ ID NO: 126)	
		QSSKSVYNKNWLS	GASTLAS	AGGYSSSSDTFA	

1J13	AA	(SEQ ID NO: 127)	(SEQ ID NO: 128)	(SEQ ID NO: 129)
	NA	CAGGCCCTTCAGAGTGTTTATGATA ACAATGCTTTTATCC	GGTGCATCCACTCTGGCATCT	CAATGTACTTATTATGTTAGTAGTTATCAA AATGAT
		(SEQ ID NO: 130)	(SEQ ID NO: 131)	(SEQ ID NO: 132)
	AA	QALQSVYDNNALS	GASTLAS	QCTYYVSSYQND
		(SEQ ID NO: 133)	(SEQ ID NO: 134)	(SEQ ID NO: 135)
1G24	NA	CAGGCCAGTGAGAGCATTGGCAAT GCATTAGCC	AGGGCATCCACTCTGGCATCT	CAAAGCTATGATGATAGTAGTAGTAGTAGT TTTTTTTATGCT
		(SEQ ID NO: 136)	(SEQ ID NO: 137)	(SEQ ID NO: 138)
	AA	QASESIGNALA	RASTLAS	QSYDDSSSSSFFYA
		(SEQ ID NO: 139)	(SEQ ID NO: 140)	(SEQ ID NO: 141)
4A7	NA	CAGTCCAGTAAGAGTGTTTATAAATA AGAACTGGTTTATCC	GGTGCATCCACTCTGGCATCT	GCAGGCGGTTATAGTAGTAGTGATACG TTTGCT
		(SEQ ID NO: 142)	(SEQ ID NO: 143)	(SEQ ID NO: 144)
	AA	QSSKSVYNKNWLS	GASTLAS	AGGYSSSSDTFA
		(SEQ ID NO: 145)	(SEQ ID NO: 146)	(SEQ ID NO: 147)

Table 22

Standard IgG Antibody VH CDRs

Ab	Type	CDR1	CDR2	CDR3
7M5	NA	AGCCATGCAATGATC	ACCATTGGGAGTCGTGATACTATATATTAT GCGAGGCTGGGCGAAAGGC	AACGCCTTG
		(SEQ ID NO: 148)	(SEQ ID NO: 149)	(SEQ ID NO: 150)
	AA	SHAMI	TIGSRDTIYYASWAKG	NAL
		(SEQ ID NO: 151)	(SEQ ID NO: 152)	(SEQ ID NO: 153)
1J14	NA	AGCTACGACATGAGC	ATTATTTATGCTAGTGGTAGCACATACTAC GCGAGCTGGGCGAAAGGC	GACCCTGCTGGTTATAGCATTAGCTTTGGC TTG
		(SEQ ID NO: 154)	(SEQ ID NO: 155)	(SEQ ID NO: 156)
		SYDMS	IIYASGSTYYASWAKG	DPAGYSISFGL

7F15	AA	(SEQ ID NO: 157)
	NA	AGCTACAACATGGGC
		(SEQ ID NO: 160)
	AA	SYNMG
		(SEQ ID NO: 163)
5I9	NA	AGCTACGACATGAGC
		(SEQ ID NO: 166)
	AA	SYDMS
		(SEQ ID NO: 169)
1J15	NA	AATTATTATATTTGC
		(SEQ ID NO: 172)
	AA	NYYIC
		(SEQ ID NO: 175)
4G17	NA	AACTATTATATTTGT
		(SEQ ID NO: 178)
	AA	NYYIC
		(SEQ ID NO: 181)
4A15	NA	AACTACTACATCTGC
		(SEQ ID NO: 184)
	AA	NYYIC
		(SEQ ID NO: 187)
1J16	NA	AACTACTACATCTGC
		(SEQ ID NO: 190)
	AA	NYYIC
		(SEQ ID NO: 193)
1C18	NA	AATTATTATATATGT
		(SEQ ID NO: 196)
		NYYIC

(SEQ ID NO: 158)	(SEQ ID NO: 159)
ATCATTGGTGCTAGTGATAGCGCATTGTAC GCGAGCTGGGCAAAAGGC	GGTGGTCTTGGTTTGAGTACTGGTTTTGCGT TG
(SEQ ID NO: 161)	(SEQ ID NO: 162)
IIGASDSALYASWAKG	GGLGLSTGFAL
(SEQ ID NO: 164)	(SEQ ID NO: 165)
TATATTGCTACTGATGGTAGGCCATATTAC GCGAGCTGGGCGAAAGGC	GGGGGGTATGCTGGTGGCTTG
(SEQ ID NO: 167)	(SEQ ID NO: 168)
YIATDGRPYYASWAKG	GGYAGGL
(SEQ ID NO: 170)	(SEQ ID NO: 171)
TGCATTGACAATGCTAATGGTAGGACTTAC TACGCGAGCTGGGCGAAAGGC	TCATTGTCTACTCCCTTG
(SEQ ID NO: 173)	(SEQ ID NO: 174)
CIDNANGRTYYASWAKG	SLSTPL
(SEQ ID NO: 176)	(SEQ ID NO: 177)
TGCATTGACAATGTTAATGGTAGGACCTAC TACGCGAGCTGGGCGAAAGGC	TCCTTGGCTACTCCCTTG
(SEQ ID NO: 179)	(SEQ ID NO: 180)
CIDNVNGRTYYASWAKG	SLATPL
(SEQ ID NO: 182)	(SEQ ID NO: 183)
TGCATTGACAATGTTAATGGTAGGACTTAC TACGCGAGCTGGGCGAAAGGC	TCCTTGGCTACTCCCTTG
(SEQ ID NO: 185)	(SEQ ID NO: 186)
CIDNVNGRTYYASWAKG	SLATPL
(SEQ ID NO: 188)	(SEQ ID NO: 189)
TGCATTGACAATATTAATGGTAGGACTTAC TACGCGAGCTGGGCGAAAGGC	TCCTTGGCTACTCCCTTG
(SEQ ID NO: 191)	(SEQ ID NO: 192)
CIDNINGRTYYASWAKG	SLATPL
(SEQ ID NO: 194)	(SEQ ID NO: 195)
TGTATTGATAACGCTAATGGTAGGACTTAC TACGCGACCTGTGGCGAAAGGC	TCATTGTCTACTAACTTG
(SEQ ID NO: 197)	(SEQ ID NO: 198)
CIDNANGRTYYATWAKG	SLSTNL

7H4	AA	(SEQ ID NO: 199)	(SEQ ID NO: 200)	(SEQ ID NO: 201)
	NA	AGCTACTACTACATATGC	TGTATTGGTGGTGGTAATACCGATGCCACT GCCTACCGCGAGGGTGGGGCGAAAGGC	GGCGGTCCTGATAATAATGTCCAATTTAAC TTG
		(SEQ ID NO: 202)	(SEQ ID NO: 203)	(SEQ ID NO: 204)
	AA	SYYYIC	CIGGGNTDATAYARWAKG	GGPDNNVQFNL
		(SEQ ID NO: 205)	(SEQ ID NO: 206)	(SEQ ID NO: 207)
1H24	NA	AATTATTATATTTGC	TGCATTGACAATAGTAATGGTAGGACTTAC TACGCCGAGCTGGGCGAAAGGC	TCATTGTCTACTCCCTTG
		(SEQ ID NO: 208)	(SEQ ID NO: 209)	(SEQ ID NO: 210)
	AA	NYYYIC	CIDNSNGRTYYASWAKG	SLSTPL
		(SEQ ID NO: 211)	(SEQ ID NO: 212)	(SEQ ID NO: 213)
1C3	NA	AACTACTACATCTGC	TGTATTGACAATGCTAATGGTAGGACTTAC TACGCGAGCTGGGCGAAAGGC	TCCTTGGCTACTCCCTTG
		(SEQ ID NO: 214)	(SEQ ID NO: 215)	(SEQ ID NO: 216)
	AA	NYYYIC	CIDNANGRTYYASWAKG	SLATPL
		(SEQ ID NO: 217)	(SEQ ID NO: 218)	(SEQ ID NO: 219)
5H20	NA	AGCAGTGCAGTGACC	TTCCTCCAAGCTGGGGATGGTAGCGCATAC TACGCGAGCTGGGCGAAAGGC	CATAAGGGTAATAGTTACGTGCCTAACTTG
		(SEQ ID NO: 220)	(SEQ ID NO: 221)	(SEQ ID NO: 222)
	AA	SSAVT	FLQAGDGSAYYASWAKG	HKGNSYVPNL
		(SEQ ID NO: 223)	(SEQ ID NO: 224)	(SEQ ID NO: 225)
1I14	NA	AGCTATGCAATGAGC	AGCATTGGTGGTGGTAGCGCAGTCTAC GCGAGCTGGGCGAAAGGC	GGATTTTATAGTATAGACTTG
		(SEQ ID NO: 226)	(SEQ ID NO: 227)	(SEQ ID NO: 228)
	AA	SYAMS	SIGGGGSAVYASWAKG	GFYSIDL
		(SEQ ID NO: 229)	(SEQ ID NO: 230)	(SEQ ID NO: 231)
1H17	NA	AGCGGCCAACTCATGTGC	TGCATTGGTTCTGGTAGTAATGCTATTAGC ACTTTCTACGCGAGCTGGGCGCAAGGC	GTGGGCTCCGATGACTATGGGTGACTCTGAT GTTTTTGATCCC
		(SEQ ID NO: 232)	(SEQ ID NO: 233)	(SEQ ID NO: 234)
	AA	SGQLMC	CIGSGSNAISTFYASWAQG	VGSDDYGDSDVFDP
		(SEQ ID NO: 235)	(SEQ ID NO: 236)	(SEQ ID NO: 237)
7B20	NA	AGCGCCTACTACATATGC	TGTATTGGTGGTGTTAATCGCGTTGCCACT GCCTACGCGACCTGGGCGAAAGGC	GGCGGTCCTGATAATAATGTCCAATTTAAC TTG
		(SEQ ID NO: 238)	(SEQ ID NO: 239)	(SEQ ID NO: 240)
		SAYYIC	CIGGVNRVATAYATWAKG	GGPDNNVQFNL

7J6	AA	(SEQ ID NO: 241)	(SEQ ID NO: 242)	(SEQ ID NO: 243)
	NA	AGGTACTACTACAGTTGC	TGTGTTGGTGGTGTTAATCGCGATGCCACT GCCTACGCGACCTGGGCGAAAGGC	GGCGGTCCTGATAATAATGTCCAATTTAAC TTG
		(SEQ ID NO: 244)	(SEQ ID NO: 245)	(SEQ ID NO: 246)
	AA	RYYYSC	CVGGVNRDATAYATWAKG	GGPDNNVQFNL
		(SEQ ID NO: 247)	(SEQ ID NO: 248)	(SEQ ID NO: 249)
1D2	NA	AATTATTATATTTGT	TGTATTGACAATGTTAATGGTAGGACTTAC TACGCGAGCTGGGCGAAAGGC	TCATTGTCTACTCCCTTG
		(SEQ ID NO: 250)	(SEQ ID NO: 251)	(SEQ ID NO: 252)
	AA	NYYIC	CIDNVNGRTYYASWAKG	SLSTPL
		(SEQ ID NO: 253)	(SEQ ID NO: 254)	(SEQ ID NO: 255)
4G8	NA	AGCAGCTACTTCATGTGC	TGCATTGCTGTTGGTAGTAGTGGTAGCACT TACTACGCGAGCTGGGCGAAAGGC	GTGGGCTACGATGACTATGGTGACTCTGAT GCTTTTGATCCC
		(SEQ ID NO: 256)	(SEQ ID NO: 257)	(SEQ ID NO: 258)
	AA	SSYFMC	CIAVGSSGSTYYASWAKG	VGYDDYGDSDAFDP
		(SEQ ID NO: 259)	(SEQ ID NO: 260)	(SEQ ID NO: 261)
714	NA	AGGTACTACTACAGTTGC	TGTGTTGGTGGTGTTAATCGCGATGCCACT GCCTACGCGACCTGGGCGAAAGGC	GGCGGTCCTGATAATAATGTCCAATTTAAC TTG
		(SEQ ID NO: 262)	(SEQ ID NO: 263)	(SEQ ID NO: 264)
	AA	RYYYSC	CVGGVNRDATAYATWAKG	GGPDNNVQFNL
		(SEQ ID NO: 265)	(SEQ ID NO: 266)	(SEQ ID NO: 267)
1123	NA	AGCAGCTACTTCATGTGC	TGCATTGGTTCTGGTAGTAGTGGTATTAGC ACTTTCTACGCGAGCTGGGCGCAAGGC	GTGGGCTACGATGACTATGGTGACTCTGAT GCTTTTGATCCC
		(SEQ ID NO: 268)	(SEQ ID NO: 269)	(SEQ ID NO: 270)
	AA	SSYFMC	CIGSGSSAISTFYASWAQG	VGYDDYGDSDAFDP
		(SEQ ID NO: 271)	(SEQ ID NO: 272)	(SEQ ID NO: 273)
1J13	NA	AGCTATGGAATGACT	TACATTTGGACTGATGGGAGGACATACTAC GCAAACTGGGCGAAAGGC	CCCTTTGATGGTAATTATAGGGACATC
		(SEQ ID NO: 274)	(SEQ ID NO: 275)	(SEQ ID NO: 276)
	AA	SYGMT	YIWTDGRTYYANWAKG	PFDGNYRDI

