

ANTIBODIES

Field of Invention

5 The present invention relates to antibodies which are specific for CD45. The antibodies include antibodies which are monospecific or biparatopic for CD45. The antibodies may be, for example, used to kill or deplete CD45-positive target cells, particularly for the treatment of cancer or autoimmune diseases which are mediated by CD45-positive cells or prior to the transplant of cells.

10 Background of Invention

CD45, the first and prototypic receptor-like protein tyrosine phosphatase, is expressed on nucleated hematopoietic cells and plays a central role in the regulation of cellular responses. CD45 has also been known as PTPRC, T200, Ly5, leucocyte
15 common antigen (LCA), and B220. CD45 is the most abundant cell surface protein expressed on the surface of T and B cells. It is essential for B and T cell development and activation. Studies of CD45 mutant cell lines, CD45-deficient mice, and CD45-deficient humans initially demonstrated the essential role of CD45 in T and B cell antigen receptor signal transduction and lymphocyte development. It is now known that CD45 also
20 modulates signals emanating from integrin and cytokine receptors. In contrast to its positive role in antigen receptor signalling, CD45 acts as a negative regulator of integrin mediated signalling for instance in macrophages. CD45 may also play a role in regulating haematopoiesis and interferon-dependent antiviral responses. CD45 can also play a role in cell survival.

25 CD45 comprises a highly and variably glycosylated extracellular domain of approximately 400 to 550 amino acids, followed by a single transmembrane domain and a long intracellular domain of 705 amino acids, containing two tandemly repeated phosphatase domains. The regulation of CD45 expression and the expression of multiple alternative splicing isoforms (which alternatively splice exons 4, 5 and 6 from the CD45
30 gene and are designated A, B and C) critically regulates phosphatase activity and differential signal transduction. CD45 affects cellular responses by controlling the relative threshold of sensitivity to external stimuli. Perturbation of this function may contribute to autoimmunity, immunodeficiency, and malignancy.

All CD45 isoforms display tyrosine phosphatase activity which is mediated by the cytoplasmic domain of the molecule comprising the two tandem repeats of phosphatase domains D1 and D2, with each containing a highly conserved HC(X)₅R motif. All of the tyrosine phosphatase activity of CD45 is thought to arise from the D1 domain, with the
5 D2 domain possibly involved in regulation. One of the primary targets for CD45 tyrosine phosphatase are Src-family kinases, reflecting the role of CD45 in cell signalling. Depending on where the phosphatase activity of CD45 acts it may activate or down-regulate the activity of such Src-family kinases.

CD45 is an attractive target for the treatment of cancer and other therapeutic
10 settings. Given the importance of CD45, there is an ongoing need for agents that can target CD45 and be used for the treatment of the above diseases.

Summary of the Invention

15 The present invention provides antibodies specific for CD45. The antibodies provided include monospecific and biparatopic antibodies specific for CD45. Both may be used in the killing and/or depletion of cells expressing CD45.

In particular, the invention provides an antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the
20 following light and heavy chain variable regions:

(a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 33, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 35 and 39 to 42; and

(b) a heavy chain variable region comprising a CDR1 comprising a sequence
25 selected from any one of SEQ ID NOs: 46, 52, 53 and 54, a CDR2 comprising a sequence selected from any one of SEQ ID NOs: 47, 55, 56, and 57, and a CDR3 comprising the sequence of SEQ ID NO: 48.

The invention also provides an antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light
30 and heavy chain variable regions:

(a') a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96; and

(b') a heavy chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 102 to 105.

The light and heavy chain variable regions may be humanised. The antibody or antigen-binding fragment may be biparatopic by way of comprising a first variable domain specific for CD45 from the first group of antibodies set forth above (i.e. the antibodies with the (a) and (b) chains) and a second variable domain specific for CD45 from the second group of antibodies set forth above (i.e. the antibodies with the (a') and (b') chains). A constant region of the antibody or antigen-binding fragment thereof may comprise a modification or modifications to reduce or eliminate binding to an Fc receptor. The antibody or antigen-binding fragment may bind specifically to both human CD45 and cynomolgus monkey CD45.

The invention also provides a pharmaceutical composition comprising an antibody as set forth herein and a pharmaceutically acceptable carrier or diluent. The pharmaceutical composition may be used for killing or depleting CD45-expressing cells in a subject, for example in treating a blood cancer, such as leukaemia, lymphoma or multiple myeloma.

Brief Description of the Figures

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Figure 1 compares the ability of monospecific (monoclonal) 17415, 17552 and 4133 antibodies to kill human PBMCs (Peripheral Blood Mononuclear Cells) expressing CD45. A VR5604 isotype control antibody not binding to CD45 was also included.

Figure 2 provides sequence alignments for the various sequences involved in the humanisation of the original rabbit 17415 antibody light chain variable region. The CDRs of the rabbit antibody were grafted onto either a human IGKV1-9 acceptor framework (top set of alignments) or an IGKV4-1 acceptor framework (bottom set of alignments). A number of donor framework residues were also transferred and some CDR modifications included. The CDRs are shown in bold and underlined. Amino acid residues that diverge from the original CDRs or the human donor frameworks are shown shaded in bold and italics. The graft variants generated are shown below the acceptor sequences.

Figure 3 provides sequence alignments for the various sequences involved in the humanisation of the original rabbit 17415 antibody heavy chain variable region. The CDRs of the rabbit antibody were grafted onto a human IGHV3-72 acceptor framework.

A number of donor framework residues were also transferred and some CDR modifications also included. The CDRs are shown in bold and underlined. Amino acid residues that diverge from the original CDRs or the human donor frameworks are shown shaded in bold and italics. The graft variants generated are shown below the acceptor
5 sequence.

Figure 4 provides sequence alignments for the various sequences involved in the humanisation of the original rabbit 17552 antibody, with the top set of alignments for the light chain humanisation onto a human IGKV1-8 acceptor framework and the bottom set of alignments for the heavy chain humanisation into a human IGHV4-4 acceptor
10 framework. A number of donor framework residues were also transferred and some CDR modifications also included. The CDRs are shown in bold and underlined. Amino acid residues that diverge from the original CDRs or the human donor frameworks are shown shaded in bold and italics. The graft variants generated are shown below the acceptor sequences.

Figure 5 shows the results of a human lymphocyte depletion assays comparing:
15 **(A)** 17415 IgG LALA heavy chain graft variant H1 paired with 17415 light chain graft variants L1 to L5, and **(B)** 17415 IgG LALA heavy chain graft variants H1 to H4 paired with 17415 light chain graft variant L1.

Figure 6 shows the results of human lymphocyte assays for: **(A)** 17415 light chain
20 graft variants L1 to L5 paired with the chimeric 17415 heavy chain comprising the original rabbit 17415 heavy chain variable region versus chimeric 17415 light and heavy chains comprising the original rabbit 17415 light and heavy chain variable regions, and **(B)** a chimeric 17415 light chain comprising the original rabbit 17415 light chain variable region paired with 17415 heavy chain graft variants H1 to H4 versus chimeric 17415
25 light and heavy chains comprising the original rabbit 17415 light and heavy chain variable regions.

Figure 7 shows the results of human T cell depletion assays for: **(A)** 17415 heavy
chain graft variant H5 paired with 17415 light chain graft variants L6, L7, L13, L14,
L15, and L16, and **(B)** 17415 heavy chain graft variant H6 paired with the same light
30 chain graft variants.

Figure 8 shows the results for human Jurkat cell killing for antibodies in the IgG1 LALA format as follows: **(A)** 17415 light chain variable region graft variants L1 to L5 paired with 17415 heavy chain graft variant H1, **(B)** 17415 light chain variable region graft variant L1 paired with 17415 heavy chain graft variants H1 to H4, **(C)** a 17415

chimeric light and heavy chain antibody comprising the original variable regions of the original rabbit 17415 antibody was compared with antibodies having the chimeric light chain paired with each of 17415 heavy chain graft variants H1 to H4, and **(D)** a 17415 chimeric light and heavy chain antibody comprising the original variable regions of the original rabbit 17415 antibody was compared with antibodies having the 17415 light chain variants L1 to L5 paired with the chimeric heavy chain.

Figure 9 shows the results for a human T cell depletion assay for IgG1 LALA antibodies comprising 17415 light chain graft variants 7, 15 or 16 and 17415 heavy chain graft variant H6; and 17552 light chain graft variant 1 and 17552 heavy chain graft variants 1 or 4.

Figure 10 shows the results for a human Jurkat cell depletion assay for IgG1 LALA antibodies comprising 17415 light chain graft variants 7, 15 or 16 and 17415 heavy chain graft variant H6.

Figure 11 shows results for binding of humanised 17415 IgG1 LALA grafts containing 17415 light chain graft variants 7, 15, and 16 paired with 17415 heavy chain graft variant 6 to human CD45

Figure 12 shows results for binding to human CD45 of various biparatopic 17415/17552, 17415/5604 and 5604/17552 antibody variants and monospecific 5604 antibody for the various antibodies indicated including those with 17415 light chain graft variants 7 **(A)**, 15 **(B)**, and 16 **(C)**.

Figure 13 shows results for binding to cyno CD45 of various biparatopic 17415/17552, 17415/5604 and 5604/17552 antibody variants and monospecific 5604 antibody for the various antibodies indicated including those with 17415 light chain graft variants 7 **(A)**, 15 **(B)**, and 16 **(C)**.

Figure 14 shows the results of a human T cell depletion assay for various biparatopic 17415/17552, 17415/5604 and 5604/17552 antibody variants comprising light chain graft variants 7 **(A)**, 15 **(B)**, and 16 **(C)**.

Figure 15 shows the results of a human Jurkat cell depletion assay for various biparatopic 17415/17552, 17415/5604 and 5604/17552 antibody variants comprising light chain graft variants 7 **(A)**, 15 **(B)**, and 16 **(C)**.

Figure 16 shows the results of a cynomolgus T cell depletion assay for 17415 and 17552 monospecific (monoclonal) IgG1 antibodies compared to the biparatopic 17415/17552 IgG1 antibody and the biparatopic 4133/6294 IgG1 antibody.

Figure 17 shows the comparison of the ability of biparatopic 17415-17552 IgG1 and 4133-6294 IgG1 antibodies to kill human PBMCs (Peripheral Blood Mononuclear Cells) expressing CD45. A VR5604 isotype control antibody (not binding to CD45) was also included.

5 **Figure 18** shows the comparison of the ability of biparatopic 17415-17552 IgG1 and YTH24.5-YTH54.12 IgG1 antibodies to reduce T cells numbers in a PBMC population. The results for an isotype (5604 IgG1) control are also included.

10 **Figure 19** shows the results for human Jurkat cell killing for biparatopic 4133-6294 IgG1 and 17415-17552 IgG1 antibodies. A VR5604 isotype control antibody (not binding to CD45) was also included.

15 **Figure 20** shows levels of induction of cytokines: (A) IFN γ , (B) IL-6 and (C) TNF α in whole blood by the biparatopic 17415gL15gH6-17552gL1gH4 IgG1 LALA antibody, or the monospecific (monoclonal) 17415gL15gH6 IgG1 LALA antibody, compared to controls with irrelevant specificity (5604 IgG1 LALA), Campath or PBS.

20 **Figure 21** shows a comparison of the ability of biparatopic 17415-17552 IgG1 and YTH24.5-YTH54.12 IgG1 antibodies to kill Jurkat cells. A VR5604 isotype control antibody (not binding to CD45) was also included.

25 **Figure 22** shows the percentage reduction of T-cells in T-cell lines (A) Peers, (B) SUPT11, and (C) SUDHL1 for biparatopic 17415gL15gH6-17552gL1gH4 IgG1 LALA and monospecific (monoclonal) 17415gL15gH6 IgG1 LALA antibodies.

30 **Figure 23** shows the percentage reduction of B-cells in B-cell lines (A) Ramos and (B) DOHH2 for biparatopic 17415gL15gH6-17552gL1gH4 and monospecific (monoclonal) 17415gL15gH6 IgG1 LALA antibodies.

35 **Figure 24** shows the percentage reduction of T-cells in PBMC derived from (A) healthy volunteers 336BB + 330CD, and from (B) a T-cell leukaemia patient 4368POS by either the biparatopic 17415gL15gH6-17552gL1gH4 IgG1 LALA antibody or monospecific (monoclonal) 17415gL15gH6 IgG1 LALA antibody compared with a control.

40 **Figure 25** shows the percentage reduction of B-cells in PBMC derived from (A) healthy volunteers 336BB + 330CD, and from (B) a B-cell leukaemia patient 4650ADG by either the biparatopic 17415gL15gH6-17552gL1gH4 IgG1 LALA antibody or monospecific (monoclonal) 17415gL15gH6 IgG1 LALA antibody compared with a control.

Figures 26 and 27 provide respectively a summary of experimental results for some of the preferred monospecific and biparatopic antibodies of the invention.

Figures 28, 29 and 30 provide respectively the amino acid sequence of the full length human CD45 (SEQ ID NO: 127), the extracellular domains D1 to D4 of human CD45 (SEQ ID NO: 128), and full length cynomolgus monkey CD45 (SEQ ID NO: 129).

Figure 31 provides the full heavy chain (top) and light chain (bottom) sequences for the VR17415gL15gH6 IgG1 LALA antibody, with the constant region sequences of each shown in italics. The heavy chain constant region can be subdivided, going from N terminus to C terminus, into the CH1 region (underlined), Hinge region (not underlined), CH2 region (underlined), and the CH3 region (not underlined).

Figure 32 shows the full heavy and light chain sequences for the VR17415gL15gH6 x VR17552gL1gH4 IgG1 LALA biparatopic antibody. The heavy and light chain sequences for the VR17415gL15gH6 are the top and second from top sequences shown. The heavy and light chain sequences for the VR17552gL1gH4 specificity are shown as the second from bottom and bottom sequences. For all of the sequences the constant region sequence is shown in italics. The heavy chain constant regions can be subdivided, going from N terminus to C terminus, into the CH1 region (underlined), Hinge region (not underlined), CH2 region (underlined), and the CH3 region (not underlined). The two heavy chains have “knobs-into-holes” modifications with the mutations for that shown shaded.

Figure 33 provides an example of preferred heavy and light chain constant region sequences to be employed in an antibody of the present invention, with the constant region being an IgG1 LALA modified constant region. The Table provides the amino acid sequence for the heavy chain with and without “knobs-into-holes” modifications. The “knobs-into-holes” modifications may be employed in the case of biparatopic antibodies to promote heterodimer formation and so biparatopic antibody formation versus monospecific antibody formation.

Detailed Description of the Invention

The present invention provides, amongst other things, antibodies specific for CD45, in particular antibodies which are monospecific for CD45 and antibodies which are biparatopic for CD45. The antibodies are useful for targeting cells expressing CD45, particularly for depleting and/or killing cells expressing CD45. In a preferred

embodiment, the antibodies provided are able to specifically bind both human and monkey CD45. In a particularly preferred embodiment, the antibodies are able to specifically bind both human CD45 and cynomolgus monkey CD45. Such antibodies are particularly useful as they can be studied in preclinical trials using cynomolgus monkeys.

- 5 In one embodiment, an antibody of the invention is monospecific for CD45, i.e. has a single specificity for CD45. In one particularly preferred embodiment, the antibody provided is an antibody which is biparatopic for CD45, combining in a single molecule two of the monospecific binding specificities for CD45 set out herein. In a further preferred embodiment, biparatopic antibodies which bind to human and cynomolgus monkey cells are provided. In a particularly preferred embodiment antibodies biparatopic for CD45 which bind to and kill both human and cynomolgus monkey cells are provided.

More details of the antibodies and their uses are provided below.

CD45 molecules

- 15 The antibodies of the present invention are specific for CD45. As explained above, CD45 is a member of the protein tyrosine phosphatase (PTP) family. PTPs are known to be signalling molecules that regulate a variety of cellular processes including cell growth, differentiation, mitotic cycle, and oncogenic transformation. CD45 contains an extracellular domain, a single transmembrane segment and two tandem intracytoplasmic catalytic domains, and thus belongs to receptor type PTP. Various isoforms of CD45 exist: CD45RA, CD45RB, CD45RC, CD45RAB, CD45RAC, CD45RBC, CD45RO, CD45R (ABC). CD45 splice variant isoforms A, B and C are expressed differentially on many leucocyte subsets. Despite the existence of different isoforms of CD45, they share common sequences that means all of the isoforms can be targeted by a single antibody.

- 25 The intracellular (COOH-terminal) region of CD45 contains two PTP catalytic domains, and the extracellular region is highly variable due to alternative splicing of exons 4, 5, and 6 (designated A, B, and C, respectively), plus differing levels of glycosylation. The CD45 isoforms detected are cell type, maturation and activation state-specific. In general, the long form of the protein (A, B or C) is expressed on naïve or resting B cells and the mature or truncated form of CD45 (RO) is expressed on activated or mature/memory B cells.

The human sequence for CD45 is available in UniProt entry number P08575 and provided herein in SEQ ID NO: 127, or amino acids 24-1304 of SEQ ID NO: 127, lacking the signal peptide. The amino acid sequence of human CD45 domains 1-4 of the extracellular domain is provided in SEQ ID NO: 128.

5 The murine version of CD45 is provided in UniProt entry P06800.

The cynomolgus monkey version of CD45 is provided herein as SEQ ID NO: 129 (Figure 21).

In one embodiment, the CD45 bound by antibody of the present invention is a mammalian CD45. In one particularly preferred embodiment CD45 refers to human
10 CD45 and natural variants and isoforms thereof. In one preferred embodiment, an antibody of the present invention is able to bind all isoforms of CD45 expressed by a given species, for example, an antibody may bind all human isoforms of CD45. Preferably, an antibody of the present invention can bind both human and cynomolgus monkey CD45, preferably all isoforms of human and cynomolgus monkey CD45.

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Antibodies - Overview

An antibody of the present invention has at least one specificity for CD45. The “specificity” of an antibody denotes the target to which the antibody binds. The portion
20 of an antibody that binds to its target may be referred to as the antigen-binding site or in some circumstances as the paratope of an antibody. The portion of the antigen bound by the antibody may be referred to as the epitope. Specificity for an antibody may be set out in terms the antigen bound or at the level of which epitope of an antigen is bound. Biparatopic antibodies are a subset of bispecific antibodies as bispecific antibodies may
25 recognise two different epitopes on different antigens or two different epitopes on the same antigen. In the latter instance they are biparatopic antibodies. The number of binding sites that an antibody has may be referred to as its valency, with each valency representing one antigen-binding site of the antibody.

The antibodies provided by the present invention are specific for CD45. An
30 antibody of the present invention therefore comprises at least one antigen-binding site, i.e. paratope, specific for CD45. An antibody which recognises a single epitope of CD45 may be referred to as an antibody which is monospecific for CD45. An antibody which recognises two different epitopes of CD45 may be referred to as an antibody which is biparatopic for CD45. The present invention provides both antibodies which are

monospecific for CD45 and antibodies that are biparatopic for CD45. CD45 specific biparatopic antibodies of the invention can be monovalent for each epitope or multivalent for each epitope.