	AA	(SEQ ID NO: 277)	(SEQ ID NO: 278)	(SEQ ID NO: 279)
1G24	NA	AACTACTACATCTGC	TGTATTGATAATGCCAATGGTCGGGACTTAC TACGCCGAACTGGGCGAAAGGC	TCCTTGGCTACTCCCTTG
		(SEQ ID NO: 280)	(SEQ ID NO: 281)	(SEQ ID NO: 282)
	AA	NYYIC	CIDNANGRTYYANWAKG	SLATPL
		(SEQ ID NO: 283)	(SEQ ID NO: 284)	(SEQ ID NO: 285)
4A7	NA	AGCAGCTACTTCATGTGC	TGCATTGGTGTTGGTGGTAGTGGTAGCACT TACTACGCGAACTGGGCGAAAGGC	GTGGCCTACGATGACGATGGTGACTCTGAT GCTTTTGATCCC
		(SEQ ID NO: 286)	(SEQ ID NO: 287)	(SEQ ID NO: 288)
	AA	SSYFMC	CIGVGGSGSTYYANWAKG	VAYDDDGDSDAFDP
		(SEQ ID NO: 289)	(SEQ ID NO: 290)	(SEQ ID NO: 291)

Table 23 Standard IgG Antibody Variable Region Sequences

Ab	Type	LC V-region	HC V-region
7M5	NA	GATGTTGTGATGACCCAGACTCCAGCCTCCGTGTCTGAACCTG TGGGAGGCCACAGTCACCATCAAGTGCCAGGCCAGTCAGAGCA TTAGGAATGATATTTTGGTGGCAGCAGAAACCAGGGCAGCC TCCCAAGCTCCTGATCTATGCTGCATCCAAACTGGCCTCTGGGG TCCCATCGCGGTTCAGCGGCAGTGGATCTGGGACAGAGTTCAC TCTCACCATCAGCGACCTGGAGTGTGCCGATGCTGCCACTTACT ACTGTCAATGCAGTTATGTTAGTAGTGGTACTTATGGAAA TGTTTTCGGCGGAGGGACCGAGGTGGTGGTCAAA (SEQ ID NO: 292)	CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTAACGCCTGGAGGATC CCTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTAGCCATG CAATGATCTGGGTCCGCCAGGCTCCAGGGGAGGGGCTGGAATGGAT CGGAACCATTGGGAGTCGTGATACTATATATTATGCGAGCTGGGCG AAAGGCCGATTCACCATCTCCAAAAACCTCGTCGACCACAATGGATC TGAAAATGACCAGTCTGACAATCGAGGACACGGCCACACCTATTTCTG TGTCAGAAAACGCCTTGTGGGGCCCAGGCACCCTGGTCACC GTCTCCTCA (SEQ ID NO: 293)
	AA	DVVMTQTPASVSEPVGGTVTIKCQASQSIRNELFWWQQKPGQPPKLL IYAASKLASGVPSRFSGSGSGTEFTLTISDLECADAATYYCQCSYVSSS GTYGNVFGGGTEVVVK (SEQ ID NO: 294)	QSLEESGGRLVTPGGSLTLICTVSGIDLSSHAMIWVRQAPGEGLEWIGTIGSR DTIYYASWAKGRFTISKTSSTTMDLKMTSLTIEDTATYFCVRNALWGPGTL VTVSS (SEQ ID NO: 295)
1J14	NA	GCCTATGATATGACCCAGACTCCAGCCTCCGTGGAGGCAGCTGTG GGAGGCACAGTCACCATCAATTGCCAGGCCAGTGAAAGCATTAGCA ACTACTTATCCTGGTATCAGCAGAAACCAGGGCAGCCTCCCAAGC TCCTGATCTACTGGGCATCCACTCTGGGGTCTCATCGCG GTTCAAAGGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGC GGCGTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAACAGGGTT ATAGTAGTAATGTTGATAATCTTTTCGGCGGAGGGACCG AGGTGGTGGTCAAA (SEQ ID NO: 296)	CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCCC TGACACTCACCTGCACAAAGTCTCTGGATTCTCCCTCAGCAGCTACGACATG AGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGAATTA TTTATGCTAGTGGTAGCACATACTACGCGAGCTGGGCGAAAGGCCGATT CACCATCTCCAAAACCTCGACCACGGTGGATCTGAAAATCGCCAGTCCG ACAACCGAGGACACGGCCACCTATTTCTGTGCCAGAGACCCTGGTT ATAGCATTTAGCTTTGGCTTGTGGGGCCCAGGCACCCTGGTCACCGTCTC CTCA (SEQ ID NO: 297)
	AA	AYDMTQTPASVEAAVGGTVTINCQASESISNYLSWYQQKPGQPP KLLIYWASTLASGVSSRFKGSGSGTQFTLTISGVECADAATYYCQQ GYSSSNVDNLFGGGTEVVVK (SEQ ID NO: 298)	QSLEESGGRLVTPGTPLTLTCKVSGFSLSSYDMSWVRQAPGKGLEWIGH YASGSTYYASWAKGRFTISKTSTTVDLKIASPTTEDTATYFCARDPAGYS ISFGLWGPGTLVTVSS (SEQ ID NO: 299)

7F15	NA	GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAG CTGTGGGAGGCACAGTCAGTCATCAGTTGCCAGTCCAGTC AGAGTGTTTGGCATAAACGACTACTTATCCTGGTATCAAC AGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGT GCATCCACTTTGGCATCTGGGGTCCCATCGCGGTTCAAA GGCAGTGGATCTGGGACACAGTTCACTCACCATCAGC GGCGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCA GGCGGTTATGGACGTAGTAGTGATTGAAAATGGTTTCGGCGG AGGGA CCGAGGTGGTGGTCAAA
		(SEQ ID NO: 300)
	AA	AAVLTQTPSPVSAAVGGTVSISCQSSQSVWHNDYLSWYQQ KPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFSLTISGVQCD DAATYYCAGGYGRSSENGFGGGTEVVVK
		(SEQ ID NO: 302)
519	NA	GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAG CTGTGGGAGGCACAGTCAGCATCAGTTGCCAGTCCAGTA AGAGTGTTTATAATAACAACTGGTTAGCCTGGTATCAGC AGAAACCAGGGCAGCCTCCTGATCTACGAT GCATCGACTCTAGATTCTGGGGTCTCATCGGCGGTTCAAA GGCAGTGGATCTGGGACACAGTTCACTCTCACCATCAG CGACGTGCAGTGTGACGATGGTGCCACTTACTACTGTGT AGGCGGTTATAGTAGTCGTAGTGATAATGGTTTCGGCG GAGGGACCGAGGTGGTGGTCAAA
		(SEQ ID NO: 304)
	AA	AAVLTQTPSPVSAAVGGTVSISCQSSKSVYNNNWLAWYQ QKPGQRPKLLIYDASTLDSGVSSRFKGSGSGTQFTLTISDVQ CDDGATYYCVGGYSSRSDNGFGGGTEVVVK
		(SEQ ID NO: 306)
1J15	NA	GACATTGTGATGACCCAGACTCCAGCCTCCGTGGAGGC AGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCCA GTCAGAGTATTAGTACTTATCCTGGTATCAGCAGA AACCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCA TCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAGAGGC AGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGA CCTGGAGTGTGCCGATGCTGCCACTTATTACTGTCAAAG CTATGATGATAGTAGTGATAATAATTTTTTTATGGTTTCG GCGGAG GGACCGAGGTGGTGGTCAGA
		(SEQ ID NO: 308)
	AA	CAGGAGCAGCTGAAGGAGTCCGGAGGAGGCCTGGTTGCGCCTGGAG GAACCCTGACACTCACCTGCGCAGTCTCTGGATTCTCCCTCAGTAGCTA CAACATGGGCTGGGTCCGCCAGGCTCCAGGGGAGGGGCTGGAATAC ATCGGAATCATTGGTGTCTAGTGATAGCGCATTGTACGCGAGCTGGGC AAAAGGCCGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTG AAAATCACCAGTCCGACAACCGAGGACACGGCCACCTATTTCTGTGC CAGAGGTGGTCTTGGTTTTGACTGGTTTTTGCGTTGTGGGGCCCAG GCACCCTGGTCACCGTCTCCTCA
		(SEQ ID NO: 310)
	AA	QEQLKESGGGLVAPGGTLTLTCAVSGFSLSSYNMGWVRQAPGEGLEYI GIIGASDSALYASWAKGRFTISKTSTTVDLKITSPTTEDTATYFCARGGL GLSTGFALWGPGTLVTVSS
		(SEQ ID NO: 312)
	NA	CAGTCGGCTGGAGGAGTCCGGGGGTCGCCTGGTAACGCCTGGAGGAC CCCTGACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGCAGCTACGA CATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATC GGATATATTGGTACTACTGATGGTAGGCCATATTACGCGAGCTGGGCGAA AGGCCGATTCACCATCTCCAAAACCCTCGTCGACCACGGTGGATCTGAA AATCACCAGTCCGACAACCGAGGACACGGCCACCTATTTCTGTGTCAG AGGGGGGTATGGCTGGTGGCTTGTGGGGCCCAGGCACCCTGGTCACC GTTTCCTCA
		(SEQ ID NO: 305)
	AA	QSLEESGGRLVTPGGPLTLTCTVSGFSLSSYDMSWVRQAPGKGLEWIGY IATDGRPYYASWAKGRFTISKPSSTTVDLKITSPTTEDTATYFCVRGGYA GGLWGPGTLVTVSS
		(SEQ ID NO: 307)
	NA	CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGAT CCCTGACACTCACCTGCACAGCTTTTGGATTCACCCTCAATAATTATT ATATTTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGACTGGAGTGGAT CGCATGCATTGACACAATGCTAATGGTAGGACTTACTACGCGAGCTGGG CGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTGACTC TACAAATGACCAGTCTGACAGCCGCGGACACGGCCACCTATTTCTGTG CGAGGTCATTGTCTACTCCCTTGTGGGGCCCAGGCACCCTGGTCACCG TCTCCTCA
		(SEQ ID NO: 309)

4G17	AA	DIVMTQTPASVEAAVGGTVTIKCQASQSISSYLSWYQQKP GQPPKLLIYRASTLASGVPSRFRGSGSGTQFTLTISDLECAD AATYYCQSYDDSSDNNFFYGFGGGTEVVVR
		(SEQ ID NO: 310)
	NA	GACATTGTGATGACCCAGACTCCAGCCTCCGTGGAGGC AGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCCA GTCAGAACATTGATAGTTACTTAGCCTGGTATCAGCAG AAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGC ATCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAG GCAGTGGATCTGGGACAGAGTTCACTCTCACCATCAGC GACCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCA AAGCTATGATGATAGTAGTTTTTTTTATGG TTTCGGCGGA GGGACCGAGGTGGTGGTCAAA
		(SEQ ID NO: 312)
	AA	DIVMTQTPASVEAAVGGTVTIKCQASQNIDSYLAWYQQKP GQPPKLLIYRASTLASGVPSRFKGSGSGTEFTLTISDLECADA ATYYCQSYDDSRSSSFFYGFGGGTEVVVK
		(SEQ ID NO: 314)
4A15	NA	GACATTGTGATGACCCAGACTCCAGCCTCCGTGGAGGC AGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCCA GTCAGAACATTAATAGTTACTTAGCCTGGTATCAGCAGA AACCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCA TCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGC AGTGGATCTGGGACAGAGTTCACTCTCACCATCAGCGA CCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAG CTATGATGATAGTAGGAGTATTAGTTTTTTTTATGCTTTCG GCGGAG GGACCGAGGTGGTGGTCAAA
		(SEQ ID NO: 316)
	AA	DIVMTQTPASVEAAVGGTVTIKCQASQNINSYLAWYQQK PGQPPKLLIYRASTLASGVPSRFKGSGSGTEFTLTISDLECAD AATYYCQSYDDSRSISFFYAFGGGTEVVVK
		(SEQ ID NO: 318)
	NA	GACATTGTGATGACCCAGACTCCAGCCTCCGTGGAGGC AGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCCA GTCAGAGCATTAGTAGCTACTTAGCCTGGTATCACCAGA AACCAGGGCAGCCTCCCAAGCTCCTGATCTATGCTGCATC CACTCTGGCATCTGGGGTCCCATCGCGGTTCGAAGGCA GTGGATCTGGGACACAGTTCACTCTCACCATCAGCGAC CTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAGC

1J16		TATGATGATAGTAGGAGTAGTAGTTTTTTTTATGCTTTC GGCGGA GGGACCGAGGTGGTGGTCAAA	GTCTCCTCA
		(SEQ ID NO: 320)	(SEQ ID NO: 321)
	AA	DIVMTQTPASVEAAVGGTVTIKCQASQSISSYLAWYHQKP GQPPKLLIYAASTLAS GVPSRFEGSGSGTQFTLTISDLECADAATYYCQSYDDSRSS SFFYAFGGGTEVVV K	QSLEESGGGLVQPEGSLTVTCTAFGVTLTNYYICWVRQAPGKGLEWIGC IDNINGR TYYASWAKGRFTISKTSSTTGTLQMTSLTAADTATYFCARSLATPLWGP GTLVTVSS
		(SEQ ID NO: 322)	(SEQ ID NO: 323)
1C18	NA	GACATTGTGATGACCCAGACTCCAGCCTCCGTGGAGGC AGCTGTGGGAGGCA CAGTCACCATCAAGTGCCAGGCCAGTCAGAGCATTAGC AGTTGGTTATCCTGG TATCAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGCTCT ACAGGGCAACCA CTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAGT GGATCTGGGACACA GTTCACTCTCACCATCAGCGACCTGGAGTGTGCCGATG CTGCCACTTATTACT GTCAAAGTTATGATGATAGTAGTAGTAATTTTTTTTA TGCTTTCGGCGGAG GGACCGAGGTGGTGGTCAGA	CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGAT CCCTGAC ACTCACCTGCACAGCTTCTGGATTCACCCTCAATTATTATATATG TTGGGT TCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGATCGGCATGTATTGAT AACGCTA ATGGTAGGACTTACTACGCGACCTGGGCGAAAGGCCGATTCACCATC TCCAAA ACCTCGTCGACCACGGTGACTCTGGCAAAATGCCCAGTCTGACAGCCGCG GACAC GGCCACCCTATTTCTGTGCGAGGTCATTGTCTACTAACTTGTGGGGCCCA GGCAC CCTGGTCACCGTCTCCTCA
		(SEQ ID NO: 324)	(SEQ ID NO: 325)
	AA	DIVMTQTPASVEAAVGGTVTIKCQASQSISSWLSWYQQKP GQPPKLLLYRATTLA SGVPSRFKGSGSGTQFTLTISDLECADAATYYCQSYDDSSS SNFFYAFGGGTEVV VR	QSLEESGGGLVQPEGSLTLTCTASGFTLTNYYICWVRQAPGKGLEWIAC IDNANGR TYYATWAKGRFTISKTSSTTVTLQMPSLTAADTATYFCARSLSTNLWGP GTLVTVSS
		(SEQ ID NO: 326)	(SEQ ID NO: 327)
7H4	NA	CAAGCCGTGGTGACCCAGACTCCATCGTCCGTGTCTGC AGCTGTGGGAGGCACAGTCACCATCAGTTGCCAGTCA GTCAGAGGTGTTTATAGTAACAACCTCTTATCTTGGTATC AGCAGAAACCAGGGCAGCCTCCTAAGCTCCTGATCTAC AAGGCATCCACTCTGGCATCTGGGGTCCCATCGCGGTTTG CAGCGAAGTACAGTGTGACGATGCTGCCACTTATTACTG TCAAGGCTATTATAGTGGTGTTATTTATGTTCGGCGG AGGGACCG AGGTGGTGGTCAAA	CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCAAGCCTGGGGCAT CCCTGACACTCACCTGCACAAAGCCTCTGGGTCAGACTTCAGAAGCTAC TACTACATATGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGT GGGTCGCATGTATTGGTGGTGGTAATACCGATGCCACTGCCTACGCGA GGTGGGCGAAAAGGCCGATTCACCATCTCCAAAACCTCGGCGACCACGG TGGCTCTCCCAAATGACCAGTCTGACAGCCGCGGACACGGCCACCTATT TCTGTGTGAGAGGCGGTCCTGATAATAATGTCCAATTTAACTTTGTGGG GCCCAGGCACCCTGGTCACCGTCTCCTCA
		(SEQ ID NO: 328)	(SEQ ID NO: 329)

1H24	AA	QAVVTQTPSSVSAAVGGTVTISCQSSQSVYSNNLLSWYQQK PGQPPKLLIYKASTLASGVPSRLKGSGSGTQFTLTISEVQCD DAATYYCQGYYSGVIYMFGGGTEVVVK	QSLEESGGGLVKPGASLTLTCKASGSDFRSYYYICWVRQAPGKGLEWV ACIGGGNTDATAYARWAKGRFTISKTSATTVALQMTSLTAADTATYFC VRGGPDNNVQFNLWGPGTLVTVSS
		(SEQ ID NO: 330)	(SEQ ID NO: 331)
	NA	GACATTGTGATGACCCAGACTCCATTCTCCGTGGAGGC AGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCA GTCAGAGCATTAGTAGTTACTTATCCTGGTATCGGCAGA AACCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCA TCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGC AGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGA CCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAG CTATGATGATAGTAGTAGTAATTTTTTTTATGGTTTCG GCGGAG GGACCGAGGTGGTGGTCAGA	CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGAT CCCTGACACTCACCTGCACAGCTTCTGGATTCACCCTCAATAATTATTA TATTTGCTGGGTCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGATCG CATGCATTGACAATAGTAATGGTAGGACTTACTACGCGAGCTGGGCG AAAGGCCGTTTCACCATCTCCAAAACCTCGTCGACCACGGTGACTCTG CAAATGACCAGTCTGACAGCCGCGGACACGGCCACCTATTTCTGTGCG AGGTCATTGTCTACTCCCTTGTGGGGCCCAGGCACCCTGGTCACCGTC TCCTCA
		(SEQ ID NO: 332)	(SEQ ID NO: 333)
	AA	DIVMTQTPFSVEAAVGGTVTIKCQASQSISSYLSWYRQKP GQPPKLLIYRASTLASGVPSRFKGSGSGTQFTLTISDLECAD AATYYCQSYDDSSSNNFFYGFGGGTEVVVR	QSLEESGGGLVQPEGSLTLTCTASGFTLNNYYICWVRQAPGKGLEWIAC IDNSNGRTYYASWAKGRFTISKTSSTTVTLQMTSLTAADTATYFCARSL STPLWGPGTLVTVSS
		(SEQ ID NO: 334)	(SEQ ID NO: 335)
1C3	NA	GACATTGTGATGACCCAGACTCCATCCTCCGTGGAGGC AGCTGTGGGAGGCACAGTCACCATCAAGTGCCAGGCCA GTCAGAACATTAGTAGCTACTTAGCCTGGTATCAGCAG AAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCC ATCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAG GCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCG ACCTGGAGTGTGCCGATGCTGCCACTTACTACTGTCAAAG CTATGATGATAGTAGGAGTAGTAATTTTTTTTATGCTTTCG GCGGAGGGACCGAGGTGGTGGTCAAA	CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGAT CCCTGACACTCACCTGCACAGCTTTTGGAGTCACCCTCACTAACTACTA CATCTGCTGGGTCCGCCAGGCTCCAGGAAAGGGGCTGGAGTGGGTCG GGTGTATTGACAATGCTAATGGTAGGACTTACTACGCGAGCTGGGCG AAAGGCCGATTCACCATCTCCAAGACCTCGTCGACCACAGGGACTCT GCAAATGACCAGTCTGACACAGCCGCGGACACGGCCACCTATTTCTGTGC GAGGTCCTTGGGGCCCAGGCACCCTGGTCACCG TCTCCTCA
		(SEQ ID NO: 336)	(SEQ ID NO: 337)
	AA	DIVMTQTPSSVEAAVGGTVTIKCQASQNISSYLAWYQQKP GQPPKLLIYRASTLASGVPSRFKGSGSGTQFTLTISDLECAD AATYYCQSYDDSRSSNFFYAFGGGTEVVVK	QSLEESGGGLVQPEGSLTLTCTAFGVTLTNYYICWVRQAPGKGLEWVG CIDNANGRTYYASWAKGRFTISKTSSTTGTLQMTSLTAADTATYFCARS LATPLWGPGTLVTVSS
		(SEQ ID NO: 338)	(SEQ ID NO: 339)