In one preferred embodiment, an antibody of the present invention specifically
5 binds to CD45, but does not significantly bind to non-CD45 proteins. In one embodiment, such specificity is just in relation to the antigen-binding sites of the antibody which recognise CD45, but the antibody may have other antigen-binding sites with other specificities. For example, in one embodiment, an antibody of the present invention has at least one antigen-binding site which is specific for a molecule other than
10 CD45, as well as that specific for CD45. In one embodiment, the further specificity is for serum albumin. In an alternative embodiment, all of the specificities of an antibody of the present invention are for CD45.

In one embodiment, the antibody specifically binds to CD45 from at least one species, but not necessarily to CD45 from all species. Preferably, an antibody of the
15 invention specifically binds human CD45. In a more preferred embodiment, an antibody of the invention specifically binds human CD45 and also CD45 from at least one or more other species. Preferably, the antibody binds specifically to CD45 from a species used in animal studies which will help in the development of the antibody as a therapeutic. In a particularly preferred embodiment, an antibody of the invention specifically binds both
20 human and monkey CD45. In an especially preferred embodiment, an antibody of the invention specifically binds both human and cynomolgus monkey CD45. In a further particularly preferred embodiment, it kills and/or depletes both CD45-expressing human cells and CD45-expressing cynomolgus monkey cells.

In one embodiment, an antibody of the present invention shows trans binding,
25 that is it binds more than one molecule of CD45 at the same time. Such trans binding typically results in cross-linking of CD45 and hence represents one preferred embodiment of the present invention. In one embodiment, an antibody of the invention displays cis binding of CD45 so that it binds specifically just one molecule of CD45 with its binding sites.

30 In one particularly preferred embodiment, an antibody of the present invention specifically binds CD45 and induces multimerisation of CD45. In one embodiment it may be able to multimerise CD45 on the surface of a target cell. CD45 multimers are in particular higher order structures of more than one CD45. In one embodiment, an antibody of the present invention specifically binds an extracellular portion of CD45 and

induces CD45 multimerisation on the surface of a target cell. In a particularly preferred embodiment, a multimer of CD45 comprises at least three CD45 molecules. In one embodiment, a multimer of CD45 may comprise at least three, four, five, six, seven, or more CD45 molecules joined together by antibodies of the present invention. Techniques
5 such as mass photometry may be used to identify multimers of CD45 complexed with antibodies of the present invention and hence to gauge the ability of an antibody of the present invention to generate multimers of CD45.

A degree of specificity (or specific) for a target molecule, in particular for CD45, as employed herein may refer to where the partners or a relevant part thereof in the
10 interaction only recognise each other or have significantly higher affinity for each other in comparison to non-partners, for example at least 10 times, at least 100 times, at least 1000 times, at least 10,000 times, at least 100,000 times or at least 1,000,000 times higher affinity than for example a background level of binding or binding to another unrelated protein (e.g. hen egg white lysozyme). In one embodiment, such degrees of
15 specificity are for CD45. In another embodiment, such specificity is not only for CD45, but also for a particular epitope of CD45 bound by an antigen-binding site, and in particular a paratope, of the antibody, as compared to other epitopes of CD45.

In one embodiment, the affinity of an antibody as measured by dissociation constant (K_D) is about 100 nM or less such as about 50 nM or less, 20 nM or less, 10 nM
20 or less, 1 nM or less, 500 pM or less, 250 pM or less, 200 pM or less, 100 pM or less. In one embodiment, the K_D is 50 pM or less. In one embodiment, at least one paratope of the antibody has such an affinity for CD45. In another embodiment, the antibody has two paratopes, each having a different specificity for CD45, where all of the paratopes individually have such an affinity for CD45. In one embodiment, that is the overall
25 avidity of the antibody for CD45. In one embodiment, the K_D of a paratope for CD45 may be less than 1 μ M, less than 750 nM, less than 500 nM, less than 250 nM, less than 200 nM, less than 150 nM, less than 100 nM, less than 75 nM, less than 50 nM, less than 10 nM, less than 1 nM, less than 0.1 nM, less than 10 pM, less than 1 pM, or less than 0.1 pM. In some embodiments, the K_D is from about 0.1 pM to about 1 μ M. In one
30 embodiment, an antibody of the invention overall has that level of affinity for CD45. Affinity is typically measured using a surface plasmon resonance assay such as a Biacore assay.

17415 derived antibodies*(a) Overview of 17415 derived antibodies and variable regions*

Preferred antibodies of the invention are antibodies that comprise at least one
5 antigen-binding site derived from the original rabbit 17415 antibody described herein and
in particular a pair of the humanized light and heavy chain variable regions derived from
the original rabbit 17415 antibody described herein. The original rabbit 17415 antibody
has a light chain variable region of SEQ ID NO: 1 and a heavy chain variable region
having the amino acid sequence of SEQ ID No: 15. The light chain variable region CDRs
10 of the original rabbit 17415 antibody are a LCDR1, LCDR2, and LCDR3 having
respectively the sequences of SEQ ID NOs: 33, 34, and 35. The heavy chain variable
region CDRs of the original rabbit 17415 are a HCDR1, HCDR2, and HCDR3 having
respectively the sequences of SEQ ID NOs: 46, 47, and 48.

Reference to a “17415 derived antibody” includes any of the specific 17415
15 derived sequences set out herein, as well as variants of such antibodies. Reference to
such “17415 derived” sequences include the humanized light and heavy chain variable
regions shown in Figures 2 and 3, as well as variable regions with the CDR sets of
CDR1, CDR2, and CDR3 shown in those Figures. The specific graft variants shown in
Figures 2 and 3 are particularly preferred. The pairings of the light and heavy chain
20 variable region graft variants generated in the Examples of the present application are
also preferred.

The original rabbit 17415 antibody generated and antibodies comprising the graft
variants described herein for the 17415 antibody are particularly effective at killing or
depleting cells expressing CD45. They also have the further advantage that they are able
25 to specifically bind both human CD45 and cynomolgus monkey CD45, making them
particularly suitable for development as a therapeutic. Preferably, they are able to kill or
deplete both human and cynomolgus monkey cells expressing CD45.

In one embodiment, an antibody comprises one or more 17415 derived antigen-
binding sites specific for CD45. In a particularly preferred embodiment, it comprises one
30 or more antigen-binding sites formed from a pair of humanized 17415 derived light and
heavy chain variable regions.

In one embodiment, the antibody is monospecific for CD45, so that the 17415
derived antigen-binding site or sites present in the antibody are the only ones specific for
CD45 that the antibody comprises. In another embodiment, an antibody of the invention

has a plurality of paratopes binding to CD45, with one of those paratopes specific for CD45 being provided by a 17415 derived antigen-binding site. In an especially preferred embodiment, an antibody of the invention is biparatopic for CD45, with one of the paratope being a 17415 derived antigen-binding site. A particularly preferred antibody of
5 the present invention which is biparatopic for CD45 is one that comprises one paratope which is 17415 derived and the other which is a 17552 derived paratope specific for CD45.

Accordingly, in one particularly preferred embodiment, the present invention provides an antibody or antigen-binding fragment thereof comprising at least one
10 variable domain specific for CD45 comprising the following light and heavy chain variable regions:

- (a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 33, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 35 and 39 to 42; and
15
- (b) a heavy chain variable region comprising a CDR1 comprising a sequence selected from any one of SEQ ID NOs: 46, 52, 53 and 54, a CDR2 comprising a sequence selected from any one of SEQ ID NOs 47, 55, 56, and 57, and a CDR3 comprising the sequence of SEQ ID NO: 48.

In one preferred embodiment, the light chain variable region comprises a LCDR1, LCDR2 and LCDR3 of SEQ ID NOs 33, 34, and 35 respectively. In another
20 embodiment, they comprise the sequences of SEQ ID Nos 33, 34 and 39. In another embodiment they comprise the sequences of SEQ ID Nos 33, 34 and 40. In another embodiment they comprise the sequences of SEQ ID Nos 33, 34 and 41. In another embodiment they comprise the sequences of SEQ ID Nos 33, 34 and 42.

In one preferred embodiment, the heavy chain variable region comprises a HCDR1, HCDR2 and HCDR3 of SEQ ID NOs 46, 47, and 48 respectively. In another
25 embodiment they comprise the sequences of SEQ ID Nos 52, 55 and 48. In another embodiment they comprise the sequences of SEQ ID Nos 53, 56 and 48. In another embodiment they comprise the sequences of SEQ ID Nos 54, 57 and 48.

The humanization of the original rabbit 17415 antibody is described herein. In one preferred embodiment, the 17415 derived antigen-binding site is a humanized antigen-binding site. In a particularly preferred embodiment, 17415 derived paratopes have been generated by transferring the CDRs from the original rabbit 17415 variable region (the donor sequence) into framework regions from a second antibody (the acceptor sequence).
30

The humanization may entail also transferring some framework residues as well as the CDRs from the donor sequence.

Preferred human light chain variable regions for acting as acceptors sequences for the framework include IGKV1-9 and IGKV4-1. A particularly preferred acceptor sequence is one derived from IGKV4-1. In respect of the heavy chain, a particularly preferred acceptor sequence for the CDRs is an IGHV3-72 acceptor framework. In one preferred embodiment, a 17415 derived paratope comprises a light chain variable region based on an IGKV4-1 acceptor framework and a heavy chain variable region based on an IGHV3-72 acceptor framework.

Table 1 below summarizes the original 17415 light and heavy chain variable regions, the framework acceptor sequences used and specific graft variants generated derived from the original 17415 antibody. Figures 2 and 3 also provide alignments of the various original donor and acceptor sequences, as well as the specific graft variants generated. Individual amino acids that are shaded and in italics indicate those residues which are more than just a straight transfer of the CDR sequences of the original 17415 antibody to the human acceptor framework, for instance which are either donor framework residues that have been transferred as well as the CDR sequences or which are CDR residues that are different to those of the original 17415 CDR.

Table 1 - 17415 antibody and humanized graft variants

17415 - LIGHT CHAINS								
Antibody Chain	Overall variable region sequence	CDR1	CDR2	CDR3	FW1	FW2	FW3	FW4
Light 17415 (donor)	SEQ ID NO: 1 DVVLTQTP ASVSEPVG GTVTIKCQ ASQSIYSN LAWYQQKP GPPKLLI YAASNLAS GVSSRFKG SGSGTEFT LTISGVQC ADAATYYC	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 35 QCTNYGSG YVNT	SEQ ID NO: 58 DVVLTQTP ASVSEPVG GTVTIKC	SEQ ID NO: 59 WYQQKPGQ PPKLLIY	SEQ ID NO: 60 GVSSRFKGS SGSGTEFTLT ISGVQCADA ATYYC	SEQ ID NO: 61 FGGGTEVV VK

	QCTNYGSG YVNTFEGG TEVVVK							
IGKV1-9 (acceptor)	SEQ ID NO: 2 DIQLTQSP SFLSASVG DRVITICR ASQGISSY LAWYQQKP GKAPKLLI YAASTLQS GVPSRFSG SGSGTEFT LTISSLQP EDFATYYC QQLNSYPL TFGGGTKV EIK	SEQ ID NO: 36 RASQGISS YLA	SEQ ID NO: 37 AASTLQS	SEQ ID NO: 38 QQLNSYPL T	SEQ ID NO: 62 DIQLTQSP SFLSASVG DRVITIC	SEQ ID NO: 63 WYQQKPGK APKLLIY	SEQ ID NO: 64 GVPSRFSGS SGSGTEFTLT ISSLPEDF ATYYC	SEQ ID NO: 65 EGGGTKVE IK
17415gL1	SEQ ID NO: 3 DVVLQTSP SFLSASVG DRVITICQ ASQSIYSN LAWYQQKP GKAPKLLI YAASNLAS GVPSRFRKG SGSGTEFT LTISSLQP EDFATYYC QCTNYGSG YVNTFEGG TKVEIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 35 QCTNYGSG YVNT	SEQ ID NO: 66 DVVLQTSP SFLSASVG DRVITIC	SEQ ID NO: 63 WYQQKPGK APKLLIY	SEQ ID NO: 70 GVPSRFRKGS SGSGTEFTLT ISSLPEDF ATYYC	SEQ ID NO: 65 EGGGTKVE IK
17415gL2	SEQ ID NO: 4 DVVLQTSP SFLSASVG DRVITICQ ASQSIYSN LAWYQQKP GKAPKLLI YAASNLAS GVPSRFRKG SGSGTEFT LTISSLQP EDFATYYC QSTNYGSG YVNTFEGG TKVEIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 39 QSTNYGSG YVNT	SEQ ID NO: 66 DVVLQTSP SFLSASVG DRVITIC	SEQ ID NO: 63 WYQQKPGK APKLLIY	SEQ ID NO: 70 GVPSRFRKGS SGSGTEFTLT ISSLPEDF ATYYC	SEQ ID NO: 65 EGGGTKVE IK
17415gL3	SEQ ID NO: 5 DVVLQTSP SFLSASVG DRVITICQ ASQSIYSN	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 40 QATNYGSG YVNT	SEQ ID NO: 66 DVVLQTSP SFLSASVG DRVITIC	SEQ ID NO: 63 WYQQKPGK APKLLIY	SEQ ID NO: 70 GVPSRFRKGS SGSGTEFTLT ISSLPEDF ATYYC	SEQ ID NO: 65 EGGGTKVE IK

	LAWYQQKP GKAPKLLI YAASNLAS GVPSRFGK SGSGTEFT LTISSLQP EDFATYYC QATNYGSG YVNTFGGG TKVEIK							
17415gL4	SEQ ID NO: 6 DVVLQTSP SFLSASVG DRVTTTCQ ASQSIYSN LAWYQQKP GKAPKLLI YAASNLAS GVPSRFGK SGSGTEFT LTISSLQP EDFATYYC QVTNYGSG YVNTFGGG TKVEIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 41 QVTNYGSG YVNT	SEQ ID NO: 66 DVVLQTSP SFLSASVG DRVTTTC	SEQ ID NO: 63 WYQQKPGK APKLLIY	SEQ ID NO: 70 GVPSRFGKS SGSGTEFTLT ISSLQPEDF ATYYC	SEQ ID NO: 65 FGGGTKVE IK
17415gL5	SEQ ID NO: 7 DVVLQTSP SFLSASVG DRVTTTCQ ASQSIYSN LAWYQQKP GKAPKLLI YAASNLAS GVPSRFGK SGSGTEFT LTISSLQP EDFATYYC QQTNYGSG YVNTFGGG TKVEIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 42 QQTNYGSG YVNT	SEQ ID NO: 66 DVVLQTSP SFLSASVG DRVTTTC	SEQ ID NO: 63 WYQQKPGK APKLLIY	SEQ ID NO: 70 GVPSRFGKS SGSGTEFTLT ISSLQPEDF ATYYC	SEQ ID NO: 65 FGGGTKVE IK
17415gL6	SEQ ID NO: 8 DVVLQTSP SSLSASVG DRVTTTCQ ASQSIYSN LAWYQQKP GQAPKLLI YAASNLAS GVPSRFGK SGSGTEFT LTISSLQP EDAATYYC QCTNYGSG YVNTFGGG TEVVIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 35 QCTNYGSG YVNT	SEQ ID NO: 67 DVVLQTSP SSLSASVG DRVTTTC	SEQ ID NO: 69 WYQQKPGQ APKLLIY	SEQ ID NO: 71 GVPSRFGKS SGSGTEFTLT ISSLQPEDA ATYYC	SEQ ID NO: 72 FGGGTEVV IK

17415gL7	SEQ ID NO: 9 DVVLTSQP SSLASVG DRVTITCQ ASQSIYSN LAWYQOKP GQAPKLLI YAASNLAS GVPSRFKG SGSGTEFT LTISSLQP EDAATYYC QSTNYGSG YVNTFGGG TEVVIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 39 QSTNYGSG YVNT	SEQ ID NO: 68 DVVLTSQP SSLASVG DRVTITC	SEQ ID NO: 69 WYQOKPGQ APKLLIY	SEQ ID NO: 71 GVPSRFKGS SGSGTEFTLT ISSLQPEDA ATYYC	SEQ ID NO: 72 FGGGTEVV IK
IGKV4-1 (acceptor)	SEQ ID NO: 10 DIVMTQSP DSLAVSLG ERATINCK SSQSVLYS SNNKNYLA WYQOKPGQ PPKLLIYW ASTRESGV PDRFSGSG SGTDFTLT ISSLQAED VAVYQCQQ YYSTPLTF GGGTKVEI K	SEQ ID NO: 43 KSSQSVLY SSNNKNYLA	SEQ ID NO: 44 WASTRES	SEQ ID NO: 45 QQYYSTPL T	SEQ ID NO: 73 DIVMTQSP DSLAVSLG ERATINC	SEQ ID NO: 74 WYQOKPGQ PPKLLIY	SEQ ID NO: 75 GVPDRFSGS SGTDFTLT ISSLQAEDV AVYYC	SEQ ID NO: 76 FGGGTKVE IK
17415gL13	SEQ ID NO: 11 DVVLTSQP DSLAVSLG ERVITNCQ ASQSIYSN LAWYQOKP GKAPKLLI YAASNLAS GVPSRFKG SGSGTEFT LTISSLQA EDAATYYC QCTNYGSG YVNTFGGG TEVVIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 35 QCTNYGSG YVNT	SEQ ID NO: 77 DVVLTSQP DSLAVSLG ERVITNC	SEQ ID NO: 74 WYQOKPGK APKLLIY	SEQ ID NO: 79 GVPSRFKGS SGSGTEFTLT ISSLQAEDA ATYYC	SEQ ID NO: 81 FGGGTEVV IK
17415gL14	SEQ ID NO: 12 DVVLTSQP DSLAVSLG ERATINCK ASQSIYSN LAWYQOKP GKAPKLLI YAASNLAS GVPSRFKG	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 35 QCTNYGSG YVNT	SEQ ID NO: 78 DVVLTSQP DSLAVSLG ERATINC	SEQ ID NO: 74 WYQOKPGK APKLLIY	SEQ ID NO: 80 GVPSRFKGS SGTDFTLT ISSLQAEDV ATYYC	SEQ ID NO: 76 FGGGTKVE IK