5H20	NA	GCGCAAGCGCTGACCCAGACTCCATCCCCTGTGTCTGC AGCTGTGGGAGGCACAGTCACCATCAGTTGCCAGTCCA GTGAGAGCGTTTATAATCACAACTGGTTAGGCTGGTATC AGCAGAAACCAGGGCAGCCTCCCAAACTCCTGATCTAT GATGCATCCACTCTGGCATCTGGGGTCCCATCGCGGTTCA AAGGCAGTGGATCTGGGACACAGTTCACTCTCACAATCA GCGAAAGTCAGTGTGACGATGCTGCCATTTACTACTGTCA AGGCTATTATCAAACTAGTGTTTGGGCTTTCGGCGGAGG GACCG AGGTGGTGGTCAAA (SEQ ID NO: 340)
	AA	AQALTQTPSPVSAAVGGTVTISCQSSESVYNHNWLGWYQQ KPGQPPKLLIYDASTLASGVPSRFKGSGSGTQFTLTISESQCD DAATYYCQGYYQTSVWAFGGGTEVVVK (SEQ ID NO: 342)
1114	NA	GATGTTGTGATGACCCAGACTCCAGCCTCCGTGGAGGC AACTGTGGGAGGCACAGTCACCATCAAGTGCCAGTCCA GTCAGAGTGTTTATGATAACAATGCTTTAGCCTGGTATC AGCAGAATGCAGGACAGCGTCCCAGACTCCTGATCTAT GGTGCATCCACTCTGGCATCTGGGGTCCCATCGGCGGTTCA GTGCCAGTGGATCTGGGACAGAGTTCACTCTCACCATCAG CGACCTGGAGTGTGCCGATGCCGCCACTTACTACTGTCA ATGTACTTATTATGTTAGTAGTTATCAAAATGATTTCGG CGGAG GGACCGAGGTGGTGGTCAAA (SEQ ID NO: 344)
	AA	DVVMTQTPASVEATVGGTVTIKCQSSQSVYDNNALAWYQ QNAGQRPRLLIYGASTLASGVPSRFSASGSGTEFTLTISDLEC ADAATYYCQCTYYVSSYQNDFGGTEVVVK (SEQ ID NO: 346)
1H17	NA	GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAG CTGTGGGAGGCACAGTCAGCGCCAGTTGCCAGTCCAGTA AGAGTGTTTATAATAAGAACTGGTTATCCTGGTTTCAGC AGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTG CATCCACTCTGGGCATCTGGGGTCCCATCGCGGTTCAAAG GCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCG ACGTGCAGTGTGACGATGCTGCCACTTACTACTGTCAG GCGGTTATAGTAGTAGTGATACATTTGCTTTCGGCG GAGGG ACCGAGGTGGTGGTCAAG (SEQ ID NO: 348)	CAGTCGGTGGAGGAGGAGTCCGGGGGTCGCCTGGTCACGCCTGGGACACCC CTGACACTCACCTGCACAGTTTCTGGAGTCGACCTCAGTAGCAGTGCA GTGACCTGGGTCCGCCAGGCTCCAGGGATGGGACTGGAATACATCGGA TTCCTCCAAGGCTGGGGATGGTAGCGCATACTACGCGAGCTGGGCGAAA GGCCGATTCACCATCTCCAAAACCTCGTCGACCACAGTGGATCTGAAA ATGACCAGTCTCACAACCGAGGACACGGCCACCTATTTCTGTGCCAGA CATAAGGGTAATAGTTACGTGCCTAACTTGTGGGGCCCAGGCACCCT GGTCACCGTCTCCTCA (SEQ ID NO: 341)
	AA	QSVEESGGRLVTPGTPLTLTCTVSGVDLSSSAVTWVRQAPGMGLEYIGFL QAGDGSAYYASWAKGRFTISKTSSTTVDLKMTSLTTEDTATYFCARHKG NSYVPNLWGPG TLVTVSS (SEQ ID NO: 343)
	NA	CAGTCGGTGGAGGAGGAGTCCGGGGGTCGCCTGGTCACGGCCTGGGACACCC CTGACACTCACCTGCACAATCTCTGGATTCTCCCTCAGTAGCTATGCAA TGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGA AGCATTGGTGGTGGTAGCGCAGTCTACGCGAGCTGGGCGAAAGGC CGATTCACCATCTCCAAAACCTCGACCACGGTGGATCTGAGAATCACC AGTCCGACAACCGAGGACACGGCCATGTATTTCTGTGGCGCAGGGGATT TTATAGTATAGACTTGTGGGGCCCAGGCACCCTGGTCACCGTCTCCT CA (SEQ ID NO: 345)
	AA	QSVEESGGRLVTPGTPLTLTCTISGFSLSSYAMSWVRQAPGKGLEWIGSI GGGGSAVYASWAKGRFTISKTSTTVDLRITSPTTEDTAMYFCGRGFYSI DLWGPGTLVTVSS (SEQ ID NO: 347)
	NA	CAGGAGCAGCTGGTGGAGTCCGGGGGAGGCCTGGTCAAGCCTGGGG CATCCCTGACACTCACCTGCAAAGCCTCTGGATTCTCCTTCAGTAGCGGC CCAACTCATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGT GGATCGCATGCATTGGTTCTGGTAGTAATGCTATTAGCACTTTCTAC GCGAGCTGGGCGCAAGGCCGATTCACCATCTCCAAATCCTCGTCGA CCACGGTGACTCTGCAATTGACCAGTCTGACACGCCGCGGACACGGCC ACCTATTTCTGTGCGAGAGTGGCGCTCCGATGACTATGGTGACTCTGAT GTTTTTGATCCCTGGGCCCAGGCACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 349)

7B20	AA	AAVLTQTPSPVSAAVGGTVSASCQSSKSVYNKNWLSWFQ QKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFTLTISDV QCDDAATYYCAGGYSSSSDTFAFGGGTEVVVK	QEQLVESGGGLVKPGASLTLTCKASGFSFSSGQLMCWVRQAPGKGLE WIACIGSGSNAISTFYASWAQGRFTISKSSTTVTLQLTSLTAADTATYYF CARVGSDDYGDSDVFDPWGPGTLVTVSS
		(SEQ ID NO: 350)	(SEQ ID NO: 351)
	NA	GCCCAAGTGCTGACCCAGACTCCAGCCTCCGTGTCTGC AGCTGTGGGAGGCACAGTCACCATCAGTTGCCAGTCCAG TCAGAGCGTTTATAGTAGCGACCTCTTATCCTGGTATCAG CAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAA GGCATCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAA GGCAGTGGATCTGGGACACAGTTCACTCTCACAATCAGC GAACTACAGTGTGACGATGCTGCCACTTATTACTGTCAA GGCTACTATAGTGGTGTGGTTTATATTTTCGGCGGAGGG ACCGAGGTGGTGGTCAAG	CAGTCGTTGGAGGAGGAGTCCGGGGGAGGCCTGGTCAAGCCTGGGGGCATCC CTGACACTCACCTGCACAAGCCTCTGGGTCAGACCTCAGTAGCGCCTA CTACATATGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGG GTCGCATGTATTGGTGGTGTTAATCGCGTTGCCACTGCCTACGCGAC CTGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGG TGACTCTGCAAATGACCAGTCTGACACGCCGCGGACACGGCCACTTATT TCTGTGTGAGAGGCGGTCCTGATAATAATGTCCAATTTAACTTGTGG GGCCCAGGCACCCTGGTCACCGTCTCCTCA
		(SEQ ID NO: 352)	(SEQ ID NO: 353)
	AA	AQVLTQTPASVSAAVGGTVTISCQSSQSVYSSDLLSWYQQ KPGQPPKLLIYKASTLASGVPSRFKGSGSGTQFTLTISELQC DDAATYYCQGYYSGVVYIFGGGTEVVVK	QSLEESGGGLVKPGASLTLTCKASGSDLSSAYYICWIRQAPGKGLEWVA CIGGVNRVATAYAYATWAKGRFTISKTSSTTVTLQMTSLTAADTATYFCVR GGPDNNVQFNLWGPGTLVTVSS
		(SEQ ID NO: 354)	(SEQ ID NO: 355)
7J6	NA	GCCCAAGTGCTGACCCAGACTCCAGCCTCCGTGTCTGC AGCTGTGGGAGGCACAGTCACCATCAGTTGCCAGTCCAG TCAGAGCGTTTATAGTAGCGACCTCTTATCCTGGTATCAG CAAAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAA GGCATCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAA GGCAGTGGATCTGGGACACAGTTCACTCTCACAATCAGC GAAGTACAGTGTGACGATGCTGCCACTTATTACTGTCAA GGCTACTATAGTGGTGTGGTTTATATTTTCGGCGGAGGG ACCGAGGTGGTGGTCAAG	CAGTCGTTGGAGGAGGAGTCCGGGGGAGGCCTGGTCAAGCCTGGGGGCATCC CTGACACTCACCTGCACAAAGCCTCTGGGTCAGACCTCAGTAGGTACTA CTACAGTTGCTGGATCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGG GTCGCATGTGTTGGTGGTGTTAATCGCGATGCCACTGCCTACGCCGACC TGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTG ACTCTGCAAATGACCAGTCTGACACGCCGCGGACACGGCCACTTATTTC TGTGTGAGAGGCGGTCCTGATAATAATGTCCAATTTAACTTGTGGGG CCCAGGCACCCTGGTCACCGTCTCCTCA
		(SEQ ID NO: 356)	(SEQ ID NO: 357)
	AA	AQVLTQTPASVSAAVGGTVTISCQSSQSVYSSDLLSWYQQ KPGQPPKLLIYKASTLASGVPSRFKGSGSGTQFTLTISEVQC DDAATYYCQGYYSGVVYIFGGGTEVVVK	QSLEESGGGLVKPGASLTLTCKASGSDLSRYYYSCWIRQAPGKGLEWV ACVGGVNRDATAYATWAKGRFTISKTSSTTVTLQMTSLTAADTATYFC VRGGPDNNVQFNLWGPGTLVTVSS
		(SEQ ID NO: 358)	(SEQ ID NO: 359)

1D2	NA	GACATTGTGATGACCCAGACTCCATTCTCCGTGGAGGC AGCTGTGGTGGAGGCACAGTCACCATCAAGTGCCAGGCCA GTCAGAGCATTAGTAGTTACTTATCTTGGTATCAGCAGA AACCAGGGCAGCCTCCCAAGCTCCTGATCTACAGGGCAT CCCCTCTGGCATCTGGGGTCCCATCGCGGTTCAAAGGCAG TGGATCTGGGACACAGTTCACTCTCACCATCAGCGACCTG GAGTGTGCCGATGCTGCCACTTACTACTGTCAAAGCTAC GATGATAGTAGTAATAATTTTTTTATGGTTTCGGC GGAGGGACCGAGGTGGTGGTCAGA (SEQ ID NO: 360)
	AA	DIVMTQTPFSVEAAVGGTVTIKCQASQSISSYLSWYQQKP GQPPKLLIYRASPLASGVPSRFKGSGSGTQFTLTISDLECAD AATYYCQSYDDSSSNNFFYGFGGGTEVVVR (SEQ ID NO: 362)
4G8	NA	GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAG CTGTGGGAGGCACAGTCACCATCAATTGCCAGTCCAGTA AGAGTGTTTATAATAAGAACTGGTTATCCTGGTTTCAGC AGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTG CATCCACTCTGGGCATCTGGGGTCCCATCGCGGTTCAAAG GCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCG ACGTGCAGTGTGACGATGTTGCCACTTACTACTGTCAGG CGGTTATAGTAGTAGTGATACGTTTGCTTCGGCGG AGGGACCGAGGTGGTGGTCAAA (SEQ ID NO: 364)
	AA	AAVLTQTPSPVSAAVGGTVTINCQSSKSVYNKNWLSWFQ QKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFTLTISDV QCDDVATYYCAGGYSSSSDIFAFGGGTEVVVK (SEQ ID NO: 366)
7I4	NA	GCCCAAGTGCTGACCCAGACTCCAGCCTCCGTGTCTGC AGCTGTGGGAGGCACAGTTGCCAGTCCAG TCAGAGCCTTTATAATAGCGACCTCTTATCCTGGTATCAG CAAAAACCAGGGCAGCCTCCCAAGCTCCTGATCTACAA GGCATCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAA GGCAGTGGATCTGGGACACAGTTCACTCTCACAATCAGC GAAGTACAGTGTGACGATGCTGCCACTTATTACTGTCAA GGCTACTATAGTGGTGGTTATATTTTCGGCGGAGGG ACCG AGGTGGTGGTCAAG (SEQ ID NO: 368)	CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGGGAT CCCTGACACTCACCTGCACAGCTCTGGATTCACCCTCAATAATTATT ATATTTGTTGGGTCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGATC GCATGTATTGACAAATGGTTAATGGTAGGACTTACTACGCGAGCTGGGC GAAAGGCCGATTCACCATCTCCAGAACCTCGTCGACCACGGTGACTCT GCAAATGACCAGTCTGACACGCCGCGGACACGGCCACCCTATTTCTGTGC GAGGTCATTGTCTACTCCCTTGTGGGGCCCAGGCACCCTGGTCACCGT CTCCTCG (SEQ ID NO: 361)
		QSLEESGGGLVQPEGSLTLTCTASGFTLNNYYICWVRQAPGKGLEWIAC IDNVNGRTYYASWAKGRFTISRTSSTTVTLQMTSLTAADTATYFCARSL STPLWGPGTLVTVSS (SEQ ID NO: 363)
		CAGGAGCAGCTGGTGGAGTCCGGGGGAGGCCTGGTCCAGCCTGAGG GATCCCTGACACTCACCTGCGCAGCTCTGGATTCTCCTTCAGTAGCA GCTACTTTCATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGATCGCATGCATTGCTGTTGGTAGTGGTAGCACTTACTACGCC GAGCTGGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCA CGGTGACTCTGCAAATGACCAGTCTGACACGGCCGCGGACACGGCCACC TATTTCTGTGCGAGAGGTGGGCTACGATGACTATGGTGACTCTGATGC TTTTGATCCCTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 365)
		QEQLVESGGGLVQPEGSLTLTCAASGFSFSSSYFMCWVRQAPGKGLEWI ACIAVGSSGSTYYASWAKGRFTISKTSSTTVTLQMTSLTAADTATYFCA RVGYDDYGDSDAF DPWGPGTLVTVSS (SEQ ID NO: 367)
		CAGTCGTTGGAGGAGTCCGGGGGAGGCCTGGTCAAGCCTGGGGGCATCC CTGACACTCACCTGCAAAGCCTCTGGGTCAGACCTCAGTAGGTACTA CTACAGTTGCTGGATCCGCCAGGCTCCAGGGGAAGGGGCTGGAGTGG GTCGCATGTGTTGGTGGTGTTAATGGCGATGCCACTGGCCTACGGCGACC TGGGCGAAAGGCCGATTCACCATCTCCAAAACCTCGTCGACCACGGTG ACTCTGCAAATGACCAGTCTGACACGGCCGCGGACACGGCCACTTATTTC TGTGTGAGAGGCGGTCCTGATAATAATGTCCAATTTAACTTGTGGGG CCCAGGCACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 369)