	SGSGTDFT LTISSLQA EDVATYYC QSTNYGSG YVNTFGGG TKVEIK							
17415gL15	SEQ ID NO: 13 DVVLTQSP DSLVSLSG ERVTINCQ ASQSIYSN LAWYQQKP GKAPKLLI YAASNLAS GVPSRFGK SGSGTEFT LTISSLQA EDAATYYC QSTNYGSG YVNTFGGG TEVVIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 39 QSTNYGSG YVNT	SEQ ID NO: 77 DVVLTQSP DSLVSLSG ERVTINC	SEQ ID NO: 74 WYQQKPGK APKLLIY	SEQ ID NO: 79 GVPSRFGKGS SGSGTEFTLT ISSLQAEDA ATYYC	SEQ ID NO: 81 FGGGTEVV IK
17415gL16	SEQ ID NO: 14 DVVLTQSP DSLAVSLG ERATINCQ ASQSIYSN LAWYQQKP GKAPKLLI YAASNLAS GVPSRFGK SGSGTDFT LTISSLQA EDVATYYC QSTNYGSG YVNTFGGG TKVEIK	SEQ ID NO: 33 QASQSIYS NLA	SEQ ID NO: 34 AASNLAS	SEQ ID NO: 39 QSTNYGSG YVNT	SEQ ID NO: 78 DVVLTQSP DSLAVSLG ERATINC	SEQ ID NO: 74 WYQQKPGK APKLLIY	SEQ ID NO: 80 GVPSRFGKGS SGSGDFTLT ISSLQAEDV ATYYC	SEQ ID NO: 76 FGGGTKVE IK
17415 - HEAVY CHAINS								
Antibody Chain	Overall variable region sequence	CDR1	CDR2	CDR3	FW1	FW2	FW3	FW4
Heavy 17415 (donor)	SEQ ID NO: 15 QEQLVESG GGLVQPEG SLTLTCTA SGLSFIGN YYICWVRQ APGKGLEW IGCIYTG SGSTYYAS WAKGRFTI	SEQ ID NO: 46 GLSFIGNY YIC	SEQ ID NO: 47 CIYTGSSG STYYASWA KG	SEQ ID NO: 48 DAYTYAF	SEQ ID NO: 82 QEQLVESG GGLVQPEG SLTLTCTA S	SEQ ID NO: 83 WVRQAPGK GLEWIG	SEQ ID NO: 84 RFTISKTS TTVTQLNS LTAADTATY FCEL	SEQ ID NO: 85 WGPGLVLT VSS

	SKTSSTTV TLQLNSLT AADTATYF CELDAYTY AFWGPCTL VTVSS							
IGHV3-72 (acceptor)	SEQ ID NO: 16 EVQLVESG GGLVQPGG SLRLSCAA SGFTFSDH YMDWVRQA PGKGLEWV GRTRNKAN SYTTEYAA SVKGRFTI SRDDSKNS LYLQMNLS KTEDTAVY YCARYFDY WGQGTLLV VSS	SEQ ID NO: 49 GFTFSDHY MD	SEQ ID NO: 50 RTRNKANS YTTEYAAS VKG	SEQ ID NO: 51 YFDY	SEQ ID NO: 86 EVQLVESG GGLVQPGG SLRLSCAA S	SEQ ID NO: 87 WVRQAPGK GLEWVG	SEQ ID NO: 88 RFTISRDDS KNSLYLQMN SLKTEDTAV YYCAR	SEQ ID NO: 89 WGQGTLLV VSS
17415gH1	SEQ ID NO: 17 EVQLVESG GGLVQPGG SLRLSCTA SGLSFIGN YYICWVRQ APGKGLEW IGCIYTGS SGSTYYAS WAKGRFTI SKDSSKTS VYLQMNLS KTEDTAVY YCELDAYT YAFWGQGT LVTVSS	SEQ ID NO: 46 GLSFIGNY YIC	SEQ ID NO: 47 CIYTGSSG STYYASWA KG	SEQ ID NO: 48 DAYTYAF	SEQ ID NO: 90 EVQLVESG GGLVQPGG SLRLSCTA S	SEQ ID NO: 91 WVRQAPGK GLEWIG	SEQ ID NO: 92 RFTISKDSS KTSVYLQMN SLKTEDTAV YYCEL	SEQ ID NO: 89 WGQGTLLV VSS
17415gH2	SEQ ID NO: 18 EVQLVESG GGLVQPGG SLRLSCTA SGLSFIGN YYISWVRQ APGKGLEW IGSIYTGS SGSTYYAS WAKGRFTI SKDSSKTS VYLQMNLS KTEDTAVY YCELDAYT YAFWGQGT LVTVSS	SEQ ID NO: 52 GLSFIGNY YIS	SEQ ID NO: 55 SIYTGSSG STYYASWA KG	SEQ ID NO: 48 DAYTYAF	SEQ ID NO: 90 EVQLVESG GGLVQPGG SLRLSCTA S	SEQ ID NO: 91 WVRQAPGK GLEWIG	SEQ ID NO: 92 RFTISKDSS KTSVYLQMN SLKTEDTAV YYCEL	SEQ ID NO: 89 WGQGTLLV VSS
17415gH3	SEQ ID NO: 19 EVQLVESG GGLVQPGG SLRLSCTA SGLSFIGN YYIAWVRQ	SEQ ID NO: 53 GLSFIGNY YIA	SEQ ID NO: 56 AIYTGSSG STYYASWA KG	SEQ ID NO: 48 DAYTYAF	SEQ ID NO: 90 EVQLVESG GGLVQPGG SLRLSCTA S	SEQ ID NO: 91 WVRQAPGK GLEWIG	SEQ ID NO: 92 RFTISKDSS KTSVYLQMN SLKTEDTAV YYCEL	SEQ ID NO: 89 WGQGTLLV VSS

	APGKGLEW IGAIYTG SGSTYYAS WAKGRFTI SKDSSKTS VYLQMN SLKTEDTAVY YCELDAYT YAFWGQGT LVTVSS							
17415gH4	SEQ ID NO: 20 EVQLVESG GGLVQPGG SLRLSCTA SGLSFIGN YYIVWVRQ APGKGLEW IGVIYTG SGSTYYAS WAKGRFTI SKDSSKTS VYLQMN SLKTEDTAVY YCELDAYT YAFWGQGT LVTVSS	SEQ ID NO: 54 GLSFIGNY YIV	SEQ ID NO: 57 VIYTGSSG STYYASWA KG	SEQ ID NO: 48 DAYTYAF	SEQ ID NO: 90 EVQLVESG GGLVQPGG SLRLSCTA S	SEQ ID NO: 91 WVRQAPGK GLEWIG	SEQ ID NO: 92 RFTISKDSS KTSVYLQMN SLKTEDTAV YCEL	SEQ ID NO: 89 WGQGT LVT VSS
17415gH5	SEQ ID NO: 21 EVQLVESG GGLVQPGG SLRLSCTA SGLSFIGN YYICWVRQ APGKGLEW IGCIYTG SGSTYYAS WAKGRFTI SKDSSTSV YLQMN SLKTEDTAVY YCELDAYT YAFWGQGT LVTVSS	SEQ ID NO: 46 GLSFIGNY YIC	SEQ ID NO: 47 CIYTGSSG STYYASWA KG	SEQ ID NO: 48 DAYTYAF	SEQ ID NO: 90 EVQLVESG GGLVQPGG SLRLSCTA S	SEQ ID NO: 91 WVRQAPGK GLEWIG	SEQ ID NO: 93 RFTISKDSS TSVYLQMN SLKTEDTAV YCEL	SEQ ID NO: 89 WGQGT LVT VSS
17415gH6	SEQ ID NO: 22 EVQLVESG GGLVQPGG SLRLSCTA SGLSFIGN YYISWVRQ APGKGLEW IGSIYTG SGSTYYAS WAKGRFTI SKDSSTSV YLQMN SLKTEDTAVY YCELDAYT YAFWGQGT LVTVSS	SEQ ID NO: 52 GLSFIGNY YIS	SEQ ID NO: 55 SIYTGSSG STYYASWA KG	SEQ ID NO: 48 DAYTYAF	SEQ ID NO: 90 EVQLVESG GGLVQPGG SLRLSCTA S	SEQ ID NO: 91 WVRQAPGK GLEWIG	SEQ ID NO: 93 RFTISKDSS TSVYLQMN SLKTEDTAV YCEL	SEQ ID NO: 89 WGQGT LVT VSS

Various examples of preferred 17415 derived sequences are set out below:

(b) Preferred 17415 Light chain graft variants comprising IGKV4-1/ IGKJ4 derived framework

5 In one preferred embodiment, the light chain variable region comprises the LCDR1, LCDR2, and LCDR3 of the rabbit 17415 antibody. In an alternative preferred embodiment, the LCDR1, LCDR2, and LCDR3 are those of the rabbit 17415 antibody, except that the LCDR3 includes the mutation C90S.

In a preferred embodiment a humanized 17415 light chain variable region graft
10 variant comprises such a set of LCDR1, LCDR2, and LCDR3 derived from the rabbit 17415 antibody, with an IGKV4-1/ IGKJ4 derived framework. In one preferred embodiment, the light chain variable region comprises a CDR1 comprising a sequence of SEQ ID NO: 33, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 35 and 39 to 42, wherein
15 the framework regions are an IGKV4-1/IGKJ4 derived framework.

In one preferred embodiment, as well as the 17415 light chain LCDR1, LCDR2, and LCDR3, one or more donor residues from the 17415 rabbit antibody light chain framework are retained at one or more positions from the group comprising residues 2 (Valine, V2), 4 (Leucine, L4), 12 (serine, S12), 19 (Valine, V19), 60 (Serine, S60), 63 (Lysine, K63), 70 (Glutamic acid, E70), 83 (Alanine, A83), 85 (Threonine, T85), 106 (Glutamic acid, E106) and 108 (Valine, V108).
20

In one embodiment, donor residues 2 (Valine, V2), 4 (Leucine, L4) are retained in the light chain variable region FWR1 from the rabbit 17415 antibody. In one embodiment, donor residues (Valine, V2), 4 (Leucine, L4), 12 (serine, S12), 19 (Valine, V19), are retained from 17415 in the FWR1. In an alternate embodiment, FWR1
25 corresponds to the FWR1 acceptor sequence from IGKV4-1/IGKJ4.

In one preferred embodiment, FWR2 corresponds to the FWR2 acceptor sequence from IGKV4-1/IGKJ4.

In one embodiment, donor residues 60 (Serine, S60), 63 (Lysine, K63), 83 (Alanine, A83), and 85 (Threonine, T85) (and optionally (Glutamic acid, E70)) are
30 retained from the rabbit 17415 antibody in the light chain variable region FWR3. In one

embodiment, donor residues 60 (Serine, S60), 63 (Lysine, K63), and 85 (Threonine, T85) are retained from 17415 in the FWR3.

- In one embodiment, donor residues 106 (Glutamic acid, E106) and 108 (Valine, V108) are retained from the rabbit 17415 antibody in the light chain variable region
- 5 FWR4. In an alternate embodiment, FWR4 corresponds to the FWR4 acceptor sequence from IGKV4-1/ IGKJ4.

- Examples of preferred light chain frameworks based on the IGKV4-1 acceptor sequence include those with a FWR1, FWR2, FWR3 and FWR4 respectively of SEQ ID Nos 77, 74, 79 and 81. In another preferred embodiment they have the sequences of SEQ
- 10 ID Nos: 78, 74, 80 and 76.

Examples of particularly preferred 17415 derived light chain variable region graft variants which may be employed include those of 17415gL13 (SEQ ID No: 11), 17415gL14 (SEQ ID No: 12), 17415gL15 (SEQ ID No: 13), and 17415gL16 (SEQ ID No: 14).

- 15 (c) *Preferred 17415 light chain graft variants comprising an IGKV1-9/IGKJ4 derived framework*

- In one preferred embodiment, the light chain variable region derived from the original rabbit 17415 antibody comprises a CDR1 comprising a sequence of SEQ ID NO: 33, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR3 comprising a
- 20 sequence selected from any one of SEQ ID NOs: 35 and 39 to 42, wherein the framework regions are derived from an IGKV1-9 acceptor framework.

- In one such preferred embodiment, the light chain variable region comprises the LCDR1, LCDR2, and LCDR3 of the rabbit 17415 antibody. In an alternative preferred embodiment, the LCDR1, LCDR2, and LCDR3 are those of the rabbit 17415 antibody,
- 25 except that the LCDR3 includes the mutation C90S. Alternatively C90 can be mutated to A (Alanine), V (Valine), or Q (Glutamine).

- In one embodiment donor residues from the rabbit 17415 antibody light chain framework are retained at one or more of positions 2 (Valine, V2), 3 (Valine, V3) and 63 (Lysine, K63) in the light chain variable region. In one embodiment, all of those residues
- 30 are retained.

In another embodiment, donor residues from the rabbit 17415 antibody light chain framework are retained at one or more positions selected from 2 (Valine, V2), 3

(Valine, V3), 10 (Serine, S10), 42 (Glutamine, Q42), 63 (Lysine, K63), 83 (Alanine, A83), 106 (Glutamic acid, E106) and 108 (Valine, V108). In a preferred embodiment, all of those donor framework residues are retained.

5 In one embodiment in FWR1 donor residues from the framework are retained at positions 2 (Valine, V2), and 3 (Valine, V3). In one particularly preferred embodiment in FWR1 donor residues from the framework are retained at positions 2 (Valine, V2), 3 (Valine, V3), and 10 (Serine, S10).

10 In one embodiment, in FWR2 a donor residue at position 42 (Glutamine, Q42) is retained. In an alternative embodiment, the FWR2 is the same as the acceptor sequence and no donor residues are retained in FWR2.

In one embodiment, in FWR3 donor residues are retained at positions 63 (Lysine, K63) and 83 (Alanine, A83).

15 In one embodiment, in FWR4 donor residues are retained at positions 106 (Glutamic acid, E106) and 108 (Valine, V108). In another embodiment, the sequence of FWR4 is the same as the FWR4 of the acceptor sequence.

20 Examples of particularly preferred donor framework regions to retain are those in all of positions 2 (Valine, V2), 3 (Valine, V3), 10 (Serine, S10), 42 (Glutamine, Q42), 63 (Lysine, K63) 83 (Alanine, A83), 106 (Glutamic acid, E106) and 108 (Valine, V108). In a further preferred embodiment, the LCDR3 then includes the mutation C90S. In another embodiment, it does not.

25 In one preferred embodiment, the acceptor framework comprises a FWR1, FWR2, FWR3 and FWR4 respectively of SEQ ID Nos: 58, 59, 60 and 61. In another those FWRs comprise respectively the sequences of SEQ ID Nos: 66, 63, 70 and 65. In another embodiment they comprise respectively the sequences of SEQ ID Nos: 67, 69, 71, and 72. In another embodiment, they respectively comprise the sequences of SEQ ID Nos: 68, 69, 71 and 72.

30 Examples of preferred light chain variable region graft variants include those of 17415gL1 to 17415gL7 (respectively SEQ ID Nos: 3 to 9). An example of a particularly preferred graft variant light chain is that of SEQ ID No: 8 (17415gL6). An example of a further particularly preferred graft variant light chain is that of SEQ ID No: 9 (17415gL6). Also provided are those of SEQ ID Nos: 3 to 7 (17415gL1 to 17415gL5).

(d) *Preferred 17415 heavy chain graft variants comprising an IGV3-72 derived framework*

In a further preferred embodiment, a 17415 derived heavy chain graft variant is
5 generated using an IGHV3-72/IGHJ4 J-region acceptor framework. Hence, in one preferred embodiment, a heavy chain variable region comprises a CDR1 comprising a sequence selected from any one of SEQ ID NOs: 46, 52, 53 and 54, a CDR2 comprising a sequence selected from any one of SEQ ID NOs 47, 55, 56, and 57, and a CDR3 comprising the sequence of SEQ ID NO: 48, wherein the acceptor framework is derived
10 from IGHV3-72/IGHJ4.

In one embodiment a 17415 derived heavy chain variable region includes the HCDR1 of the rabbit 17415 antibody. In another embodiment, the final residue of the HCDR1 is changed from C (cysteine) to S (Serine), A (Alanine), or V (Valine).

In one embodiment a 17415 derived heavy chain variable region includes the
15 HCDR2 of the rabbit 17415 antibody. In another embodiment, the first residue of the HCDR2 is changed from C (cysteine) to S (Serine), A (Alanine), or V (Valine).

In one embodiment, derived heavy chain variable region includes the original HCDR3 of the rabbit 17415 antibody.

Particularly preferred CDR combinations are shown in Figure 3. Any of the
20 combinations of three heavy chain CDRs shown for an individual variant may be employed in a 17415 derived heavy chain variable region variant. In particular, in one embodiment, a 17415 derived heavy chain variant has the CDRs of 17415gH1 (SEQ ID No: 17). In another it has those of 17415gH2 (SEQ ID No: 18). In a further embodiment, it has those of 17415gH3 (SEQ ID No: 19). In a further embodiment, it has those of
25 17415gH4 (SEQ ID No: 20). In another embodiment, it has those of 17415gH5 (SEQ ID No: 21). In another embodiment, it has the CDRs of 17415gH6 (SEQ ID No: 22).

In one preferred embodiment, a 17415 derived heavy chain variant has the FWR1 of an IGHV3-72 acceptor, but a donor residue from the rabbit 17415 antibody heavy chain framework is retained at position 23 (Threonine, T23).

30 In one preferred embodiment, a 17415 derived heavy chain variant has the FWR2 of an IGHV3-72 acceptor, but a donor residue is retained at position 49 (Isoleucine, I49).

In one preferred embodiment, a 17415 derived heavy chain variant has the FWR3 of an IGHV3-72 acceptor, but donor residues are retained at positions 74 (Lysine, K74), 76 (Serine, S76), 79 (Threonine, T79), 81 (Valine, V81), 99 (Glutamic acid, E99) and 100 (Leucine, L100).

5 In one preferred embodiment, a 17415 derived heavy chain variant has the FWR4 of an IGHV3-72/ IGHJ4 acceptor, but with no further sequence changes.

Particularly preferred frameworks are shown in Figure 3. Any of the combinations of FWR1, FWR2, FWR3, and FWR4 for an individual variant shown in Figure 3 may be employed in a 17415 derived heavy chain variable region.

10 Hence, in one preferred embodiment, the acceptor framework for a 17415 derived heavy chain variable region has a FWR1, FWR2, FWR3, and FWR4 respectively comprising the sequences of SEQ ID Nos: 90, 91, 92, and 89. In another they comprise the sequences respectively of SEQ ID Nos: 90, 91, 93 and 89.

Particularly preferred 17415 derived heavy chain graft variants are shown in Figure 3. In one preferred embodiment, the heavy chain variable region of 17415gH1 (SEQ ID No: 17) may be employed. In another embodiment, that of 17415gH2 (SEQ ID No: 18) is employed. In another that of 17415gH3 (SEQ ID No: 19) is employed. In a further embodiment, that of 17415gH4 (SEQ ID No: 20) is employed. In another embodiment, that of 17415gH5 (SEQ ID No: 21) may be employed. In another
20 embodiment, that of 17415gH6 (SEQ ID No: 22) may be employed.

(e) Further preferred 17415 derived light and heavy chain pairings

In one embodiment, an antibody of the present invention comprises a 17415 derived light chain variable region and a 17415 derived heavy chain variable region. In one embodiment, the light chain variable region is any of those shown in Figure 2 and the
25 heavy chain variable region any of those shown in Figure 3. Particularly preferred light and heavy chain variable region pairings are those in the Examples of the present application. Also preferred are the CDR sets of six CDRs (LCDR1, LCDR2, and LCDR3 and HCDR1, HCDR2, and HCDR3 of the specific light and heavy chain variable region pairings employed in the Examples of the present application.

30 In particularly preferred embodiments, the light chain variable region is a graft variant of 17415gL6 (SEQ ID No: 8), 17415gL7 (SEQ ID No: 9), or 17415gL13 to 17415gL16 (respectively SEQ ID Nos 11 to 14). In particularly preferred embodiments,

the heavy chain variable region is a graft variant of 17415gH1 to H6 (respectively SEQ ID Nos: 17 to 22).

In one embodiment, 17415gL6 (SEQ ID No: 8) is paired with any of 17415gH1 to H6 (respectively SEQ ID Nos: 17 to 22). In another embodiment 17415gL7 (SEQ ID No: 9) is paired with any of 17415gH1 to H6 (respectively SEQ ID Nos: 17 to 22). In another embodiment, 17415gL13 (SEQ ID No: 11) is paired with any of 17415gH1 to H6 (respectively SEQ ID Nos: 17 to 22). In another embodiment, 17415gL14 (SEQ ID No: 12) is paired with any of 17415gH1 to H6. In another embodiment, 17415gL15 (SEQ ID No: 13) is paired with any of 17415gH1 to H6. In another embodiment, 17415gL16 (SEQ ID No: 14) is paired with any of 17415gH1 to H6 (respectively SEQ ID Nos: 17 to 22).

In one embodiment 17415gH1 (SEQ ID No: 17) is paired with any of 17415gL6 (SEQ ID No: 8), 17415gL7 (SEQ ID No: 9), or 17415gL13 to 17415gL16 (respectively SEQ ID Nos: 11 to 14). In one embodiment 17415gH2 (SEQ ID No: 18) is paired with any of 17415gL6 (SEQ ID No: 8), 17415gL7 (SEQ ID No: 9), or 17415gL13 to 17415gL16 (respectively SEQ ID Nos: 11 to 14). In one embodiment 17415gH3 (SEQ ID No: 19) is paired with any of 17415gL6, 17415gL7, or 17415gL13 to 17415gL16. In one embodiment 17415gH4 (SEQ ID No: 20) is paired with any of 17415gL6 (SEQ ID No: 8), 17415gL7 (SEQ ID No: 9), or 17415gL13 to 17415gL16 (respectively SEQ ID Nos: 11 to 14). In one embodiment 17415gH5 (SEQ ID No: 21) is paired with any of 17415gL6 (SEQ ID No: 8), 17415gL7 (SEQ ID No: 9), or 17415gL13 to 17415gL16 (respectively SEQ ID Nos: 11 to 14). In one embodiment 17415gH6 (SEQ ID No: 22) is paired with any of 17415gL6 (SEQ ID No: 8), 17415gL7 (SEQ ID No: 9), or 17415gL13 to 17415gL16 (respectively SEQ ID Nos: 11 to 14).

In one embodiment, a 17415 derived antigen binding site comprises 17415gL7 (SEQ ID No: 9) and 17415gH6 (SEQ ID No: 22) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen-binding site comprises a 17415gL15 (SEQ ID No: 13) and 17415gH6 (SEQ ID No: 22) light and heavy chain variable region pair.

In one embodiment, a 17415 derived antigen-binding site comprises a 17415gL16 (SEQ ID No: 14) and 17415gH6 (SEQ ID No: 22) light and heavy chain variable region pair.

In one embodiment, a 17415 derived antigen-binding site comprises a 17415gL1 (SEQ ID No: 3) and 17415gH1 (SEQ ID No: 17) light and heavy chain variable region

pair. In one embodiment, a 17415 derived antigen-binding site comprises a 17415gL1 (SEQ ID No: 3) and 17415gH4 (SEQ ID No: 20) light and heavy chain variable region pair.

5 In one embodiment, a 17415 derived antigen binding site comprises a 17415gL2 (SEQ ID No: 4) and 17415gH1 (SEQ ID No: 17) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL3 (SEQ ID No:5) and 17415gH1 (SEQ ID No: 17) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL4 (SEQ ID No: 6) and 17415gH1 (SEQ ID No: 17) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL5 (SEQ ID No: 7) and 17415gH1 (SEQ ID No: 17) light and heavy chain variable region pair.

15 In one embodiment, a 17415 derived antigen binding site comprises a 17415gL1 (SEQ ID No: 3) and 17415gH2 (SEQ ID No: 18) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL1 (SEQ ID No: 3) and 17415gH3 (SEQ ID No: 19) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL1 (SEQ ID No: 3) and 17415gH4 (SEQ ID No:20) light and heavy chain variable region pair.

20 In one embodiment, a 17415 derived antigen binding site comprises a 17415gL1 (SEQ ID No: 3) and 17415gH5 (SEQ ID No: 21) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL6 (SEQ ID No: 8) and 17415gH5 (SEQ ID No: 21) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL7 (SEQ ID No: 9) and 17415gH5 (SEQ ID No: 21) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL13 (SEQ ID No: 11) and 17415gH5 (SEQ ID No: 21) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL14 (SEQ ID No: 12) and 17415gH5 (SEQ ID No: 21) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL15 (SEQ ID No: 13) and 17415gH5 (SEQ ID No: 21) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL16 (SEQ ID NO: 14) and 17415gH5 (SEQ ID No: 21) light and heavy chain variable region pair.

In one embodiment, a 17415 derived antigen binding site comprises a 17415gL7 (SEQ ID No: 9) and 17415gH6 (SEQ ID No: 22) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL15 (SEQ ID No: 13) and 17415gH6 (SEQ ID No: 22) light and heavy chain variable region pair. In one embodiment, a 17415 derived antigen binding site comprises a 17415gL16 (SEQ ID No: 14) and 17415gH6 light and heavy chain variable region pair.

(f) Variants and derivatives

As well as the specific 17415 derived sequences set out herein, also provided are variants and derivatives of the specific sequences as described elsewhere herein. Such a variant will retain the ability to specifically bind CD45. It will preferably retain the ability to kill or deplete CD45 expressing cells.

17552 derived antibodies

(a) Overview of 17552 derived antibodies and variable regions

Further preferred antibodies of the invention are antibodies that comprise at least one antigen-binding site derived from the rabbit 17552 antibody described herein and in particularly a pair of the humanized light and heavy chain variable regions derived from the rabbit 17552 antibody described herein. The original light and heavy chain variable region sequences of the rabbit 17552 antibody are provided respectively as SEQ ID Nos: 23 and 27. The light chain variable region LCDR1, LCDR2, and LCDR3 of the original rabbit antibody are provided as respectively SEQ ID Nos: 94, 56, and 96. The heavy chain variable region HCDR1, HCDR2, and HCDR3 of the original rabbit antibody are provided as respectively SEQ ID Nos: 100, 101, and 102.

In one preferred embodiment, a 17552 derived antibody has the light and heavy chain CDR sets of one of those antibodies in the Examples of the present application, including the 17552 derived specificities in the biparatopic antibodies set out in Figure 17. In another preferred embodiment, it comprises the light and heavy chain variable regions of the 17552 derived specificities shown in Figure 17.

The original rabbit 17552 antibody generated and antibodies comprising the graft variants described herein have the advantage that they are able to specifically bind both human CD45 and cynomolgus monkey CD45 helping to make them particularly suitable

for development as a therapeutic. 17552 derived antigen-binding sites also have the further advantage that, when used in conjunction with a second specificity against CD45 that brings about killing or depletion of CD45, the 17552 specificity acts as a “helper” specificity, potentially boosting the efficacy of the other specificity. Hence, the 17552 derived specificity is particularly effective in antibodies with more than one paratope or specificity for CD45. In an even more preferred embodiment, the 17552 derived specificity is used as part of an antibody that is biparatopic for CD45. In an especially preferred embodiment, the present invention provides biparatopic antibodies comprising 17415 and 17552 derived specificities, with the 17415 specificity acting as a “killing” specificity and the 17552 specificity acting as a “helper” specificity.

The humanization of the original rabbit 17552 antibody is described herein. In one preferred embodiment, the 17552 derived paratope present in an antibody of the invention is a humanized antigen-binding site. In a particularly preferred embodiment, 17552 derived paratopes have been generated by transferring the CDRs from the 17552 variable region (the donor sequence) into framework regions from a second antibody (the acceptor sequence). The humanization may entail also transferring some framework residues as well as the CDRs from the donor sequence.

Hence, in one particularly preferred embodiment, the present invention provides an antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light and heavy chain variable regions:

- (a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96; and
- (b) a heavy chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO 101, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 102 to 105.

Preferred human light chain variable regions for acting as acceptors sequences for the framework include IGKV1-8/IGKJ4. In respect of the heavy chain, a particularly preferred acceptor sequence for the CDRs is an IGHV4-4/IGHJ4 acceptor framework. In one preferred embodiment, a 17552 derived paratope comprises a light chain variable region based on an IGKV1-8 acceptor framework and a heavy chain variable region based on an IGHV4-4 acceptor framework.

Table 2 below summarizes the original 17552 light and heavy chain variable regions, the framework acceptor sequences used and specific graft variants generated

- derived from the original 17552 antibody. Figure 4 also provide alignments of the various original donor and acceptor sequences, as well as the specific graft variants generated. Individual amino acids that are shaded and in italics indicate those residues which are more than just a straight transfer of the CDR sequences of the original 17552 antibody to the human acceptor framework, for instance which are either donor framework residues that have been transferred as well as the CDR sequences or which are CDR residues that are different to those of the original 17552 CDR.

Table 2 - 17552 antibody and humanized graft variants

17552 - LIGHT CHAINS								
Antibody Chain	Overall variable region sequence	CDR1	CDR2	CDR3	FW1	FW2	FW3	FW4
Light 17552 (donor)	SEQ ID NO: 23 ALVMTQTP ASVSAAVG GTVTINCQ ASQSITTA LAWYQQKP GQRPKLLI YRASTLAS GVSSRFEG SGSGTQFT LTISGVEC DDAATYYC QEYYSSSD VDNTEGGG TEVVVK	SEQ ID NO: 94 QASQSITT ALA	SEQ ID NO: 95 RASTLAS	SEQ ID NO: 96 QEYYSSSD VDNT	SEQ ID NO: 106 ALVMTQTP ASVSAAVG GTVTINC	SEQ ID NO: 107 WYQQKPGQR PKLLIY	SEQ ID NO: 108 GVSSRFEG SGSGTQFT LTISGVEC DDAATYYC	SEQ ID NO: 109 EGGGTEVV VK
IGKV1-8 (acceptor)	SEQ ID NO: 24 AIRMTQSP SSFSASTG DRVITICR ASQGISSY LAWYQQKP GKAPKLLI YAASTLQS GVPSRFSG SGSGTDFT LTISCLQS EDFATYYC QQYYSYPL TFGGGTKV EIK	SEQ ID NO: 97 RASQGISS YLA	SEQ ID NO: 98 AASTLQS	SEQ ID NO: 99 QQYYSYPL T	SEQ ID NO: 110 AIRMTQSP SSFSASTG DRVITIT	SEQ ID NO: 111 WYQQKPGKA PKLLIY	SEQ ID NO: 112 GVPSRFSG SGSGTDFT LTISCLQS EDFATYYC	SEQ ID NO: 113 EGGGTKVE IK

17552gL1	SEQ ID NO: 25 ALVMTQSP SSFSASTG DRVITTCQ ASQSITTA LAWYQQKP GKAPKLLI YRASTLAS GVPSRFEG SGSGTDFT LTISSLQS EDFATYYC QEYYSSSD VDNTFGGG TKVEIK	SEQ ID NO: 94 QASQSITT ALA	SEQ ID NO: 95 RASTLAS	SEQ ID NO: 96 QEYYSSSD VDNT	SEQ ID NO: 114 ALVMTQSP SSFSASTG DRVITTC	SEQ ID NO: 111 WYQQKPGKA PKLLIY	SEQ ID NO: 115 GVPSRFEG SGSGTDFT LTISSLQS EDFATYYC	SEQ ID NO: 113 FGGGTKVE IK
17552gL2	SEQ ID NO: 26 ALVMTQSP SSFSASTG DRVITTCQ ASQSITTA LAWYQQKP GKAPKLLI YRASTLAS GVPSRFEG SGSGTDFT LTISSLQS EDFATYYC QEYYSSSD VDNTFGGG TKVEIK	SEQ ID NO: 94 QASQSITT ALA	SEQ ID NO: 95 RASTLAS	SEQ ID NO: 96 QEYYSSSD VDNT	SEQ ID NO: 114 ALVMTQSP SSFSASTG DRVITTC	SEQ ID NO: 111 WYQQKPGKA PKLLIY	SEQ ID NO: 112 GVPSRFEG SGSGTDFT LTISSLQS EDFATYYC	SEQ ID NO: 113 FGGGTKVE IK
17552 - HEAVY CHAINS								
Antibody Chain	Overall variable region sequence	CDR1	CDR2	CDR3	FW1	FW2	FW3	FW4
Heavy 17552 (donor)	SEQ ID NO: 27 QSVESGG RLVTPGTP LTLTCTVS GFSLSDYY MIWVRQAP GKGLEIYG AISYGGSA YYLSWAKG RFTISKTS TTVDLMIT SPTTEDTA TYFCTRGI WDGGSGGP WGYFDIWG	SEQ ID NO: 100 GFSLSDYY MI	SEQ ID NO: 101 AISYGGSA YYLSWAKG	SEQ ID NO: 102 GIWDGGSG GPGYFDI	SEQ ID NO: 115 QSVESGG RLVTPGTP LTLTCTVS	SEQ ID NO: 116 WVRQAPGKG LEYIG	SEQ ID NO: 117 RFTISKTS TTVDLMIT SPTTEDTA TYFCTR	SEQ ID NO: 118 WGPGLT VSS

	PGTLVTVS S							
IGHV4-4 (acceptor)	SEQ ID NO: 28 QVQLQESG PGLVKPSG TSLTCAV SGGSISSS NWWSWVRQ PGKGLEW IGEIVHSG STNYNPSL KSRVTISV DKSKNQFS LKLSSVTA ADTAVYYC ARYFDYWG QGTILVTVS S	SEQ ID NO: 138 GGSISSSN WWS	SEQ ID NO: 139 EIVHSGST NYNPSLKS	SEQ ID NO: 51 YFDY	SEQ ID NO: 119 QVQLQESG PGLVKPSG TSLTCAV S	SEQ ID NO: 120 WVRQPPGKG LEWIG	SEQ ID NO: 121 RVTISVDK SKNQFSLK LSSVTAAD TAVYYCAR	SEQ ID NO: 122 WGQGTILVT VSS
17552gH1	SEQ ID NO: 29 EVQLQESG PGLVKPSG TSLTCTV SGFSLSDY YMIWVRQP PGKGLEYI GAISYGGS AYYLSWAK GRFTISKD SSKNQVSL KLSSVTAA DTAVYYCT RGIWDGGS GGPWGYFD IWGQGTILV TVSS	SEQ ID NO: 100 GFSLSDYY MI	SEQ ID NO: 101 AISYGGSA YYLSWAKG	SEQ ID NO: 102 GIWDGGSG GPWGYFDI	SEQ ID NO: 123 EVQLQESG PGLVKPSG TSLTCTV S	SEQ ID NO: 124 WVRQPPGKG LEYIG	SEQ ID NO: 125 RFTISKDS SKNQVSLK LSSVTAAD TAVYYCTR	SEQ ID NO: 126 WGQGTILVT VSS
17552gH2	SEQ ID NO: 30 EVQLQESG PGLVKPSG TSLTCTV SGFSLSDY YMIWVRQP PGKGLEYI GAISYGGS AYYLSWAK GRFTISKD SSKNQVSL KLSSVTAA DTAVYYCT RGIWEGGS GGPWGYFD IWGQGTILV TVSS	SEQ ID NO: 100 GFSLSDYY MI	SEQ ID NO: 101 AISYGGSA YYLSWAKG	SEQ ID NO: 103 GIWEGGSG GPWGYFDI	SEQ ID NO: 123 EVQLQESG PGLVKPSG TSLTCTV S	SEQ ID NO: 124 WVRQPPGKG LEYIG	SEQ ID NO: 125 RFTISKDS SKNQVSLK LSSVTAAD TAVYYCTR	SEQ ID NO: 126 WGQGTILVT VSS
17552gH3	SEQ ID NO: 31 EVQLQESG PGLVKPSG TSLTCTV SGFSLSDY YMIWVRQP PGKGLEYI GAISYGGS	SEQ ID NO: 100 GFSLSDYY MI	SEQ ID NO: 101 AISYGGSA YYLSWAKG	SEQ ID NO: 104 GIWDGGSG GPWGYFDI	SEQ ID NO: 123 EVQLQESG PGLVKPSG	SEQ ID NO: 124 WVRQPPGKG LEYIG	SEQ ID NO: 125 RFTISKDS SKNQVSLK	SEQ ID NO: 126 WGQGTILVT VSS

	AYYLSWAK GRFTISKD SSKNQVSL KLSSVTAA DTAVYYCT RGIWDGSG GGPWGYFD IWGQGTLV TVSS				TLSLTCTV S		LSSVTAAD TAVYYCTR	
17552gH4	SEQ ID NO: 32 EVQLQESG PGLVKPSG TLSLTCTV SGFSLSDY YMIWVRQP PGKGLIYI GAISYGGG AYYLSWAK GRFTISKD SSKNQVSL KLSSVTAA DTAVYYCT RGIWDGSG GGPWGYFD IWGQGTLV TVSS	SEQ ID NO: 100 GFSLSDYY MI	SEQ ID NO: 101 AISYGGSA YYLSWAKG	SEQ ID NO: 105 GIWDAGSG GFWGYFDI	SEQ ID NO: 123 EVQLQESG PGLVKPSG TLSLTCTV S	SEQ ID NO: 124 WVRQPPGKG LEYIG	SEQ ID NO: 125 RFTISKDS SKNQVSLK LSSVTAAD TAVYYCTR	SEQ ID NO: 126 WGQGTLVT VSS

(b) *Preferred 17552 Light chain graft variants comprising IGKV11-8 derived framework*

In one particularly preferred embodiment, a 17552 derived light chain variable region comprises the original CDRs of the 17552 light chain variable region. In particular, it comprises a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96. Such a set of CDRs may be combined with any of the framework regions set out below.

In one preferred embodiment, the 17552 derived light chain variable region has an IGKV1-8/IGKJ4 framework region, with the exception that one or more residues from the group comprising Leucine (L2), Valine (V3) and Glutamic acid (E63) are retained. In one embodiment, Leucine (L2) and Valine (V3) are retained. In one embodiment, Leucine (L2), Valine (V3) and Glutamic acid (E63) are retained.

In one embodiment, a free Cysteine residue at position 77 (C77) in FWR3 is mutated to Serine (C77S). Hence, in one embodiment Leucine (L2) and Valine (V3) are retained from the donor sequence and a (C77S) modification is also present. In another

embodiment, Leucine (L2), Valine (V3) and Glutamic acid (E63) are retained are retained from the donor sequence and a (C77S) modification is also present.