1123	AA	AQVLTQTPASVSAAVG GTVTISCQSSQSLYNSDLLSWYQQKPGQPPKLLIYKASTLA SGVPSRFKGSGSGTQFTLTISEVQCDDAATYYCQGYYSGVV YIFGGGTEVVVK (SEQ ID NO: 370)
	NA	GCCGCCGTGCTGACCCAGACTCCATCTCCCGTGTCTGCAG CTGTGGGAGGCACAGTCAGCATCAGTTGCCAGTCCAGTA AGAGTGTTTATAATAAGAACTGGTTATCCTGGTTTCAGC AGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTG CATCCACTCTGGCATCTGGGGTCCCATCGCGGTTCAAAG GCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCG ACGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCAG GCGGTTATAGTAGTAGTAGTGATACATTTGCTTTCGGCG GAGGG ACCGAGGTGGTGGTCAAA (SEQ ID NO: 372)
	AA	AAVLTQTPSPVSAAVGGTVSISCQSSKSVYNKNWLSWFQ QKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFTLTISDVQ CDDAATYYCAGGYSSSSDTFAFGGGTEVVVK (SEQ ID NO: 374)
1J13	NA	GATGTTGTGATGACCCACACTCCAGCCTCCCTCTTTGAAA CTGTGGGAGGCACAGTCACCATCAAGTGCCAGCCCTTC AGAGTGTTTATGATAACAATGCTTTATCCTGGTATCAACA AAATGCAGGACAGCGTCCCATACTCCTGATCTATGGTGCA TCCACTCTGGCATCTGGGGCCCCATCGCGGTTCAGTGCC AGTGGATCTGGGACAGATTTCACTCTCACCATCATCGAC CTGGAGTGTGCCGATGCTTCCACTTACTACTGTCAATGT ACTTATAATAGTTAGTAGTTATCAAAATGATTTCGGCGGA GGG ACCGAGGTGGTGGTCAAA (SEQ ID NO: 376)
	AA	DVVMTHTPASLFETVGGTVTIKCQALQSVYDNNALSWYQ QNAGQRPILLIYGASTLASGAPSRFSASGSGTDFTLTIIDLEC ADASTYYCQCTYYVSSYQNDFGGGTEVVVK (SEQ ID NO: 378)	QSLEESGGGLVKPGASLTLTCKASGSDLSRYYYSCWIRQAPGKGLEWV ACVGGVNRIDATAYATWAKGRFTISKTSSTTVTLQMTSLTAADTATYFC VRGGPDNNVQFNLWGPGTLVTVSS (SEQ ID NO: 371) CAGGAGCAGCTGGTGGAGTCCGGGGGAGGCCTGGTCCAGCCTAAGG GATCCCTGACACTCACCTGCGCAGCTTCTGGATTCTCCTTCAGTAGCA GCTACTTCATGTGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAG TGGATCGCATGCATTGGTTCTGGTAGTAGTGCTATTAGCACTTTCTAC GCGAGCTGGGCCGCAAGGCCGATTCACCATCTCCAAAACCTCGTCGA CCACGGTGACTCTGCAAATGACCAGTCTGACACAGCCGCGGACACGGC CACCTATTTCTGTGCGAGAGTGGGCTACGATGACTATGGTGACTCTG ATGCTTTTGATCCCTGGGGCCCAGGCACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 373) QEQLVESGGGLVQPKGSLTLTCAASGFSFSSSYFMCWVRQAPGKGLEW IACIGSGSSAISTFYASWAQGRFTISKTSSTTVTLQMTSLTAADTATYFCA RVGYDDYGDSDAFDPWGPGTLVTVSS (SEQ ID NO: 375) CAGTCGCTGGAGGAGTCCGGGGGTCGCCTGGTAACGCCTGGGACACCC CTGACACTCACCTGCACAGTCTCTGGAATCGACCTCAGTAGCTATGGA ATGACTTGGGTCCGCCAGGCTCCAGGGGAAGGGGCTGGAGTGGATCGG ATACATTTGGACTGATGGGAGGACATACTACGCAAACTGGGCGAAAG GCCGATTCACCATCTCCAAAACCTCGACCACAGTGGATCTCAAGATCA CCAGTCCGACAGCCGAGGACACGGCCACCTATTTCTGTGCCAGACCCT TTGATGGTAATTATAGGGACATCTGGGGCCCAGGCACCCTGGTCACG GTCTCCTTA (SEQ ID NO: 377) QSLEESGGRLVTPGTPLTLTCTVSGIDLSSYGMTWVRQAPGKGLEWIGY IWTDGRTYYANWAKGRFTISKTSTTVDLKITSPTAEDTATYFCARPFDG NYRIDWGPGTLVTV SL (SEQ ID NO: 379)

1G24	NA	GACATTGTGATGACCCAGACTCCAGCCTCCGTGGAGGC AGCTGTGGTGGAGGCACAGTCACCATCAAGTGCCAGGCCA GTGAGAGCATTGGCAATTGCATTAGCCTGGTATCAGCAGA AACCAGGGCAGCCTCCCAGTCTCCTGATCTACAGGGCA TCCACTCTGGCATCTGGAATCCCATCGCGGGTCAAAGG CAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCGA CCTGGAGTGTGCCGATGCTGCCACTTACTATTGTCAAAGC TATGATGATAGTAGTAGTAGTTTTTTTTATGCTTTCG GCGGA GGGACCGAGGTGGTGGTCAAA (SEQ ID NO: 380)
	AA	DIVMTQTPASVEAAVGGTVTIKCQASESIGNALAWYQQKP GQPPSLLIYRASTLASGIPSRVKGSGSGTQFTLTISDLECADA ATYYCQSYDDSSSSSFFYAFGGGTEVVVK (SEQ ID NO: 382)
4A7	NA	GCCGCCGTGCTGACCCAGACTCCATCTCCGTGTCTGCAG CTGTGGGAGGCACAGTCAGCATCAGTTGCCAGTCCAGTA AGAGTGTTTATAATAAGAACTGGTTATCCTGGTTTCAGC AGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGGTG CATCCACTCTGGGCATCTGGGGTCCCATCGCGGTTCAAAG GCAGTGGATCTGGGACACAGTTCACTCTCACCATCAGCG GCGTGCAGTGTGACGATGCTGCCACTTACTACTGTGCAG GCGGTTATAGTAGTAGTAGTGATACGTTTGCTTTCGGCG GAGGGACCGAGGTGGTGGTCAAA (SEQ ID NO: 384)
	AA	AAVLTQTPSPVSAAVGGTVSISCQSSKSVYNKNWLSWFQ QKPGQPPKLLIYGASTLASGVPSRFKGSGSGTQFTLTISGV QCDDAATYYCAGGYSSSSDTFAFGGGTEVVVK (SEQ ID NO: 386)

CLAIMS

1. An isolated antigen binding construct that binds MAGEB2, wherein the binding construct binds to an epitope comprising a sequence selected from SEQ ID NOs: 2, 3, 4, or 388 - 554.

2. The isolated antigen binding construct of claim 1, wherein the antigen binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 85; the CDRL2 comprises a sequence set forth in SEQ ID NO: 86; the CDRL3 comprises a sequence set forth in SEQ ID NO: 87; the CDRH1 comprises a sequence set forth in SEQ ID NO: 229; the CDRH2 comprises a sequence set forth in SEQ ID NO: 230; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 231.

3. The isolated antigen binding construct of claim 1, wherein the antigen binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 73; the CDRL2 comprises a sequence set forth in SEQ ID NO: 74; the CDRL3 comprises a sequence set forth in SEQ ID NO: 75; the CDRH1 comprises a sequence set forth in SEQ ID NO: 217; the CDRH2 comprises a sequence set forth in SEQ ID NO: 218; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 219.

4. The isolated antigen binding construct of claim 1, wherein the antigen binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 91; the CDRL2 comprises a sequence set forth in SEQ ID NO: 92; the CDRL3 comprises a sequence set forth in SEQ ID NO: 93; the CDRH1 comprises a sequence set forth in SEQ ID NO: 235; the CDRH2 comprises a sequence set forth in SEQ ID NO: 236; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 237.

5. The isolated antigen binding construct of claim 1, wherein the antigen binding construct comprises a light chain variable region comprising a sequence set forth in SEQ ID NO: 346 and a heavy chain variable region comprising a sequence set forth in SEQ ID NO: 347.

6. The isolated antigen binding construct of claim 1, wherein the antigen binding construct comprises a light chain variable region comprising a sequence set forth in SEQ ID NO: 338 and a heavy chain variable region comprising a sequence set forth in SEQ ID NO: 339.

7. The isolated antigen binding construct of claim 1, wherein the antigen binding construct comprises a light chain variable region comprising a sequence set forth in SEQ ID NO: 350 and a heavy chain variable region comprising a sequence set forth in SEQ ID NO: 351.

8. The isolated antigen binding construct of any of claims 1-7, wherein the antigen binding construct is a monoclonal antibody or an antibody fragment thereof.

9. The isolated antibody of claim 8, wherein the antibody is of the IgG1 or the IgG2 isotype.

10. The isolated antigen binding construct of any of claims 1-9, wherein the antigen binding construct is coupled to a labeling group.

11. A nucleic acid molecule encoding the antigen binding construct, or fragment thereof, according to any of claims 1-9.

12. A vector comprising a nucleic acid molecule according to claim 11.

13. A host cell comprising the nucleic acid molecule according to claim 12.

14. An antigen binding construct, or fragment thereof, produced by the host cell of claim 13.

15. A method of making the antigen binding construct, or fragment thereof, according to any of claims 1-9, comprising the step of preparing the antibody or fragment thereof from a host cell that secretes the antibody.

16. An isolated antibody or fragment thereof that competes for binding to MAGEB2 with an antibody or fragment thereof of any of claims 1-9.

17. A method of making an antibody that binds to MAGEB2 comprising immunizing an animal with a peptide comprising a sequence selected from SEQ ID NO: 2, 3, or 4, and isolating from said animal antibodies that bind to MAGEB2.

18. An antibody made by the method of claim 17.

19. A method for treating a tumor in a subject, said method comprising: determining the subject as responsive to treatment with an anti-MAGEB2 therapeutic by obtaining a sample from the subject, wherein the sample comprises a cell from the tumor, measuring the level of MAGEB2 in the sample using an antigen binding construct of any of claims 1-9, and determining the subject as responsive to treatment with an anti-MAGEB2 therapeutic, and administering to the subject an effective amount of the anti-MAGEB2 therapeutic.