In one preferred embodiment, the FWR1, FWR2, FWR3, and FWR4 framework sequences of the light chain variable region comprise SEQ ID Nos: 114, 111, 115, and
5 113 respectively.

In a further preferred embodiment, the FWR1, FWR2, FWR3, and FWR4 framework sequences of the light chain variable region comprise SEQ ID Nos: 114, 111, 112, and 113 respectively.

A particularly preferred 17552 derived light chain graft variants is 17552gL1
10 (SEQ ID No: 25). A further particularly preferred 17552 derived light chain graft variants is 17552gL2 (SEQ ID No: 26).

(b) Preferred 17552 heavy chain graft variants comprising IGHV4-4 derived framework

In one particularly preferred embodiment, a 17552 derived heavy chain variable
15 region comprises the original HCDR1 and HCDR2 of the 17552 heavy chain variable region. In one preferred embodiment, it also comprises the original 17552 HCDR3. Alternatively, in further preferred embodiments, the HCDR3 has an aspartic acid (D) to glutamic acid (E) sequence change at the fourth amino acid of the HCDR3. In further preferred embodiments, it has a Glycine (G) to a Serine (S) or Alanine (A) amino acid
20 sequence change at the fifth amino acid of the CDR.

In one preferred embodiment, a 17552 derived heavy chain variable region comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO 101, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 102 to 105.

25 In one embodiment, the framework region of the 17552 derived heavy chain variable region has a framework region wherein FWR1 corresponds to that of the IGHV4-4 acceptor, except that the first amino acid Glutamine (Q1) has been substituted for Glutamic acid (E1), with Threonine (T23) retained from the donor sequence.

In one embodiment, the FWR2 corresponds to that of the acceptor sequence
30 except that Tyrosine (Y47) has been retained from the donor sequence.

In one embodiment, the FWR3 corresponds to that of the acceptor sequence except that Phenylalanine (F67), Lysine (K71), Serine (S73), Valine (V78) and Threonine (T96) are retained.

In one embodiment, the FWR4 corresponds to that of the IGHV4-4/IGHJ4 acceptor sequence.

In one embodiment, one or more of the following residues are retained from the donor 17552 sequence: Threonine (T23), 47 Tyrosine (Y47), Phenylalanine (F67),
 5 Lysine (K71), Serine (S73), Valine (V78) and Threonine (T96). In one preferred embodiment, all of those residues are retained. In a further preferred embodiment, all of those residues are retained and Glutamine (Q1) has been substituted for Glutamic acid (E1).

In one particularly embodiment, the FWR1, FWR2, FWR3, and FWR4 regions
 10 have respectively the amino acid sequences of SEQ ID Nos: 123, 124, 125, and 126.

One particularly preferred 17552 derived heavy chain variable region is that of 17552gH1 (SEQ ID No: 29). Another preferred variant is 17552gH2 (SEQ ID No: 30). A further preferred variant is 17552gH3 (SEQ ID No: 31). A further preferred variant is 17552gH4 (SEQ ID No: 32).

15 (c) *Preferred 17552 derived light and heavy chain pairings*

In one preferred embodiment, an antibody of the present invention comprises 17552 derived light and heavy chain variable region, for instance a pair of any of the 17552 derived light and heavy chain variable regions set out above.

Hence, in one embodiment, an antibody or antigen-binding fragment thereof of the
 20 invention comprises at least one variable domain specific for CD45 comprising the following light and heavy chain variable regions:

- (a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96; and
- 25 (b) a heavy chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO 101, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 102 to 105.

Preferred pairings include a 17552gL1 light chain variable region (SEQ ID No: 25) and a 17552gH1 (SEQ ID No: 29) heavy chain variable region. A further preferred
 30 pair is 17552gL1 (SEQ ID No: 25) and 17552gH2 (SEQ ID No: 30). Another preferred pair is 17552gL1 (SEQ ID No: 25) and 17552gH3 (SEQ ID No: 31). Another example of a preferred pair is 17552gL1 (SEQ ID No: 25) and 17552gH4 (SEQ ID No: 32).

Further preferred pairings include a 17552gL2 (SEQ ID No: 26) light chain variable region and a 17552gH1 (SEQ ID No: 29) heavy chain variable region. A further preferred pair is 17552gL2 (SEQ ID No: 26) and 17552gH2 (SEQ ID No: 30). Another preferred pair is 17552gL2 (SEQ ID No: 26) and 17552gH3 (SEQ ID No: 31). Another example of a preferred pair is 17552gL2 (SEQ ID No: 26) and 17552gH4 (SEQ ID No: 32).

(d) *Variants and derivatives*

As well as the specific 17552 derived sequences set out herein, also provided are variants and derivatives of the specific sequences as described elsewhere herein. Such a variant will retain the ability to specifically bind CD45. It will preferably retain the ability to specifically bind to both human and cynomolgus monkey CD45.

Illustrative antibody formats

The term “antibody” is not limited to a conventional four chain IgG antibody with two identical light chains and two identical heavy chains. It includes any format which has at least one antigen-binding site formed by CDRs and in particular by a set of six CDRs. The antibodies of the present invention may be a complete antibody having full length heavy and light chains or a fragment thereof. Any reference herein to an antibody also encompasses an antigen-binding fragment of the antibody instead being employed unless stated otherwise. Examples of types of antibodies and antibody fragments include, for instance, a Fab, modified Fab, Fab', modified Fab', F(ab')₂, Fv, single domain antibody (e.g. VH or VL or VHH), scFv, bi, tri or tetra-valent antibody, Bis-scFv, diabody, triabody, tetrabody or epitope-binding fragments of any of the above (see for example Holliger and Hudson, 2005, Nature Biotech. 23(9):1126-1136; Adair and Lawson, 2005, Drug Design Reviews - Online 2(3), 209-217). In one embodiment, the antibodies of the present invention are not single domain antibodies. The methods for creating and manufacturing antibody fragments are well known in the art (see for example Verma *et al.*, 1998, Journal of Immunological Methods, 216, 165-181). Other antibody fragments for use in the present invention include the Fab and Fab' fragments described in international patent applications WO 2005/003169, WO 2005/003170 and WO 2005/003171. Multi-valent antibodies may comprise multiple specificities e.g. bispecific or may be monospecific (see for example WO 92/22853, WO 05/113605,

WO 2009/040562 and WO 2010/035012). In one particularly preferred embodiment an antibody of the present invention is an antibody which is monospecific for CD45. In a further particularly preferred embodiment, an antibody of the present invention is biparatopic for CD45. The term antibody includes monospecific, bispecific, and multi-specific antibodies. Thus, for instance, the term “antibody” specifically includes formats such as the Fab-X/Fab-Y, BYbe and TrYbe. The term “antibody” also includes antibody fragments, preferably those mentioned herein. Anywhere reference is made herein to an antibody an antigen-binding antibody fragment may be employed as well unless the specific context dictates otherwise.

Examples of possible antibody formats are known in the art, for example as disclosed in the review “The coming of Age of Engineered Multivalent Antibodies, Nunez-Prado *et al* Drug Discovery Today Vol 20 Number 5 Mar 2015, page 588-594, D. Holmes, Nature Rev Drug Disc Nov 2011:10; 798, Chan and Carter, Nature Reviews Immunology vol. 10, May 2010, 301, incorporated herein by reference. In one embodiment, an antibody of the invention may comprise, consist essentially of, or consist of any of the formats set out below. Antibodies based on sequences derived from the rabbit 17415 and 17552 antibodies described herein are particularly preferred. Hence, for any of the formats set out below, in a preferred embodiment, the antibody will comprise at least one antigen-binding site comprising humanised light and heavy chain variable regions derived from the rabbit 17415 antibody. In another preferred embodiment, for any of the formats set out below the antibody will comprise at least one antigen-binding site comprising humanised light and heavy chain variable regions derived from the rabbit 17552 antibody. In one particularly preferred embodiment, for those formats with at least two antigen-binding sites, they will comprise at least one antigen-binding site comprising humanised light and heavy chain variable regions derived from the rabbit 17415 antibody and at least one antigen-binding site comprising humanised light and heavy chain variable regions derived from the rabbit 17552 antibody.

One especially preferred antibody format is an IgG format antibody.

A “binding fragment” as employed herein refers to a fragment capable of binding a target peptide or antigen with sufficient affinity to characterise the fragment as specific for the peptide or antigen. The term “Fab fragment” as used herein refers to an antibody fragment comprising a light chain fragment comprising a V_L (variable light) domain and a constant domain of a light chain (C_L), and a V_H (variable heavy) domain and a first constant domain (CH₁) of a heavy chain. The term “Fv” refers to two variable domains,

for example co-operative variable domains, such as a cognate pair or affinity matured variable domains, i.e. a V_H and V_L pair. In one embodiment such fragments are used as an antibody molecule of the present invention. Co-operative variable domains as employed herein are variable domains that complement each other and/or both contribute to antigen binding to render the Fv (V_H/V_L pair) specific for the antigen in question.

An 'antigen-binding site' as employed herein refers to a binding region, typically a polypeptide, capable of binding a target antigen, for example with sufficient affinity to characterise the site as specific for the antigen. In one embodiment the binding site contains at least one variable domain or a derivative thereof, for example a pair of variable domains or derivatives thereof, such as a cognate pair of variable domains or a derivative thereof. Typically, this is a V_H/V_L pair. Variable regions (also referred to herein as variable domains) generally comprise 3 CDRs and a suitable framework. In one embodiment, an antigen-binding site comprises two variable regions, a light chain variable region and a heavy chain variable region and together these elements contribute to the specificity of the binding interaction of the antibody or binding fragment for CD45 and in particular for the specificity in terms of where on CD45 the binding site binds. In one particularly preferred embodiment, the V_H/V_L pair is humanised. The six CDRs provided by a light and heavy chain variable region pairing may be referred to as a "CDR set".

The residues in antibody variable domains are conventionally numbered according to a system devised by Kabat *et al.* This system is set forth in Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat *et al. (supra)*"). The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or complementarity determining region (CDR), of the basic variable domain structure. The correct Kabat numbering of residues may be determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence. The CDRs of the heavy chain variable domain are located at residues 31-35 (CDR-H1), residues 50-65 (CDR-H2) and residues 95-102 (CDR-H3) according to the Kabat numbering system. However, according to Chothia (Chothia, C. and Lesk, A.M. J. Mol. Biol., 196, 901-917 (1987)), the loop equivalent to CDR-H1 extends from residue

26 to residue 32. Thus, unless indicated otherwise ‘CDR-H1’ as employed herein is intended to refer to residues 26 to 35, as described by a combination of the Kabat numbering system and Chothia’s topological loop definition. The CDRs of the light chain variable domain are located at residues 24-34 (CDR-L1), residues 50-56 (CDR-L2) and
5 residues 89-97 (CDR-L3) according to the Kabat numbering system.

In the present application where humanisation is discussed linear amino acid numbering is used, rather than Kabat numbering. Hence, the discussion of amino acid positions in the graft variants adopts linear amino acid numbering.

10 **Derivatives, modifications, and humanization**

As well as the specific antibodies set out herein, variants and derivatives of the same are provided. Such variants and derivatives will, at a minimum, retain the ability to specifically bind to CD45. In one preferred embodiment, they will retain their biological
15 function(s) as described herein, e.g. the ability to kill or deplete target cells expressing CD45. Any of the specific assays and target cells employed herein may be used to, for instance, confirm such activity. In one particularly preferred embodiment, a variant or derivative will retain the ability to specifically bind both human and cynomolgus monkey CD45. Assays such as Biacore may be used to confirm such ability or cell-based assays.
20 Again, any of the specific methods used in the Examples of the present application may be employed to confirm ability to specifically bind human and cynomolgus CD45.

A “variant” or “derivative” as employed herein, may, for example, have one, two, three, four or five or more amino acid sequence changes compared to a specific sequence set out herein. In one embodiment, a paratope or antigen-binding site may comprise one
25 of the sets of six specific CDRs set out herein apart from a total of up to seven amino acid sequence changes across all six CDRs compared to the specific sequences. In one embodiment there are up to six amino acid sequence changes. In another embodiment, there are up to five amino acid sequence changes. In another embodiment, there are up to four amino acid sequence changes. In one preferred embodiment, there are up to three
30 amino acid sequence changes. In one more preferred embodiment, there are up to two amino acid sequence changes. In a particularly preferred embodiment, there is only one amino acid sequence change compared to the specific six CDRs set out. Any such variants will retain the ability to specifically bind human CD45. Preferably, the variant will retain the ability to specifically bind to both human and cynomolgus monkey CD45.

In another embodiment such numbers of sequence changes may be in the overall variable regions for the paratopes compared to those of the specific antibody set out herein.

Modification in the CDRs may, for example, include replacing one or more cysteines with, for example a serine residue. Asn can be the substrate for deamination and this propensity can be reduced by replacing Asn and/or a neighboring amino acid with an alternative amino acid, such as a conservative substitution. The amino acid Asp in the CDRs may be subject to isomerization. The latter can be minimized by replacing Asp and/or a neighboring amino acid with an alternative amino acid, for example a conservative substitution. Amino acid sequences may be used to eliminate or reduce undesirable properties but wherein the characterizing feature(s) is/are retained. Examples of modifications are those to remove glycosylation sites, GPI anchors, or solvent exposed lysines. These modifications can be achieved by replacing the relevant amino acid residues with a conservative amino acid substitution.

15 **Antibody constant regions and Fc region functions**

In one preferred embodiment, an antibody of the present invention does not comprise an Fc domain. In an alternative preferred embodiment, an antibody of the present invention comprises an altered Fc domain as described herein below. In a preferred embodiment an antibody of the present invention comprises an Fc domain, but the sequence of the Fc domain has been altered to remove one or more Fc effector functions. In another embodiment, the Fc region of an antibody of the present invention has been modified to optimise a particular property of the antibody, such as any of those discussed herein.

25 In one embodiment, an antibody of the present invention comprises a “silenced” Fc region. For example, in one embodiment an antibody of the present invention does not display the effector function or functions associated with a normal Fc region.

Fc domain as employed herein generally refers to $-(CH_2CH_3)_2$, unless the context clearly indicates otherwise.

30 In one embodiment, an antibody of the present invention does not comprise a $-CH_2CH_3$ fragment.

In one embodiment, an antibody of the present invention does not comprise a CH_2 domain.

In one embodiment, an antibody of the present invention does not comprise a CH₃ domain.

In one embodiment, an antibody of the present invention does not bind Fc receptors.

5 In one embodiment, an antibody of the present invention does not bind complement. In one preferred embodiment, an antibody of the present invention does not bind the first complement factor, C1q or C1. In one embodiment, an antibody of the invention does not bind those factors because, for example, it lacks an Fc region. In another embodiment, an antibody of the present invention does not bind those factors
10 because it has a modification in the constant region preventing its ability to do so. In an alternative embodiment, an antibody of the invention does not bind FcγR, but does bind complement. For example, in one embodiment, an antibody of the invention does not bind FcγR, but does bind C1q and/or C1.

In one embodiment the antibody of the present invention does not comprise an
15 active Fc region in the sense that the antibody does not trigger the release of one or more cytokines which a normal Fc region would trigger the release of. For instance, the Fc region of an antibody of the invention may not trigger the release of cytokines when it binds to an Fc receptor or may not significantly do so.

In one embodiment, antibodies of the present invention in general may comprise
20 modifications that alter serum half-life of the antibody. Hence, in another embodiment, an antibody of the present invention has Fc region modification(s) that alter the half-life of the antibody. Such modifications may be present as well as those that alter Fc functions. In one embodiment, an antibody of the present invention has modification(s) that increase or decrease serum half-life of the antibody compared to an antibody lacking
25 such modifications. In another embodiment, an antibody of the present invention comprises modification(s) that collectively both silence the Fc region and increase or decrease the serum half-life of the antibody compared to an antibody lacking such modifications.

The antibody constant region domains of an antibody of the present invention, if
30 present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. In a preferred embodiment, an antibody is one that lacks an Fc or lacks one or more effector function of an Fc region and preferably all of them. In other embodiments of the invention, the effector function(s) of the Fc region of the antibody may be still present. In one

embodiment, an antibody of the invention may comprise a human constant region, for instance IgA, IgD, IgE, IgG or IgM constant domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when the antibody molecule is intended for therapeutic uses where antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required. Particularly preferred IgG isotypes are IgG2 and IgG4. The constant region may have been modified in a preferred embodiment so that the antibody does not have effector functions. Hence, it will be appreciated that sequence variants of these constant region domains may also be used. For example, IgG4 molecules in which the serine at position 241 has been changed to proline as described in Angal *et al.*, 1993, *Molecular Immunology*, 1993, 30:105-108 may be used. Accordingly, in the embodiment, where the antibody is an IgG4 antibody, the antibody may include the mutation S241P. In another embodiment, an antibody of the invention may lack an Fc region.

15 An antibody of the invention may have, in one embodiment, a silenced Fc region. The term “silent”, “silenced”, or “silencing” as used herein refers to an antibody having a modified Fc region described herein that has decreased binding to an Fc gamma receptor (FcγR) relative to binding of an identical antibody comprising an unmodified Fc region to the FcγR (e.g., a decrease in binding to a FcγR by at least 70%, at least 80%, at least 20 90%, at least 95%, at least 98%, at least 99%, or 100% relative to binding of the identical antibody comprising an unmodified Fc region to the FcγR as measured by, e.g., BLI). In some embodiments, the Fc silenced antibody has no detectable binding to an FcγR. Binding of an antibody having a modified Fc region to an FcγR can be determined using a variety of techniques known in the art, for example but not limited to, equilibrium 25 methods (e.g., enzyme-linked immunoabsorbent assay (ELISA); KinExA, Rathanaswami *et al.* *Analytical Biochemistry*, Vol. 373:52-60, 2008; or radioimmunoassay (RIA)), or by a surface plasmon resonance assay or other mechanism of kinetics-based assay (e.g., BIACORE™ analysis or Octet™ analysis (forteBIO)), and other methods such as indirect binding assays, competitive binding assays fluorescence resonance energy 30 transfer (FRET), gel electrophoresis and chromatography (e.g., gel filtration). In another embodiment, an antibody of the present invention may have been modified to reduce or eliminate binding to the FcγR, but still allow activation of complement. In another embodiment, an antibody of the present invention may have a modified Fc region such that it does not activate cytokine release, but is still able to activate complement.

In one embodiment, the antibody heavy chain comprises a CH₁ domain and the antibody light chain comprises a CL domain, either kappa or lambda. In one embodiment, the antibody heavy chain comprises a CH₁ domain, a CH₂ domain and a CH₃ domain and the antibody light chain comprises a CL domain, either kappa or
5 lambda. Kappa light chains are preferred.

The four human IgG isotypes bind the activating Fcγ receptors (FcγRI, FcγRIIa, FcγRIIc, FcγRIIIa), the inhibitory FcγRIIb receptor, and the first component of complement (C1q) with different affinities, yielding very different effector functions (Bruhns P. *et al.*, 2009. Specificity and affinity of human FcγRs and their polymorphic
10 variants for human IgG subclasses. *Blood*. 113(16):3716-25), see also Jeffrey B. Stavenhagen, *et al. Cancer Research* 2007 Sep 15; 67(18):8882-90. In one embodiment, an antibody of the invention does not bind to Fc receptors. In another embodiment of the present invention, the antibody does bind to one or more type of Fc receptor.

Binding of IgG to the FcγRs or C1q depends on residues located in the hinge
15 region and the CH₂ domain. Two regions of the CH₂ domain are critical for FcγRs and C1q binding, and have unique sequences in IgG2 and IgG4. Substitutions into human IgG1 of IgG2 residues at positions 233-236 and IgG4 residues at positions 327, 330 and 331 have been shown to greatly reduce ADCC and CDC (Armour KL. *et al.*, 1999. Recombinant human IgG molecules lacking Fc gamma receptor I binding and monocyte triggering activities. *Eur J Immunol*. 29(8):2613-24 and Shields RL. *et al.*, 2001). High
20 resolution mapping of the binding site on human IgG1 for Fc gamma RI, Fc gamma RII, Fc gamma RIII, and FcRn and design of IgG1 variants with improved binding to the Fc gamma R. *J Biol Chem*. 276(9):6591-604). Furthermore, Idusogie *et al.* demonstrated that alanine substitution at different positions, including K322, significantly reduced
25 complement activation (Idusogie EE. *et al.*, 2000. Mapping of the C1q binding site on rituxan, a chimeric antibody with a human IgG1 Fc. *J Immunol*. 164(8):4178-84). Similarly, mutations in the CH₂ domain of murine IgG2A were shown to reduce the binding to FcγRI, and C1q (Steurer W. *et al.*, 1995. *Ex vivo* coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. *J Immunol*. 155(3):1165- 74).

30 In one embodiment the Fc region employed is mutated, in particular a mutation described herein. In one embodiment the mutation is to remove binding and/or effector function. In one preferred embodiment the antibody of the invention has been mutated so that it does not bind Fc receptors. In another preferred embodiment, an antibody of the

present invention does not comprise an Fc region and so does not display Fc effector activity for that reason. In one embodiment the Fc mutation is selected from the group comprising a mutation to remove or enhance binding of the Fc region to an Fc receptor, a mutation to increase or remove an effector function, a mutation to increase or decrease half-life of the antibody and a combination of the same. In a preferred embodiment, the modification eliminates or reduces binding to Fc receptors. In another preferred embodiment, the modification eliminates or reduces an Fc effector function. In another preferred embodiment, the modification increases or reduces serum half-life. In another preferred embodiment, the constant region of the antibody comprises a modification or modifications that reduce or eliminate Fc receptor binding, and Fc effector function, as well as increasing or reducing serum half-life. In one embodiment, where reference is made to the impact of a modification, it may be demonstrated by comparison to the equivalent antibody but lacking the modification.

In another embodiment of the present invention, an antibody may have heavy chain modifications that modify the ability to bind Protein A and in particular to eliminate Protein A binding. As discussed herein, such an approach may be preferably used to facilitate purification of bispecific antibodies. However, in other embodiments, any antibody of the invention may be modified, if it has an Fc region, to alter Protein A binding. For example, both heavy chains may include the modification. Alternatively, both heavy chains may lack the modification. In a preferred embodiment though, one has the modification and the other not.

Some antibodies that selectively bind FcRn at pH 6.0, but not pH 7.4, exhibit a higher half-life in a variety of animal models. Several mutations located at the interface between the CH₂ and CH₃ domains, such as T250Q/M428L (Hinton PR. *et al.*, 2004. Engineered human IgG antibodies with longer serum half-lives in primates. J Biol Chem. 279(8):6213-6), M252Y/S254T/T256E + H433K/N434F (Vaccaro C. *et al.*, 2005. Engineering the Fc region of immunoglobulin G to modulate *in vivo* antibody levels. Nat Biotechnol. 23(10):1283-8) and M428L/N434S (Zalevsky *et al.*, 2010, Enhanced antibody half-life improves *in vivo* activity. Nature Biotech 28:157-159), have been shown to increase the binding affinity to FcRn and the half-life of IgG1 *in vivo*. Hence, modifications may be present at M252/S254/T256 + H44/N434 that alter serum half-life and in particular M252Y/S254T/T256E + H433K/N434F may be present. However, there is not always a direct relationship between increased FcRn binding and increased half-life (Datta-Mannan A. *et al.*, 2007. Humanized IgG1 Variants with Differential Binding

Properties to the Neonatal Fc Receptor: Relationship to Pharmacokinetics in Mice and Primates. Drug Metab. Dispos. 35: 86 – 94). In one embodiment, it is desired to increase half-life. In another embodiment, it may be actually desired to decrease serum half-life of the antibody and so modifications may be present that decrease serum half-life.

5 IgG4 subclass show reduced Fc receptor (FcγRIIIa) binding. Antibodies of other IgG subclasses generally show strong binding. Reduced receptor binding in these other IgG subtypes can be affected by altering, for example replacing, one or more amino acids selected from the group comprising Pro238, Aps265, Asp270, Asn270 (loss of Fc carbohydrate), Pro329, Leu234, Leu235, Gly236, Gly237, Ile253, Ser254, Lys288,
10 Thr307, Gln311, Asn434 and His435. In one embodiment a molecule according to the present invention has an Fc of IgG subclass, for example IgG1, IgG2 or IgG3 wherein the Fc is mutated in one, two or all following positions S228, L234 and/or D265. In one embodiment the mutations in the Fc region are independently selected from S228P, L234A, L235A, L235E and combinations thereof.

15 In one embodiment, an antibody of the present invention may comprise modifications that influence whether an antibody brings about cytokine release. In particular, the L234F and K274Q modifications are shown to reduce the ability of the antibody to bring about cytokine release. Hence, in one embodiment, an antibody of the present invention may comprise modifications at L234 and/or K274 that alter cytokine
20 release and in particular the L234F and K274Q modifications. Further, the L234 residue may have an impact on platelet activation and that residue may be additionally or alternatively modified. In one embodiment of the invention, for example a L234 modification that alters platelet binding and in particular an L234F modification may be introduced. P331 is also shown to play a role in C1q binding, so in one embodiment
25 P331 may be unmodified in order to retain complement activation. In another it may be modified to reduce or eliminate complement activation; for instance, the heavy chains may comprise a P331S modification. In another embodiment, a P329 modification is present that reduces or eliminates complement binding, in particular a P329A modification. In another embodiment, the antibody may comprise one or more of the
30 modifications at positions P329, P331, K332 and/or D265. In one preferred embodiment, an antibody may comprise modifications at P329A, P331S, K332A, and D265A to influence complement binding and in particular to reduce C1q binding.

It may be desired to either reduce or increase the effector function of an Fc region. In one preferred embodiment, it is desired to decrease such effector functions. In another, it is desired to optimise it. With antibodies that target cell-surface molecules, especially those on immune cells, abrogating effector functions is typically required. In other instances, particularly where the aim is to deplete cells, it may be desirable for Fc effector functions to have been eliminated or reduced to as low a level as possible. For instance, in a particularly preferred embodiment, an antibody of the present invention is able to induce cell death in target cells expressing CD45, but does not display Fc effector functions. Hence, in one preferred embodiment, an antibody of the invention lacks an active Fc region. For instance, the antibody may not physically have an Fc region or the antibody may comprise modifications that render the Fc region inactive. The latter may be, for instance, referred to as Fc silencing. In one embodiment, the Fc silencing may mean that an antibody of the invention is less able, or does not, bring about release of one or more cytokine which an antibody with an unmodified Fc region would usually trigger release of. In one preferred embodiment, an antibody of the invention is able to stimulate cell death but does not display Fc functions. Further examples of Fc functions include the stimulation of degranulation of Mast cells and again that function may be reduced or absent in an antibody of the invention. The degree in reduction of Fc function may be, for instance, at least 65%, and, for example, at least 75%. In one embodiment, the reduction is at least 80%. In another embodiment, the reduction is at least 90%. The reduction may be, for instance, at least 95%. In one preferred embodiment, the reduction is by at least 99%. In another embodiment, the reduction may be 100%, meaning that Fc function is completely eliminated in such instances.

Numerous mutations have been made in the CH₂ domain of human IgG1 and their effect on ADCC and CDC tested *in vitro* (Idusogie EE. *et al.*, 2001. Engineered antibodies with increased activity to recruit complement. J Immunol. 166(4):2571-5). Notably, alanine substitution at position 333 was reported to increase both ADCC and CDC. Hence, in one embodiment a modification at position 333 may be present, and in particular one that alters ability to recruit complement. Lazar *et al.* described a triple mutant (S239D/I332E/A330L) with a higher affinity for FcγRIIIa and a lower affinity for FcγRIIb resulting in enhanced ADCC (Lazar GA. *et al.*, 2006). Hence, modifications at S239/I332/A330 may be present, particularly those that alter affinity for Fc receptors and in particular S239D/I332E/A330L. Engineered antibody Fc variants with enhanced effector function. PNAS 103(11): 4005–4010). The same mutations were used to

generate an antibody with increased ADCC (Ryan MC. *et al.*, 2007. Antibody targeting of B-cell maturation antigen on malignant plasma cells. *Mol. Cancer Ther.*, 6: 3009 – 3018). Richards *et al.* studied a slightly different triple mutant (S239D/I332E/G236A) with improved FcγRIIIa affinity and FcγRIIa/FcγRIIb ratio that mediates enhanced phagocytosis of target cells by macrophages (Richards JO *et al.* (2008) Optimization of antibody binding to FcγRIIIa enhances macrophage phagocytosis of tumor cells. *Mol Cancer Ther.* 7(8):2517-27). In one embodiment, S239D/I332E/G236A modifications may be therefore present.

Due to their lack of effector functions, IgG4 antibodies represent a suitable IgG subclass for receptor blocking. IgG4 molecules can exchange half-molecules in a dynamic process termed Fab-arm exchange. This phenomenon can occur between therapeutic antibodies and endogenous IgG4. In one preferred embodiment, an antibody of the present invention has a modification at S228 and in particular S228P. The S228P mutation has been shown to prevent this recombination process allowing the design of less unpredictable therapeutic IgG4 antibodies (Labrijn AF. *et al.*, 2009. Therapeutic IgG4 antibodies engage in Fab-arm exchange with endogenous human IgG4 *in vivo*. *Nat Biotechnol.* 27(8):767-71). This technology may be employed to create bispecific antibody molecules. The modifications set out herein may, in a preferred embodiment, be employed in the context of IgG4.

WO 2008/145142 discloses examples of modifications and in particular modifications for IgG4 isotype antibodies that may be employed in the present invention. In one embodiment, the heavy chains of an antibody of the present invention may comprise a human IgG4 constant region having a substitution of the Arg residue at position 409, the Phe residue at position 405 and/or the Lys residue at position 370. For example, in one preferred embodiment the heavy chains of the antibody comprise a modification at position 409 and in particular one selected from the introduction of a Lys, Ala, Thr, Met, or Leu residue at that position. In one embodiment, the modification is the introduction of a Lys, Thr, Met, or Leu residue at position 409. In another embodiment, the modification may be the introduction of a Lys, Met or Leu residue at position 409. In one embodiment, the antibody does not comprise a Cys-Pro-Pro-Cys in the hinge region. In one embodiment, the antibody shows reduced ability to induce Fab arm exchange *in vivo*. In one embodiment, the hinge region of the antibody comprises a CXPC or CPXC sequence where X is any amino acid except proline. In one embodiment, an antibody of the invention may employ the ability of a particular antibody class, antibody isotype, or

antibody allotype to display a particular property. Such natural diversity may be used to confer a particular property. For example, IgG1 has R409 whereas IgG4 has K409 at position 409 of the heavy chain which may naturally influence the ability of the antibody. A review of various naturally occurring sequence variations is provided in Jefferis *et al* (2009) mAbs, 1(4): 332-338, which is incorporated by reference in its entirety in particular in relation to the sequence variations discussed therein.

It will also be understood by one skilled in the art that antibodies may undergo a variety of post-translational modifications. The type and extent of these modifications often depends on the host cell line used to express the antibody as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, RJ. Journal of Chromatography 705:129-134, 1995). Accordingly, the C-terminal lysine of the antibody heavy chain may be absent.

In one embodiment, an antibody of the present invention may be an aglycosyl IgG, for example to bring about reduced Fc function and in particular a nearly Fc-null phenotype. In one embodiment, an antibody of the invention has a modification at N297 and in particular N297A. In one embodiment an antibody of the invention has modifications at F243 and/or F244, in particular ones that mean that the antibody is an aglycosyl IgG. In one embodiment, an antibody of the present invention may comprise the F243A and/or F244A heavy chain modifications. In another embodiment, one or more of F241, F243, V262 and V264 may be modified and particularly to amino acids that influence glycosylation. In one embodiment, an antibody of the present invention may have modifications at F241A, F243A, V262E and V264E. Such modifications are discussed in Yu *et al* (2013) 135(26): 9723-9732, which is incorporated by reference in its entirety, particularly in relation to the modifications discussed therein. Such modifications provide a way to modulate, for example, Fc receptor binding. A modification which influences the glycosylation of the antibody may be present. Further, an antibody of the invention may be produced in a cell type that influences glycosylation as a further approach for sugar engineering. In one embodiment, the fucosylation, sialylation, galactosylation, and/or mannosylation of an antibody of the present invention may be altered either by sequence modifications and/or via the type of cell used to produce the antibody.

In one embodiment, an antibody of the present invention has modifications at position 297 and/or 299. For example, in one embodiment, an antibody of the present invention comprises a N297A modification in its heavy chains, preferably N297Q or mutation of Ser or Thr at 299 to other residues. In one embodiment it has both those
5 modifications.

An example of particularly preferred constant region modifications are the Leu234Ala and Leu235Ala modifications (according to EU numbering) also known as the LALA modification. In particular, an IgG1 antibody format with a LALA modification is a preferred as a format. In a further preferred embodiment, the antibody
10 of the present invention is an IgG4 antibody with a FALA modification.

In another embodiment, an antibody of the present invention may have a modified hinge region and/or CH1 region. Alternatively, the isotype employed may be chosen as it has a particular hinge region. As described in White *et al* (2015) *Cancer Cell* 27(1): 138-148), the IgG2 CH1 and hinge regions confer particular properties, particularly in
15 relation to disulphide bridges between the heavy and light chains. The use of modifications to favour flexibility in the hinge region or reduced flexibility may also be employed, for example, in an antibody of the present invention. Approaches to alter hinge region flexibility are disclosed in Liu *et al* (2019) *Nature Communications* 10: 4206. White *et al* (2015) and Liu *et al* (2019) are incorporated by reference in their
20 entirety, particularly in relation to the modifications discussed. In one embodiment, a heavy chain of an antibody of the present invention has an IgG2 CH1 and/or hinge region and in another embodiment both heavy chains do so. In one embodiment, the antibody employed is an IgG1 antibody. In a particularly preferred embodiment, the antibody employed may be an IgG2 or IgG4 antibody with a hinge or CH1 modification, in
25 particular one with a modified hinge region, for example one engineered to alter disulphide bond formation. In another embodiment, an IgG2 or IgG4 isotype antibody is employed, as the hinge regions of those isotypes show less flexibility than an IgG3 isotype antibody. In one embodiment, an IgG4 isotype antibody is employed in a form that may be able to bring about CD32 cross-linking.

30 In another embodiment, the antibody shows the best ability sterically to bring about cross-linking of CD45 molecules.

Examples of particularly preferred heavy chain constant region sequences comprising the LALA modification are provided respectively as SEQ ID NOs: 144, 146, and 148.

The heavy chain constant region of SEQ ID NO: 148 has the so called “knob” modification, T336W. The heavy chain constant region of SEQ ID NO: 146 has the so called “hole” modifications, T366S, L366S and Y407. The “knob-into-holes” modifications promote heterodimer formation and so formation of biparatopic antibody over monospecific antibody. As such, in a particularly preferred embodiment, such heavy chain constant regions are employed for biparatopic antibodies of the invention.

An example of a particularly preferred light chain constant region sequence comprising is provided respectively as SEQ ID NOs: 145.

In one particularly preferred embodiment, the heavy chain and light chain constant regions in a monospecific antibody of the invention are those of SEQ ID NO: 144 and 145. In one embodiment those constant regions are used together with the VR17415gL15gH6 variable regions.

In another particularly preferred embodiment, the heavy chain and light chain constant regions in a biparatopic antibody of the invention are those of SEQ ID NO: 146 and 148 for the heavy chains and SEQ ID NO: 145 for the light chain. In one embodiment the constant regions used for the VR17552gL1gH4 portion of a biparatopic antibody are those of SEQ ID NOs: 148 and 145. In one embodiment the constant regions used for the VR17552gL1gH4 portion of a biparatopic antibody are those of SEQ ID NOs: 146 and 145.

In one embodiment the constant regions used for the VR17415gL15gH6 portion of a biparatopic antibody are those of SEQ ID NOs: 148 and 145. In one embodiment the constant regions used for the VR17415gL15gH6 portion of a biparatopic antibody are those of SEQ ID NOs: 146 and 145.

In one embodiment the constant regions used for the VR17552gL1gH4 portion of a biparatopic antibody are those of SEQ ID NOs: 148 and 145 and the constant regions used for the VR17415gL15gH6 portion of a biparatopic antibody are those of SEQ ID NOs: 148 and 145. In one embodiment the constant regions used for the VR17552gL1gH4 portion of a biparatopic antibody are those of SEQ ID NOs: 146 and 145 and the constant regions used for the VR17415gL15gH6 portion of a biparatopic antibody are those of SEQ ID NOs: 146 and 145.

Bispecific and biparatopic antibodies

In one preferred embodiment, an antibody of the present invention is bispecific.
In a preferred embodiment, it is a biparatopic antibody for CD45, i.e. has two
5 specificities for CD45. A variety of bispecific antibody formats are available for
favouring formation, or purification, of bispecific antibodies over monospecific
antibodies when the different heavy and light chains for the specificities are expressed
together, and these may be employed in the present invention.

In one embodiment, an antibody of the present invention may have modifications
10 that favour the formation of an antibody of the invention over unwanted species. Such
modifications are particularly preferred where an antibody of the invention comprises at
least two different antigen-binding sites recognising different epitopes. In particular, such
modifications are particularly preferred for bispecific and biparatopic antibodies. For
example, in one embodiment the production of an antibody of the invention may involve
15 two different antigen-binding sites, in particular two different paratopes, being on
different polypeptide chains and associated. Hence, it may be desirable to form
heterodimers which include both specificities in preference to homodimers which only
include one of the two. An example of an approach that favours heterodimer formation is
employing heavy chain modifications that favour two different heavy chains, rather than
20 two of the same heavy chains associating. In one embodiment one (or at least one) of the
binding partners is incapable of forming a homodimer, for example an amino acid
sequence of the binding partner is mutated to eliminate or minimise the formation of
homodimers. Examples of such modifications include so called "knobs-into-holes"
modifications. Possible knobs-into-holes modifications are set out, for instance, in
25 Merchant *et al* (1998) *Nature Biotechnology* 16(7): 677-681 and Carter *et al* (2001) *J*
Immunol Methods, 248(1-2): 7-15, which are both incorporated by reference in particular
in relation to the knobs-into-holes modifications discussed therein. Charge modifications
may be alternatively or additionally employed to favour formation of heterodimers over
homodimers, for example such modifications may be present in the heavy chains. In
30 another embodiment, charge modifications are used to bring about pairing of a particular
light chain with a particular heavy chain.