20. A method of identifying a subject as needing an anti-MAGEB2 thereapeutic comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct of any of claims 1-9; and b) identifying the subject as needing the anti-MAGEB2 therapeutic when the level of MAGEB2 is increased relative to a control.

21. A method of determining treatment for a subject with a MAGEB2 positive tumor comprising: determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct of any of claims 1-9; and determining the treatment as comprising an anti-MAGEB2 therapeutic when the level of MAGEB2 is increased, relative to a control.

22. A method of determining efficacy of treatment with an anti-MAGEB2 therapeutic in a subject comprising: determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct of any of claims 1-9 before treatment with an anti-MAGEB2 therapeutic and after treatment with an anti-MAGEB2 therapeutic; and determining the treatment as effective when the level of MAGEB2 positive tumor cells is decreased after treatment with the anti-MAGEB2 therapeutic.

23. A method of diagnosing a subject with a tumor, comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct of any of claims 1-9; and b) diagnosing the subject as having a MAGEB2 positive tumor when the level of MAGEB2 is increased relative to a control.

24. A method of identifying a subject having a MAGEB2 positive tumor comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct of any of claims 1-9; and b) identifying the subject as having a MAGEB2 positive tumor when the level of MAGEB2 is increased relative to a control.

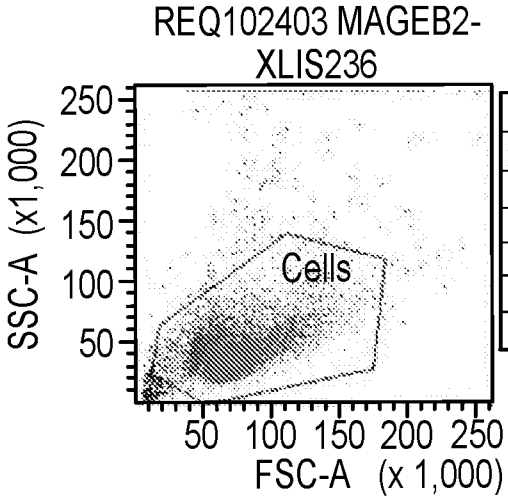
25. A method of identifying a subject as needing an anti-MAGEB2 therapeutic comprising: a) determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct of any of claims 1-9; and b) identifying the subject as needing the anti-MAGEB2 therapeutic when the level of MAGEB2 is increased relative to a control.

26. A method of determining treatment for a subject with a MAGEB2 positive tumor comprising: determining the level of MAGEB2 in a sample obtained from the subject using an antigen binding construct of any of claims 1-9; and determining the treatment as comprising an anti-MAGEB2 therapeutic when the level of MAGEB2 is increased, relative to a control.

27. The method of any of claims 19-26, wherein MAGEB2 is detected using an IHC assay.

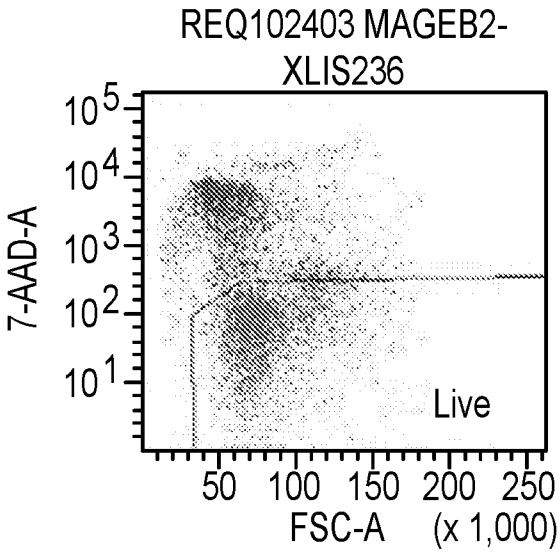
FIG. 1

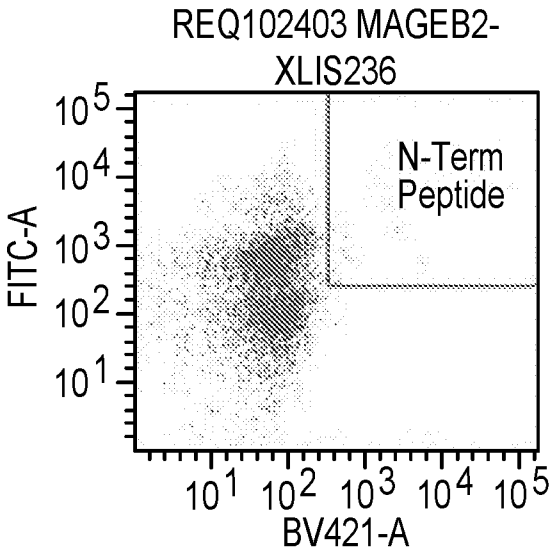
Gating Hierarchy Used in the Enrichment of MAGEB2 N-Term Helix, N-Term Peptide, and Middle Loop Region Peptide Binders

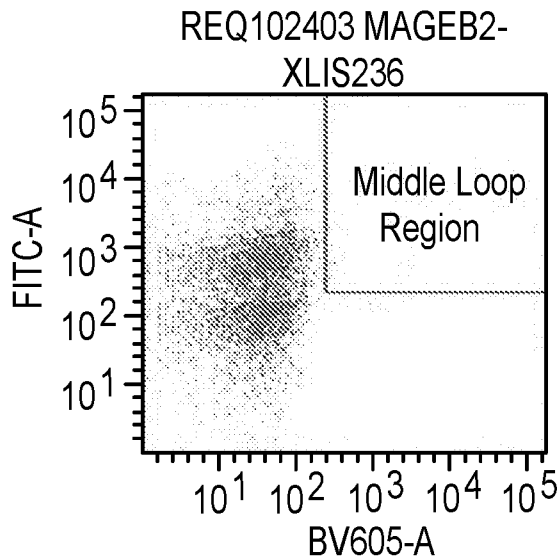


Tube: XLIS236

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
Cells	8,998	90.0	90.0
Live	5,779	64.2	57.8
N-Term Peptide	18	0.3	0.2
Middle Loop Region	7	0.1	0.1
N-Term Helix	15	0.3	0.2







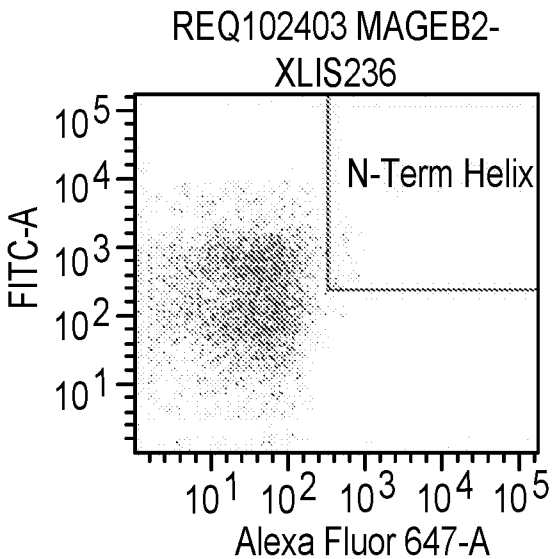
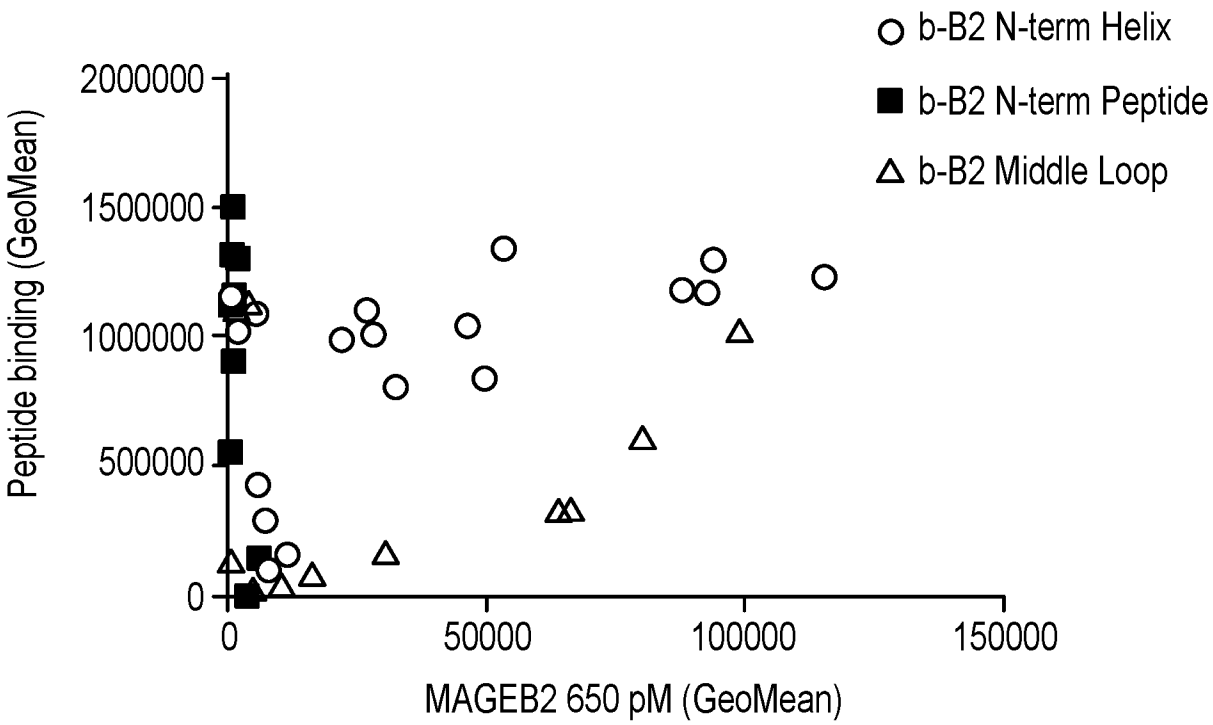


FIG. 2

ANTIBODIES THAT BIND TO FULL LENGTH MAGEB2 PROTEIN



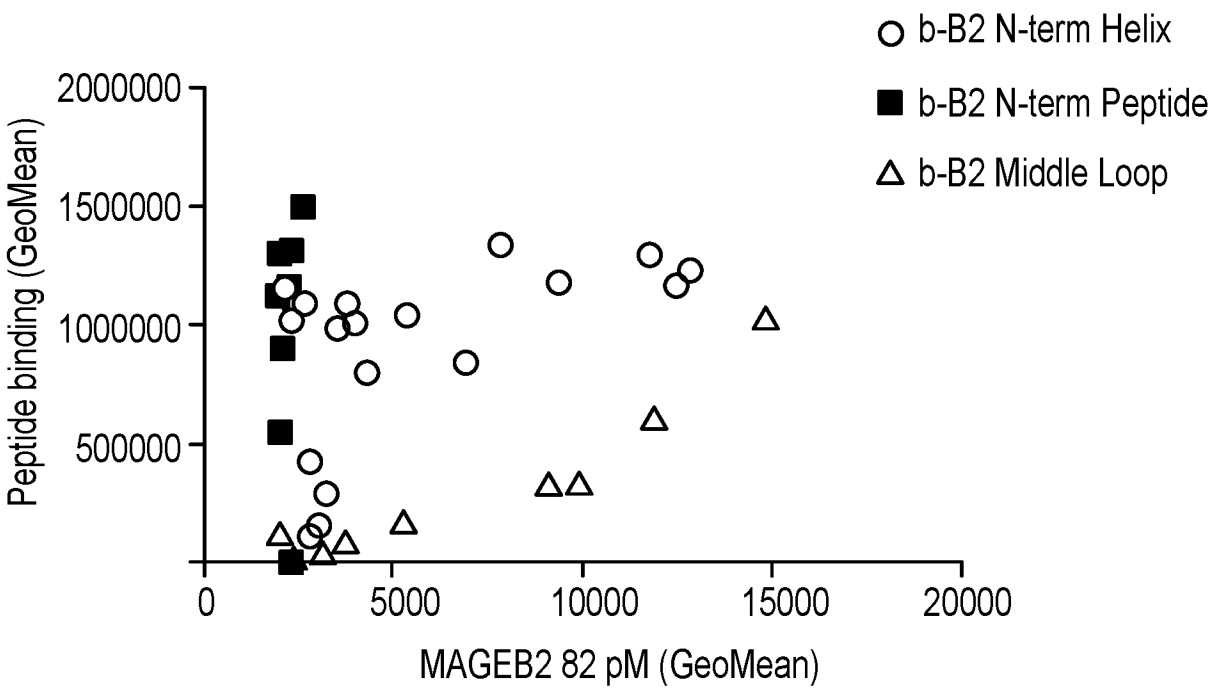


FIG. 3

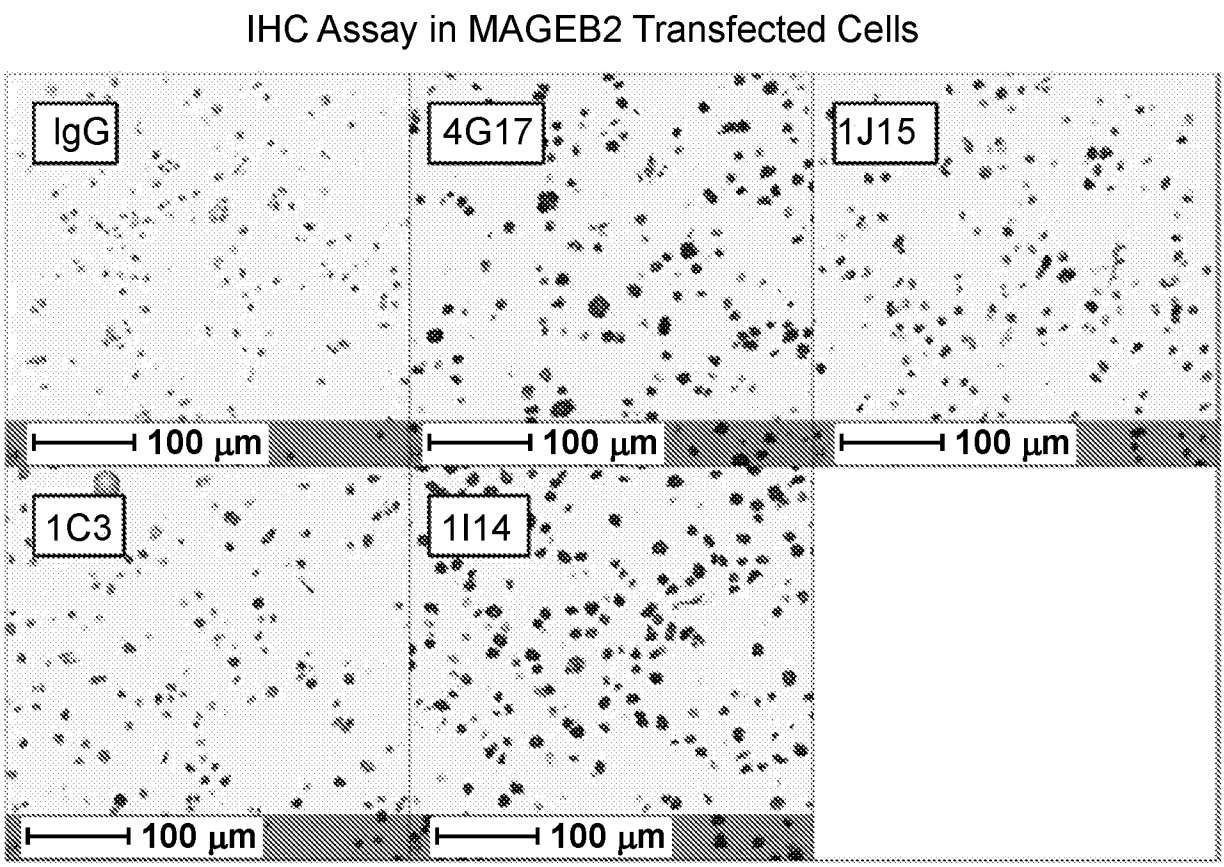

IHC Assay in MAGEB2 Transfected Cells
IgG
4G17
1J15
1C3
1I14
100 µm
100 µm
100 µm
100 µm
100 µm

FIG. 4

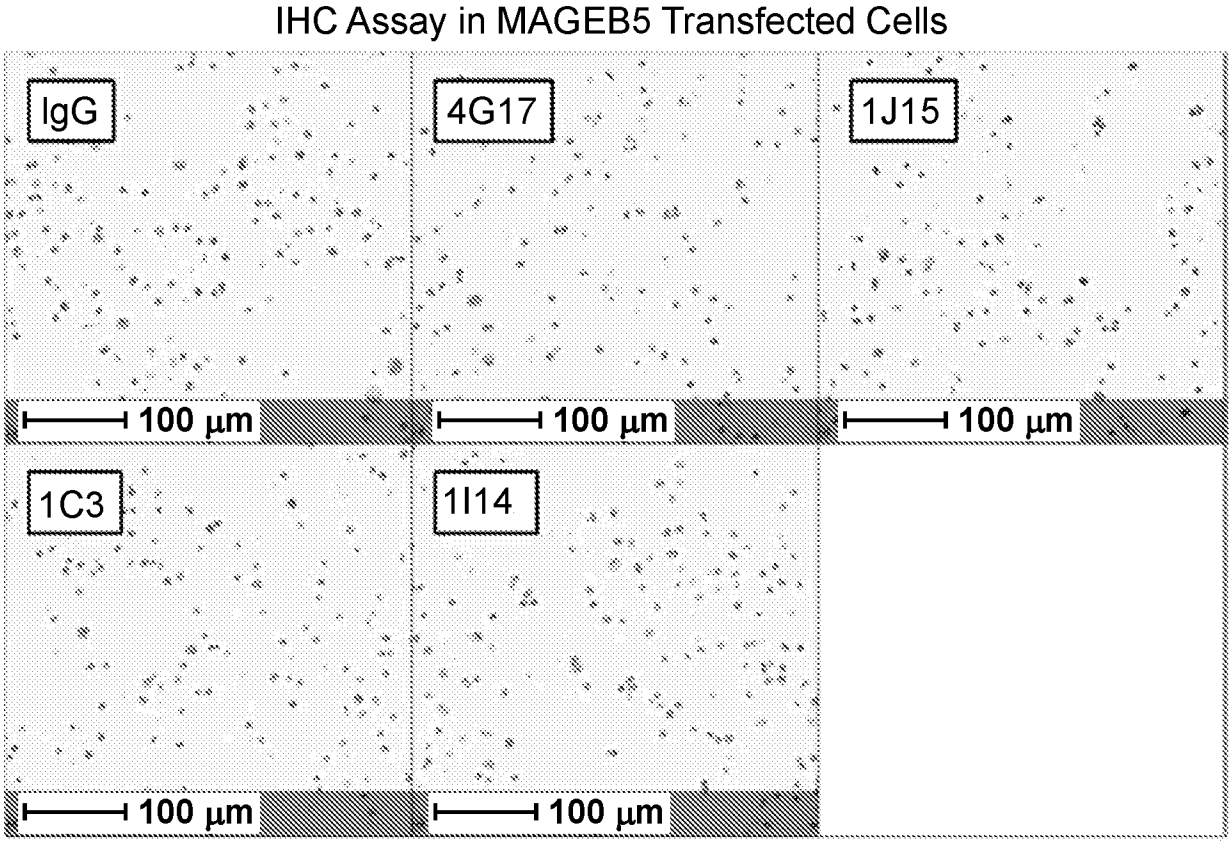

IHC Assay in MAGEB5 Transfected Cells
IgG
4G17
1J15
1C3
1I14
100 µm
100 µm
100 µm
100 µm
100 µm

FIG. 5

IHC Assay in Control Testis Tissue

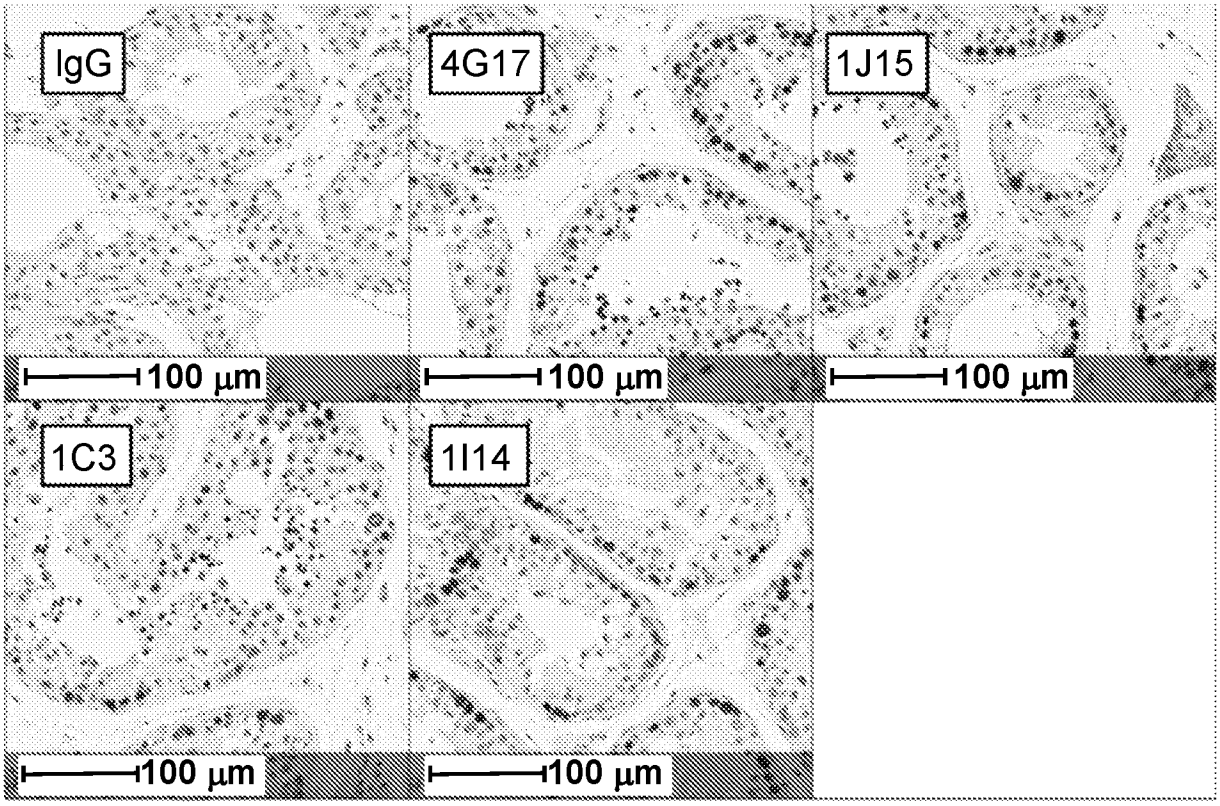


FIG. 6

IHC Assay in Normal Human Tissue

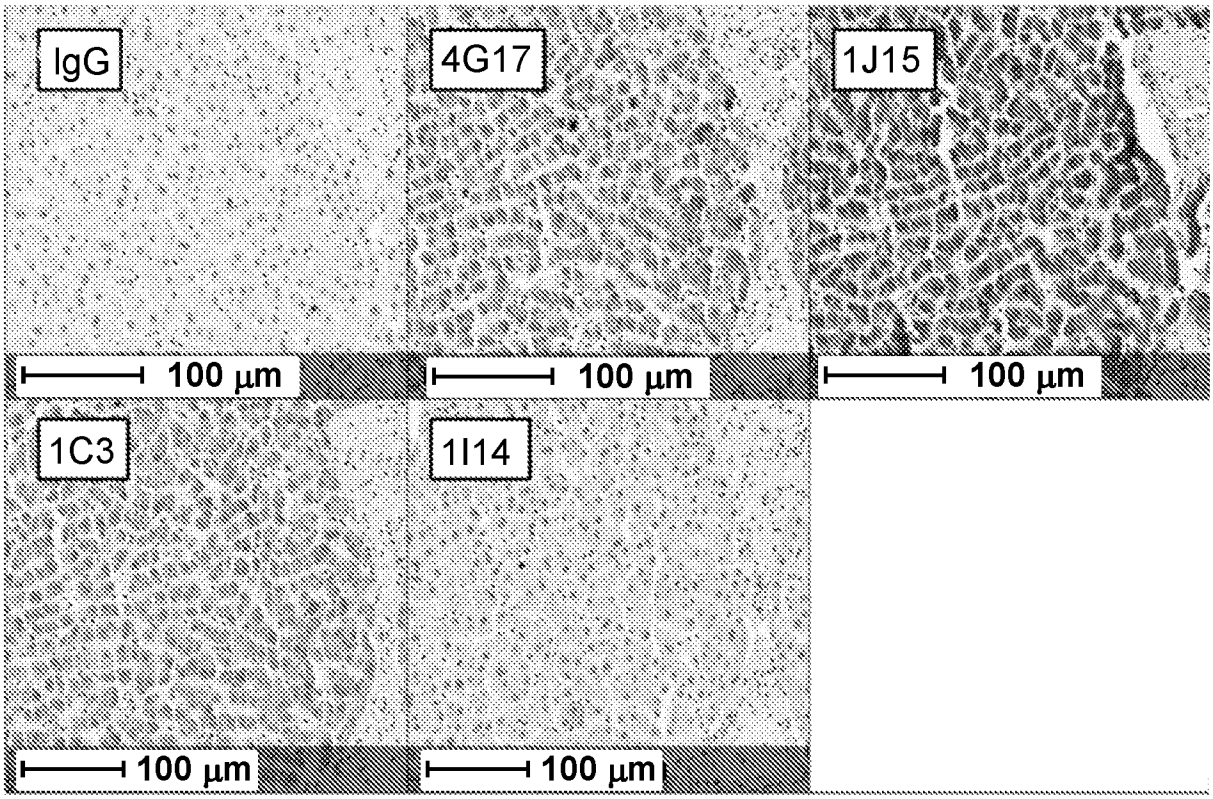
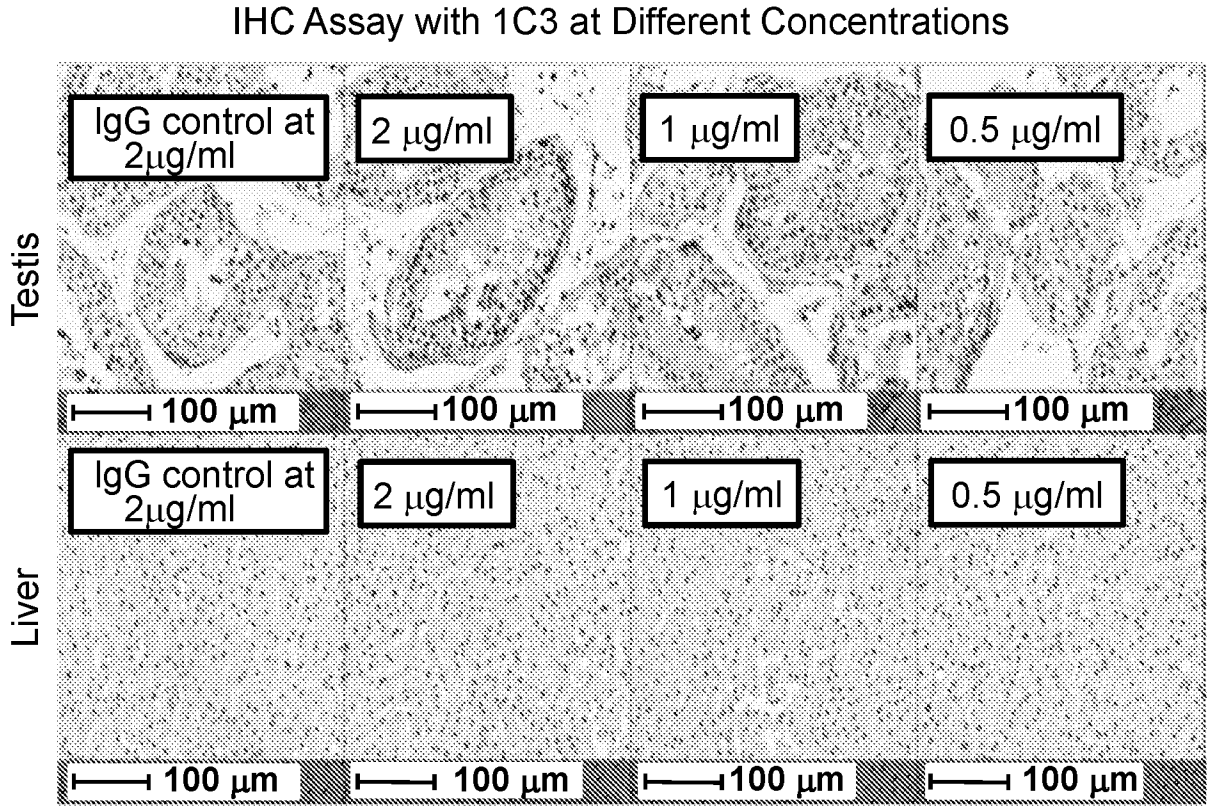


FIG. 7


IHC Assay with 1C3 at Different Concentrations
IgG control at 2μg/ml
2 μg/ml
1 μg/ml
0.5 μg/ml
Testis
Liver
100 μm

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/023034

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/30
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

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, CHEM ABS Data, 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.
X	JUNG-HEE LEE ET AL: "Identification, Expression, and Nuclear Location of Murine Mage-b2 Protein, a Tumor-associated Antigen", MOLECULES AND CELLS, SEOUL, KR, vol. 10, no. 6, 1 January 2001 (2001-01-01), pages 647-653, XP019361382, ISSN: 1016-8478 abstract, page 650, figures 1, 3, 4 -----	1,2,5, 8-15, 17-27
Y	WO 2019/204683 A1 (UNIV TEXAS [US]) 24 October 2019 (2019-10-24) paragraphs [0091]- [0093], examples, claims ----- -/--	1,2,5, 8-15, 17-27

[x] Further documents are listed in the continuation of Box C.

[x] 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
30 June 2022	31/08/2022

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	Bernhardt, Wiebke

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

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/023034

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PATTANI KAVITA M. ET AL: "MAGEB2 is Activated by Promoter Demethylation in Head and Neck Squamous Cell Carcinoma", PLOS ONE, vol. 7, no. 9, 24 September 2012 (2012-09-24), page e45534, XP055936905, DOI: 10.1371/journal.pone.0045534 Retrieved from the Internet: URL:https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0045534&type=printable> abstract, figures 4, 5	1,2,5, 8-15, 17-27
Y	RAMANATHAN SAUMYA ET AL: "Cause and Consequence of MAGEB2 expression in cancer", FASEB JOURNAL, vol. 34, no. Suppl. S1, April 2020 (2020-04), XP009537050, & ANNUAL MEETING ON EXPERIMENTAL BIOLOGY; SAN DIEGO, CA, USA; APRIL 04 -07, 2020 abstract	1,2,5, 8-15, 17-27
A	S. BUUS ET AL: "High-resolution Mapping of Linear Antibody Epitopes Using Ultrahigh-density Peptide Microarrays", MOLECULAR & CELLULAR PROTEOMICS, vol. 11, no. 12, 1 December 2012 (2012-12-01), pages 1790-1800, XP055247517, US ISSN: 1535-9476, DOI: 10.1074/mcp.M112.020800 abstract	1
A	GERSHONI JONATHAN M ET AL: "Epitope mapping - The first step in developing epitope-based vaccines", BIODRUGS, ADIS INTERNATIONAL LTD, NZ, vol. 21, no. 3, 1 January 2007 (2007-01-01), pages 145-156, XP009103541, ISSN: 1173-8804, DOI: 10.2165/00063030-200721030-00002 pages 148-149	1

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/023034

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUDIKOFF S ET AL: "Single amino acid substitution altering antigen-binding specificity", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, vol. 79, no. 6, 1 March 1982 (1982-03-01), pages 1979-1983, XP002683593, ISSN: 0027-8424, DOI: 10.1073/PNAS.79.6.1979 abstract ----- WINKLER K ET AL: "Changing the antigen binding specificity by single point mutations of an anti-p24 (HIV-1) antibody", THE JOURNAL OF IMMUNOLOGY, WILLIAMS & WILKINS CO, US, vol. 165, no. 8, 15 October 2000 (2000-10-15), pages 4505-4514, XP002579393, ISSN: 0022-1767 abstract -----	1,2,5, 8-15, 17-27 1,2,5, 8-15, 17-27

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2022/023034

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [ ] forming part of the international application as filed:

      [ ] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/023034

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. [X] Claims Nos.: 16
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:
see FURTHER INFORMATION sheet PCT/ISA/210

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. [X] 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.:
2, 5(completely); 1, 8-15, 17-27(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: 2, 5(completely); 1, 8-15, 17-27(partially)

An isolated antigen binding construct that binds MAGEB2, wherein the binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 85; the CDRL2 comprises a sequence set forth in SEQ ID NO: 86; the CDRL3 comprises a sequence set forth in SEQ ID NO: 87; the CDRH1 comprises a sequence set forth in SEQ ID NO: 229; the CDRH2 comprises a sequence set forth in SEQ ID NO: 230; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 231; products and methods related thereto

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2. claims: 3, 6(completely); 1, 8-15, 17-27(partially)

An isolated antigen binding construct that binds MAGEB2, wherein the binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 73; the CDRL2 comprises a sequence set forth in SEQ ID NO: 74; the CDRL3 comprises a sequence set forth in SEQ ID NO: 75; the CDRH1 comprises a sequence set forth in SEQ ID NO: 217; the CDRH2 comprises a sequence set forth in SEQ ID NO: 218; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 219; products and methods related thereto

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3. claims: 4, 7(completely); 1, 8-15, 17-27(partially)

An isolated antigen binding construct that binds MAGEB2, wherein the binding construct comprises a CDRL1, a CDRL2, a CDRL3, a CDRH1, a CDRH2, and a CDRH3, wherein the CDRL1 comprises a sequence set forth in SEQ ID NO: 91; the CDRL2 comprises a sequence set forth in SEQ ID NO: 92; the CDRL3 comprises a sequence set forth in SEQ ID NO: 93; the CDRH1 comprises a sequence set forth in SEQ ID NO: 235; the CDRH2 comprises a sequence set forth in SEQ ID NO: 236; and the CDRH3 comprises a sequence set forth in SEQ ID NO: 237; products and methods related thereto

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 16

Claim 16 defines the antibody as "compet[ing] for binding to MAGE2B with an antibody or fragment thereof of any of claims [...] ". This definition is considered as an unusual parameter. As the preceding antibodies are supposedly novel, the prior art will not provide any comparative data with those antibodies. Consequently, in addition to the fact that such unusual parameters might disguise novelty, it is impossible to carry out a search in the prior art for an antibody which competes with an hitherto unknown antibody.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/023034

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2019204683 A1	24-10-2019	CA 3097399 A1	24-10-2019
		CN 112236447 A	15-01-2021
		EP 3784255 A1	03-03-2021
		JP 2021521776 A	30-08-2021
		KR 20210003767 A	12-01-2021
		US 2021363215 A1	25-11-2021
		WO 2019204683 A1	24-10-2019