In one embodiment, such approaches for favouring heterodimer formation are
used in combination with a common light chain approach. In another embodiment, it may
be that rather favouring the formation of heterodimer over homodimers, modifications

are present that mean the heterodimers can be separated from the homodimers more easily, for instance by chromatography. Again, such an approach may be, in some embodiments, employed with a common light chain approach. In another embodiment, the portions of the antibody carrying a particular paratope against CD45 are only able to
5 associate with those portions of the antibody which comprise the different paratope of the antibody.

Incapable of forming homodimers as employed herein, refers to a low or zero propensity to form homodimers. Low as employed herein refers to 20%, 10%, 5% or less, such as 4, 3, 2, 1, 0.5% or less aggregate.

10 Heavy chain modifications may also be employed so that one heavy chain has a different affinity for a binding agent compared to the other. For example, the two different heavy chains may have different affinity for Protein A. In one embodiment, one heavy chain has a modification that eliminates Protein A binding or is of an isotype that does not bind Protein A, whilst the other heavy chain does still bind Protein A. Whilst
15 such an approach does not alter the proportion of heterodimer formed, it does allow the purification of the heterodimeric antibody from either of the homodimeric antibodies based on Protein A affinity. An antibody of the present invention may have modifications at positions 95 and 96 of one of the heavy chains that influence Protein A binding. Examples of such modifications that may be employed include employing a H95R
20 modification for one heavy chain or the H95R and Y96F modifications both in the IMGT exon numbering system. Those modifications are the H435R modification and H435R and Y436F modification in the EU numbering system. In one embodiment, an antibody of the present invention may also have modifications at D16, L18, N44, K52, V57 and V82. In one embodiment, such modifications are present in the heavy chain as well as
25 one or more of the D16E, L18M, N44S, K52N, V57M and V82I modifications in the IMGT numbering system. In one embodiment, such modifications are employed where the IgG is IgG1, IgG2 or IgG4. In a particularly preferred embodiment, they are employed for one of the two heavy chains where both heavy chains are the IgG4 isotype. The approach of such modifications to influence Protein A binding is described in, for
30 instance, US 2010/0331527 A1, which is incorporated by reference in its entirety and in particular in relation to the modifications it discloses that relate to Protein A binding.

In a further embodiment, the isotype of the heavy chains employed may be chosen based on their ability to bind Protein A. For example, in humans IgG1, IgG2, and IgG4 in their wild type form all bind Protein A, whereas wild type human IgG3 does not.

In a particularly preferred embodiment, both heavy chains are IgG4, but one has modification(s) to reduce or eliminate Protein A binding. That means the heterodimeric form of the antibody will be able to be separated from the unwanted homodimeric forms more readily based on Protein A affinity.

5 In one embodiment, modifications to promote heterodimer formation may be combined with those that allow purification of the heterodimer. In one embodiment, the modifications may be at positions F405 and K409. For example, one example of a pair of modifications that may be introduced into the two heavy chains to favour heterodimer formation are F405L and K409R. Those modifications may be employed on their own or
10 in combination with heavy chain modifications allowing preferential purification of the heterodimer. In one embodiment, one heavy chain has modifications at positions 405, 409, 435, and 436 and the other heavy chain at position 409. In one embodiment, one heavy chain has the F405L modification with the other having the K409R, H435R and Y436F modifications. In another embodiment, one heavy chain has the F405L, H435R
15 and Y436F modification and the other heavy chain has the K409R modification. Examples of such approaches are described in Steinhardt *et al* (2020) *Pharmaceutics*, 12, 3, which is incorporated by reference in its entirety, in particular in relation to the bispecific antibody formats and heavy chain modifications described. In another embodiment, approaches concerned with the light chain may be employed and in
20 particular in addition to the approaches for the heavy chain discussed above. In one embodiment, the Roche Cross-Mab approach is applied. In another embodiment a common light chain may be employed so that the same light chain is employed for both specificities. Various bispecific antibody formats are reviewed in Spiess *et al* (2015) *Molecular Immunology* 67: 95-106 and may be employed in the present invention.

25 In one particularly preferred embodiment, a biparatopic antibody of the present invention is an IgG format antibody. In one preferred embodiment, it is an IgG1 or IgG4 antibody. In one particularly preferred embodiment, it is an IgG1 format antibody whose constant region comprises LALA modifications. In one particularly preferred embodiment, it is an IgG4 format antibody whose constant region comprises FALA
30 modifications.

17415 x 17552 derived biparatopic antibodies

In one preferred embodiment, an antibody of the present invention is biparatopic
35 for CD45. In one embodiment, the antibody may also comprise at least one specificity for

a molecule other than CD45, which is in addition to the two specificities for CD45. For example, the antibody may comprise a further specificity which is specific for a blood protein or a cell specific surface protein. A particularly preferred specificity is one against serum album. In an alternative embodiment, an antibody of the present invention
5 is biparatopic for CD45 and does not comprise any further specificities.

In an especially preferred embodiment, an antibody of the present invention is biparatopic for CD45 as it comprises a 17415 derived specificity for CD45 and also comprises a 17552 derived specificity for CD45. It may be the case that the 17552 derived specificity can be thought of as the “helper” specificity and the 17415 derived
10 specificity can be thought of as the “killing” specificity.

Any pairing of 17415 and 17552 derived graft variants may be employed. For example, any pair from Figures 2 to 4 may be employed. In one embodiment, the 17415 specificity is from Table 1 and the 17552 specificity is from Table 2. Particularly preferred combinations are those utilized in the Examples and Figures of the present
15 application.

In one embodiment, a preferred biparatopic is a 17415gL7gH6 x 17552gL1gH1 biparatopic. In another embodiment, a preferred biparatopic is a 17415gL7gH6 x 17552gL1gH4 biparatopic. In one embodiment, the biparatopic comprises the CDRs of those combinations. In another it will comprise the entire light and heavy chain variable
20 regions of those combinations.

In one embodiment, a preferred biparatopic is a 17415gL15gH6 x 17552gL1gH1 biparatopic. In another embodiment, a preferred biparatopic is a 17415gL15gH6 x 17552gL1gH4 biparatopic. In one embodiment, the biparatopic comprises the CDRs of those combinations. In another it will comprise the entire light and heavy chain variable
25 regions of those combinations.

In one embodiment, a preferred biparatopic is a 17415gL16gH6 x 17552gL1gH1 biparatopic. In another embodiment, a preferred biparatopic is a 17415gL16gH6 x 17552gL1gH4 biparatopic. In one embodiment, the biparatopic comprises the CDRs of those combinations. In another it will comprise the entire light and heavy chain variable
30 regions of those combinations.

In one embodiment, a preferred biparatopic is a 17415gL7gH6 x 17552gL1gH1 biparatopic. In one embodiment, a preferred biparatopic is a 17415gL7gH6 x 17552gL1gH4 biparatopic. In one embodiment, the biparatopic comprises the CDRs of

those combinations. In another it will comprise the entire light and heavy chain variable regions of those combinations.

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5 17552gL1gH4 biparatopic. In one embodiment, the biparatopic comprises the CDRs of those combinations. In another it will comprise the entire light and heavy chain variable regions of those combinations.

In one embodiment, a preferred biparatopic is a 17415gL16gH6 x 17552gL1gH1 biparatopic. In one embodiment, a preferred biparatopic is a 17415gL16gH6 x
10 17552gL1gH4 biparatopic. In one embodiment, the biparatopic comprises the CDRs of those combinations. In another it will comprise the entire light and heavy chain variable regions of those combinations.

In one particularly preferred embodiment, a biparatopic antibody of the present invention is an IgG format antibody. In one preferred embodiment, it is an IgG1 or IgG4
15 antibody. In one particularly preferred embodiment, it is an IgG1 format antibody whose constant region comprises LALA modifications. In one particularly preferred embodiment, it is an IgG4 format antibody whose constant region comprises FALA modifications. Hence, those formats may be used for any of the 17415 and 17552 derived antibodies set out herein which comprise humanised variable regions and in particular
20 any of the combinations of light and heavy chain variable regions set out above.

The SEQ ID Nos for the light and heavy chain variable region sequences for the above biparatopic antibodies can be found by referring to Tables 1 and 2 and in particular by referring to the SEQ ID NOs of the graft variants as set out in those Tables. Also preferred are biparatopic antibodies that comprise the CDR sets or variable region
25 pairings of the specific biparatopic antibodies described in the Examples of the present application.

Particularly preferred biparatopic antibodies of the invention are those with the CDR sets of the biparatopic antibodies shown in Figure 17. Further particularly preferred biparatopic antibodies of the invention are those with the light and heavy chain variable
30 regions of the biparatopic antibodies shown in Figure 17.

In one particularly preferred embodiment, where a VR17415 derived specificity is provided the VR17415gL15gH6 IgG1 LALA antibody the sequence for the light and heavy chains of which are in Figure 31 is provided. Hence, in one particularly preferred embodiment, the antibody will comprise the heavy chain sequence of SEQ ID NO: 140

and the light chain sequence of SEQ ID NO: 141. In one embodiment, where the antibody is a monospecific VR17415gL15gH6 IgG1 LALA antibody that comprises two such heavy chains and two such light chains. In one embodiment, where the antibody is biparatopic, one specificity of the antibody will comprise a heavy and light chain pair of
5 SEQ ID NOs: 140 and 141. Variants as discussed herein of such a specific antibody are also provided.

In one particularly preferred embodiment, where a VR17552 derived specificity is employed the VR17552gL1gH4 IgG1 LALA antibody is employed. Hence, in one particularly preferred embodiment, the antibody will comprise the heavy chain sequence
10 of SEQ ID NO: 142 and the light chain sequence of SEQ ID NO: 143. In one embodiment, where the antibody is a monospecific VR17552gL1gH4 IgG1 LALA antibody the antibody will comprise two such heavy chains and two such light chains. In one embodiment, where the antibody is biparatopic, one specificity of the antibody will comprise a heavy and light chain pair of SEQ ID NOs: 142 and 143. Variants as
15 discussed herein of such a specific biparatopic antibody are also provided.

In one particularly preferred embodiment a biparatopic antibody is provided with the light and heavy chain sequences given in Figure 32 so the VR17415gL15gH6 is provided respectively by the heavy and light chain sequences of SEQ ID NOs: 147 and 141, whilst the VR17552gL1gH4 specificity is provided respectively by the heavy and
20 light chain sequences of SEQ ID Nos: 142 and 143. Variants as discussed herein of such a specific biparatopic antibody are also provided.

Antibody generation and screening

25 The present section describes various methods which may be employed to generate variants. In the case of an antibody which is biparatopic for CD45, whilst a biparatopic antibody comprising both a 17415 derived and a 17552 derived CD45 specificity is preferred, the presently described methods may also be used to identify
30 further CD45 specificities to pair with a 17415 derived specificity or a 17552 derived specificity.

In one embodiment, the antibodies of the present invention or antibody/fragment components thereof have an enhanced potency, optionally wherein the antibodies of the present invention or antibody/fragment components thereof also have an enhanced
35 efficacy compared to other known antibodies for CD45. By 'enhanced potency', we

include the meaning that the same level of cell killing can be achieved by an antibody of the present invention at a lower concentration/titre compared to an antibody for CD45 of the prior art. In one embodiment reference to an 'enhanced efficacy' means that a higher maximum effect can be achieved by an antibody of the present invention compared to an antibody for CD45 of the prior art.

In one embodiment, the antibodies of the present invention or antibody/fragment components thereof are processed to provide improved affinity for a target antigen or antigens and in particular for CD45. Such variants can be obtained by a number of affinity maturation protocols including mutating the CDRs (Yang *et al.*, J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks *et al.*, Bio/Technology, 10, 779-783, 1992), use of mutator strains of *E. coli* (Low *et al.* J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten *et al.* Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson *et al.*, J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Cramer *et al.* Nature, 391, 288-291, 1998). Vaughan *et al.* (*supra*) discusses these methods of affinity maturation. Binding domains for use in the present invention may be generated by any suitable method known in the art, for example CDRs may be taken from non-human antibodies including commercially available antibodies and grafted into human frameworks or alternatively chimeric antibodies can be prepared with non-human variable regions and human constant regions etc.

Examples of CD45 antibodies are known in the art and a paratope from such antibody may be employed in an antibody of the present invention which has more than one specificity for CD45 or screened for suitability using the methods described herein, and subsequently modified if necessary, for example humanised, using the methods described herein. Therapeutic anti-CD45 antibodies have been described in the art, for example anti-CD45 antibodies disclosed in US2011/0076270.

The skilled person may generate antibodies for use in the antibodies of the invention using any suitable method known in the art. Antigen polypeptides, for use in generating antibodies for example for use to immunize a host or for use in panning, such as in phage display, may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems or they may be recovered from natural biological sources. In the present application, the term "polypeptides" includes peptides, polypeptides and proteins. These are used interchangeably unless otherwise specified. The antigen polypeptide may in some instances be part of a larger protein such as a fusion protein for example fused to an affinity tag or similar. In one embodiment,

the host may be immunised with a cell transfected with CD45, for instance expressing CD45 on its surface.

Antibodies generated against an antigen polypeptide may be obtained, where immunisation of an animal is necessary, by administering the polypeptides to an animal, preferably a non-human animal, using well-known and routine protocols, see for example Handbook of Experimental Immunology, D. M. Weir (ed.), Vol 4, Blackwell Scientific Publishers, Oxford, England, 1986). Many warm-blooded animals, such as rabbits, mice, rats, sheep, cows, camels or pigs may be immunized. However, mice, rabbits, pigs and rats are generally most suitable. Monoclonal antibodies may be prepared by any method known in the art such as the hybridoma technique (Kohler & Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al* 1983, Immunology Today, 4:72) and the EBV-hybridoma technique (Cole *et al* Monoclonal Antibodies and Cancer Therapy, pp77-96, Alan R Liss, Inc., 1985).

Antibodies may also be generated using single lymphocyte antibody methods by cloning and expressing immunoglobulin variable region cDNAs generated from single lymphocytes selected for the production of specific antibodies by, for example, the methods described by Babcook, J. *et al* 1996, Proc. Natl. Acad. Sci. USA 93(15):7843-7848; WO 92/02551; WO 2004/051268 and WO 2004/106377. The antibodies for use in the present invention can also be generated using various phage display methods known in the art. In one preferred embodiment, an antibody of the present invention has at least two different paratopes specific for CD45 and it may be that antibodies recognising one paratope of CD45 are first raised and then, for instance, two of those antibodies are used to generate an antibody of the present invention able to specifically bind at least two different paratopes of CD45. It may be, for instance, that multiple antibodies against CD45 are raised using the methods discussed herein and then screened for desirable properties, such as binding affinities. Then the best candidates may be used to generate an antibody of the present invention.

In one example, the antigen-binding sites, and in particular the variable regions, of the antibodies according to the invention are humanised. Humanised (which include CDR-grafted antibodies) as employed herein refers to molecules having one or more complementarity determining regions (CDRs) from a non-human species and a framework region from a human immunoglobulin molecule. It will be appreciated that it may only be necessary to transfer the specificity determining residues of the CDRs rather than the entire CDR (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). In a

preferred embodiment though, the whole CDR or CDRs is/are transplanted. Humanised antibodies may optionally further comprise one or more framework residues derived from the non-human species from which the CDRs were derived. As used herein, the term "humanised antibody molecule" refers to an antibody molecule wherein the heavy and/or light chain contains one or more CDRs (including, if desired, one or more modified CDRs) from a donor antibody (e.g. a murine monoclonal antibody) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody). For a review, see Vaughan *et al*, Nature Biotechnology, 16, 535-539, 1998. In one embodiment, rather than the entire CDR being transferred, only one or more of the specificity determining residues from any one of the CDRs described herein above are transferred to the human antibody framework (see for example, Kashmiri *et al.*, 2005, Methods, 36, 25-34). In one embodiment only the specificity determining residues from one or more of the CDRs described herein above are transferred to the human antibody framework. In another embodiment, only the specificity determining residues from each of the CDRs described herein above are transferred to the human antibody framework.

When the CDRs or specificity determining residues are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Suitably, the humanised antibody according to the present invention has a variable domain comprising human acceptor framework regions as well as one or more of the CDRs provided herein. Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat *et al supra*). For example, KOL and NEWM can be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain. Alternatively, human germline sequences may be used; these are available at: <http://www2.mrc-lmb.cam.ac.uk/vbase/list2.php>.

In a humanised antibody molecule of the present invention, the acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains. The framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues

in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody (see Reichmann *et al* 1998, Nature, 332, 323-324). Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967. Derivatives of frameworks may have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids replaced with an alternative amino acid, for example with a donor residue. Donor residues are residues from the donor antibody, i.e. the antibody from which the CDRs were originally derived, in particular the residue in a corresponding location from the donor sequence is adopted.

Donor residues may be replaced by a suitable residue derived from a human receptor framework (acceptor residues).

In one embodiment the invention extends to an antibody sequence disclosed herein, in particular humanised sequences disclosed herein.

In one example the binding domains are humanised.

In one example one or more CDRs provided herein may be modified to remove undesirable residues or sites, such as cysteine residues or aspartic acid (D) isomerisation sites or asparagine (N) deamidation sites. In one example an Asparagine deamidation site may be removed from one or more CDRs by mutating the asparagine residue (N) and/or a neighbouring residue to any other suitable amino acid.

The skilled person is able to test variants of CDRs or humanised sequences in any suitable assay such as those described herein to confirm activity is maintained.

Specific binding to antigen may be tested using any suitable assay including for example ELISA or surface plasmon resonance methods such as BIAcore where binding to antigen (CD45) may be measured. Such assays may use isolated natural or recombinant CD45 or a suitable fusion protein/polypeptide. In one example, binding is measured using recombinant CD45 (SEQ ID NO: 127 or amino acids 23-1304 of SEQ ID NO: 127) by, for example, surface plasmon resonance, such as BIAcore. Alternatively, the proteins may be expressed on a cell, such as a HEK cell and affinity measured employing a flow cytometry-based affinity determination. In one embodiment, where it is desired to determine the properties of one antigen-binding site, in particular paratope, on its own, an antibody is generated with just that paratope. For example, the same format antibody as an antibody of the invention with two different specificities is generated, but with just one of the specificities for CD45 present. In one embodiment, antibodies for each of the paratopes against CD45 from an antibody of the present

invention with at least two paratopes may be generated, for instance to allow the affinity of each paratope to be determined or to determine whether or not the paratopes display cross-blocking against each other. In one embodiment the ability to specifically bind the extracellular region of CD45 is measured, for instance using the protein of SEQ ID NO: 5 113. In one embodiment, monovalent antibodies, such as ScFv are generated to perform the comparison.

Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, 10 New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987, Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991, the BLAST™ software available from NCBI (Altschul, S.F. *et al.*, 1990, J. Mol. Biol. 215:403-410; Gish, W. & 15 States, D.J. 1993, Nature Genet. 3:266-272. Madden, T.L. *et al.*, 1996, Meth. Enzymol. 266:131-141; Altschul, S.F. *et al.*, 1997, Nucleic Acids Res. 25:3389-3402; Zhang, J. & Madden, T.L. 1997, Genome Res. 7:649-656,).

The present invention also extends to novel polypeptide sequences disclosed herein and sequences at least 80% similar or identical thereto, for example 85% or 20 greater, such 90% or greater, in particular 95%, 96%, 97%, 98% or 99% or greater similarity or identity. In one embodiment a sequence may have at least 99% sequence identity to at least one of the specific sequences provided herein. "Identity", as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity", as used herein, indicates that, at 25 any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- 30 - phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains).

It will be appreciated that this aspect of the invention also extends to variants of these anti-CD45 antibodies including those in which amino acids have been mutated in the CDRs to remove one or more isomerisation, deamidation, glycosylation site or cysteine residue as described herein above.

5

Preferred antibodies with FALA, LALA and knob-in-hole modifications

Please note that the amino acid residue positions given in this section are indicated using EU numbering rather than Kabat numbering.

10 In one particularly preferred embodiment of the invention, the antibody employed comprises heavy chains with FALA or LALA modifications. In particular, FALA and LALA modifications alter Fc receptor binding.

In a further preferred embodiment, the antibody comprises a modification in the hinge region of the antibody and in particular a modification at position 228, preferably
15 228P. In one embodiment, an antibody has a heavy chain comprising modifications at position 228, 234, and 235. In a particularly preferred embodiment, the heavy chains of an antibody of the present invention will comprise S228P, F234A, and L235A FALA modifications. In an especially preferred embodiment of the present invention the antibody provided will be an IgG4(P) isotype antibody and comprise such modifications.

20 In another particularly preferred embodiment, an antibody of the present invention will comprise so called “knob-in-hole” modifications. In one embodiment, one heavy chain of the antibody comprises a modification at T366 and the other at T366, L368, and Y407 and in particular to make complementary shapes in the two different heavy chains that mean they preferentially pair, rather than two identical heavy chains
25 pairing. In particular, the heavy chain for one specificity may have a T366W “knob” modification, whilst the other has T366S, L368A, Y407V “hole” modifications. In a particularly preferred embodiment, the antibody of the present invention is an IgG4 isotype antibody and has such modifications.

In another especially preferred embodiment of the present invention, the FALA,
30 hinge, and “knob-in-hole” modifications are combined. In a preferred embodiment, they are combined in the context of an IgG4 isotype antibody. In one embodiment, one heavy chain of the antibody has modifications at positions 228, 234, 235 and 355. In another embodiment, one heavy chain comprises modifications at positions 228, 234, 235, 366, 368 and 407. For example, in one embodiment one heavy chain has S228P, F234A,

L235A, T366W modifications (so both FALA and a “knob” modification) and preferably the other has S228P, F234A, L235A, T366S, L368A, Y407V modifications (so both FALA and “hole” modifications).

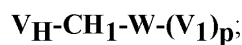
In an especially preferred embodiment, an antibody of the present invention is a FALA IgG4(P) antibody. In a further especially preferred embodiment, it is a FALA knobs-in-holes IgG4(P) antibody. In another it is a LALA knobs-in-holes IgG4(P) antibody.

In another embodiment, the above formats may be combined with other formats/modifications discussed herein. For example, they may also include the modifications discussed herein to remove Protein A binding at positions 95 and 96. In a further embodiment, they may include a common light chain and may also comprise the Protein A binding modification as well.

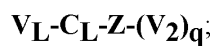
Further preferred antibody formats including BYbe and TrYbe

In one aspect, there is provided an antibody molecule comprising or consisting of:

a) a polypeptide chain of formula (VII):



b) a polypeptide chain of formula (VIII):



wherein:

V_H represents a heavy chain variable domain;

CH_1 represents a domain of a heavy chain constant region, for example domain 1 thereof;

W represents a bond or linker, for example an amino acid linker, except if p or q is zero in which case they will also be zero;

Z represents a bond or linker, for example an amino acid linker;

V_1 represents a dab, scFv, dsscFv or dsFv;

V_L represents a variable domain, for example a light chain variable domain;

CL represents a domain from a constant region, for example a light chain constant region domain, such as Ckappa;

V_2 represents a dab, scFv, dsscFv or dsFv;

p is 0 or 1;

q is 0 or 1; and

when p is 1 q is 0 or 1 and when q is 1 p is 0 or 1 i.e. p and q do not both represent 0,

where at least two of the antigen-binding sites of the antibody are different

- 5 paratopes against CD45, each recognising different epitopes against CD45, wherein at least one of the V_H and V_L are a pair of 17415 derived variable regions or a pair of 17552 derived variable regions.

In one example the binding domains specific for CD45 are selected from at least two of V₁, V₂ or V_H/V_L.

- 10 In one embodiment q is 0 and p is 1.

In one embodiment q is 1 and p is 1.

In one embodiment V₁ is a dab and V₂ is a dab and together they form a single binding domain of a co-operative pair of variable regions, such as a cognate V_H/V_L pair, which are optionally linked by a disulphide bond.

- 15 In one embodiment V_H and V_L are specific to CD45.

In one embodiment the V₁ is specific to CD45.

In one embodiment the V₂ is specific to CD45.

In one embodiment the V₁ and V₂ together (e.g. as binding domain) are specific to CD45 and V_H and V_L are specific to CD45.

- 20 In one embodiment the V₁ is specific to CD45.

In one embodiment the V₂ is specific to, CD45.

In one embodiment the V₁ and V₂ together (e.g. as one binding domain) are specific to CD45 and V_H and V_L are specific to CD45.

- 25 In one embodiment the V₁ is specific to CD45, V₂ is specific to CD45 and V_H and V_L are specific to CD45.

V₁, V₂, V_H and V_L in the constructs above may each represent a binding domain and incorporate any of the sequences provided herein.

W and Z may represent any suitable linker, for example W and Z may be independently SGGGSGGGGS (SEQ ID NO: 67) or SGGGGTGGGGS (SEQ ID

- 30 NO:114).

In one embodiment, when V_1 and/or V_2 are a dab, dsFv or a dsscFv, the disulfide bond between the variable domains V_H and V_L of V_1 and/or V_2 is formed between positions V_{H44} and V_{L100} .

In a preferred embodiment of the invention, an antibody of the invention is in the BYbe antibody format. A BYbe format antibody comprises a Fab linked to only one scFv or dsscFv, as described for example in WO 2013/068571, and Dave *et al*, (2016) *Mabs*, 8(7): 1319-1335. Hence, for example in one preferred embodiment in the formula given above one of $(V_1)_p$ and $(V_2)_q$ will be a ScFv or a dsscFv and the other will be nothing, so that the BYbe format antibody comprises a Fab and only one scFv or dsscFv. For whichever of $(V_1)_p$ and $(V_2)_q$ is zero the corresponding W or Z will also be nothing and the other will be a bond or a linker. Preferably the BYbe format antibody comprises a Fab and dsscFv. In such BYbe format antibodies the two antigen-binding sites may be preferably both specific for CD45, with the two corresponding to the two different paratopes for different epitopes of CD45.

In a further especially preferred embodiment of the invention the antibody is in the TrYbe format. A TrYbe format comprises a Fab linked to two scFvs or dsscFvs, each scFv or dsscFv binding the same or a different target (e.g., one scFv or dsscFv binding a therapeutic target and one scFv or dsscFv that increases half-life by binding, for instance, albumin). Such antibody fragments are described in WO 2015/197772. In respect of the formula given above, for a TrYbe antibody, p and q will both be one, with V_1 and V_2 being independently selected from a ScFv and dsscFv. In a preferred embodiment, V_1 and V_2 will both be a ScFv. In another embodiment, V_1 and V_2 will both be a dsscFv. In another embodiment one of V_1 and V_2 will be a ScFv and the other a dsscFv. At least two of the antigen-binding sites of the TrYbe will be specific for CD45, with the antibody comprising two different paratopes each specific for a different epitope of CD45. In one particularly preferred embodiment, the third antigen-binding site will be specific for albumin and in particular one of V_1 and V_2 will be specific for albumin. For example, V_H/V_L may be specific for CD45 (e.g. for a first epitope of CD45), one of V_1 and V_2 may be specific for CD45 (e.g. for a second epitope of CD45), and the other of V_1 and V_2 may be specific for albumin.

In one preferred embodiment, an antibody of the invention will comprise at least one paratope specific for albumin. In one embodiment, the antibody will be a TrYbe format antibody comprising the two paratopes specific for a different epitope of CD45

and also a third paratope specific for albumin. Examples of albumin binding antibody sequences which may be used to specifically bind albumin include those disclosed in WO 2017/191062 the entirety of which is incorporated by reference, particularly so far as it relates to albumin binding sequences. Hence, an antibody of the invention may comprise
5 a paratope from one of the albumin specific antibodies in WO 2017/191062.

In an alternative embodiment, an antibody as discussed above has only one specificity for CD45, rather than at least two different ones. For example, one of the antigen binding sites of the antibody may be specific for CD45. In another embodiment, two of the antigen binding sites are specific for CD45, but have the same specificity. In a
10 further embodiment, all three antigen-binding sites of an antibody set out above have the same specificity for CD45. In another embodiment, two of the antigen-binding sites have the same specificity for CD45 and the third is specific for serum albumin.

In a particular preferred embodiment, at least one of the variable regions in the above molecules will be a 17415 or 17552 variable region. In one embodiment, at least
15 one antigen-binding domain will be present comprising a 17415 derived light and heavy chain variable region. In one embodiment, at least one antigen-binding domain will be present comprising a 17552 derived light and heavy chain variable region. In one embodiment, both a 17415 derived antigen-binding site and a 17552 derived antigen-binding site will be present.

20

Disulphide bridges

Where one or more pairs of variable regions in the antibody of the present invention comprise a disulphide bond between VH and VL this may be in any suitable
25 position such as between two of the residues listed below (unless the context indicates otherwise Kabat numbering is employed in the list below). Wherever reference is made to Kabat numbering the relevant reference is Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

In one embodiment, when V1 and/or V2 are a dsFv or a dsscFv in the formulae
30 discussed above, the disulfide bond between the variable domains VH and VL of V1 and/or V2 is between two of the residues listed below (unless the context indicates otherwise Kabat numbering is employed in the list below). Wherever reference is made to Kabat numbering the relevant reference is Kabat *et al.*, 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.

In one embodiment the disulfide bond is in a position selected from the group comprising:

- V_H37 + V_L95C see for example Protein Science 6, 781-788 Zhu *et al* (1997);
 - V_H44 + V_L100 see for example; Biochemistry 33 5451-5459 Reiter *et al* (1994);
5 or Journal of Biological Chemistry Vol. 269 No. 28 pp.18327-18331 Reiter *et al* (1994); or Protein Engineering, vol.10 no.12 pp.1453-1459 Rajagopal *et al* (1997);
 - V_H44 + V_L105 see for example J Biochem. 118, 825-831 Luo *et al* (1995);
 - V_H45 + V_L87 see for example Protein Science 6, 781-788 Zhu *et al* (1997);
 - 10 • V_H55 + V_L101 see for example FEBS Letters 377 135-139 Young *et al* (1995);
 - V_H100 + V_L50 see for example Biochemistry 29 1362-1367 Glockshuber *et al* (1990);
 - V_H100b + V_L49;
 - V_H98 + V_L 46 see for example Protein Science 6, 781-788 Zhu *et al* (1997);
 - 15 • V_H101 + V_L46;
 - V_H105 + V_L43 see for example; Proc. Natl. Acad. Sci. USA Vol. 90 pp.7538-7542 Brinkmann *et al* (1993); or Proteins 19, 35-47 Jung *et al* (1994), and
 - V_H106 + V_L57 see for example FEBS Letters 377 135-139 Young *et al* (1995)
- and a position corresponding thereto in variable region pair located in the molecule.
- 20 In one embodiment, the disulphide bond is formed between positions V_H44 and V_L100.

The amino acid pairs listed above are in the positions conducive to replacement by cysteines such that disulfide bonds can be formed. Cysteines can be engineered into these desired positions by known techniques. In one embodiment an engineered cysteine
25 according to the present disclosure refers to where the naturally occurring residue at a given amino acid position has been replaced with a cysteine residue.

Introduction of engineered cysteines can be performed using any method known in the art. These methods include, but are not limited to, PCR extension overlap mutagenesis, site-directed mutagenesis or cassette mutagenesis. Cassette mutagenesis can
30 be performed based on Wells *et al*, 1985, Gene, 34:315-323. Alternatively, mutants can be made by total gene synthesis by annealing, ligation and PCR amplification and cloning of overlapping oligonucleotides.

WO 2015/197772 sets out in detail preferred locations for disulphide bridges in relation to BYbe and TrYbe format antibodies.

As discussed herein, alteration of the ability of residues in the hinge regions of antibodies is one potential way to influence binding to CD45 and may be employed in the present invention.

Tether Format Antibodies

In one embodiment, an antibody of the invention may comprise two parts brought together by a heterodimeric tether. For example, an antibody of the invention may comprise two parts with each comprising a different antibody fragment having a different paratope for CD45 and also the tether region which allows it to form the overall antibody molecule with the other half of the antibody. In one embodiment, the antibody of the invention is in the Fab-X/Fab-Y antibody format (also referred to as the Fab-Kd-Fab format) as for example described in WO 2017/093402, see e.g. Figure 3. The Fab-X/Fab-Y antibody format is particularly useful for screening because it allows permutations of different paratopes for CD45 to be rapidly screened.

Hence, in one embodiment an antibody molecule according to the present invention is an antibody comprising at least two different paratopes specific for different epitopes of CD45 having the formula A-X:Y-B wherein:

A-X is a first fusion protein;

Y-B is a second fusion protein;

X:Y is a heterodimeric-tether;

: is a binding interaction between X and Y;

A is a first protein component of the antibody selected from a Fab or Fab' fragment;

B is a second protein component of the antibody selected from a Fab or Fab';

X is a first binding partner of a binding pair independently selected from an antigen or an antibody or binding fragment thereof; and

Y is a second binding partner of the binding pair independently selected from an antigen or an antibody or a binding fragment thereof;

with the proviso that when X is an antigen Y is an antibody or binding fragment thereof specific to the antigen represented by X and when Y is an antigen X is an antibody or binding fragment thereof specific to the antigen represented by Y.

Illustrative examples of albumin antibodies and sequences

5 An antibody with an antigen-binding site specific for albumin used in the present invention may have the following CDR sequences:

SEQ ID NO: 130 - CDRH1	GIDLSNYAIN
SEQ ID NO: 131 - CDRH2	IIWASGTTFYATWAKG
SEQ ID NO: 132 - CDRH3	TVPGYSTAPYFDL
10 SEQ ID NO: 133 - CDRL1	QSSPSVWSNFLS
SEQ ID NO: 134 - CDRL2	EASKLTS
SEQ ID NO: 135 - CDRL3	GGGYSSISDTT

15 Examples of albumin binding specificities that may be adapted for use include those of WO 05/117984 and WO 2017/191062 which are incorporated by reference both in their entirety and in relation to albumin binding antibody specificities.

Effector Molecules

20 An antibody of the invention may be conjugated to an effector molecule. Hence, if desired in particular an antibody, for use in the present invention may be conjugated to one or more effector molecule(s). It will be appreciated that the effector molecule may comprise a single effector molecule or two or more such molecules so linked as to form a single moiety that can be attached to the antibodies. Where it is desired to obtain an

25 antibody according to the present invention linked to an effector molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody, is linked either directly or via a coupling agent to the effector molecule. Techniques for conjugating such effector molecules to antibodies are well known in the art (see, Hellstrom *et al.*, Controlled Drug Delivery, 2nd Ed., Robinson *et al.*, eds., 1987, pp. 623-

30 53; Thorpe *et al.*, 1982, Immunol. Rev., 62:119-58 and Dubowchik *et al.*, 1999, Pharmacology and Therapeutics, 83, 67-123). Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 89/00195, WO 89/01476 and WO 03/031581. Alternatively, where the effector molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for

example as described in WO 86/01533 and EP0392745. In one embodiment the antibodies, of the present invention may comprise an effector molecule. The term effector molecule as used herein includes, for example, antineoplastic agents, drugs, toxins, biologically active proteins, for example enzymes, antibody or antibody fragments, synthetic or naturally occurring polymers, nucleic acids and fragments thereof e.g. DNA, RNA and fragments thereof, radionuclides, particularly radioiodide, radioisotopes, chelated metals, nanoparticles and reporter groups such as fluorescent compounds or compounds which may be detected by NMR or ESR spectroscopy.

Examples of effector molecules may include cytotoxins or cytotoxic agents including any agent that is detrimental to (*e.g.* kills) cells. Examples include combrestatins, dolastatins, epothilones, staurosporin, maytansinoids, spongistatins, rhizoxin, halichondrins, roridins, hemiasterlins, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Effector molecules also include, but are not limited to, antimetabolites (*e.g.* methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.* mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.* daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.* dactinomycin (formerly actinomycin), bleomycin, mithramycin, anthramycin (AMC), calicheamicins or duocarmycins), and anti-mitotic agents (*e.g.* vincristine and vinblastine).

Other effector molecules may include chelated radionuclides such as ^{111}In and ^{90}Y , Lu^{177} , Bismuth 213 , Californium 252 , Iridium 192 and Tungsten 188 /Rhenium 188 ; or drugs such as but not limited to, alkylphosphocholines, topoisomerase I inhibitors, taxoids and suramin. Other effector molecules include proteins, peptides and enzymes. Enzymes of interest include, but are not limited to, proteolytic enzymes, hydrolases, lyases, isomerases, transferases. Proteins, polypeptides and peptides of interest include, but are not limited to, immunoglobulins, toxins such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin, a protein such as insulin, tumor necrosis factor (TNF), α -interferon, β -

interferon, nerve growth factor, platelet derived growth factor or tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, *e.g.* angiostatin or endostatin, or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor and immunoglobulins.

Other effector molecules may include detectable substances useful for example in diagnosis. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ^{125}I , ^{131}I , ^{111}In and ^{99}Tc .

In another embodiment, the effector molecule may increase or decrease the half-life of the antibody *in vivo*, and/or reduce immunogenicity and/or enhance delivery across an epithelial barrier to the immune system. Examples of suitable effector molecules of this type include polymers, albumin, albumin-binding proteins or albumin binding compounds such as those described in WO 05/117984. Where the effector molecule is a polymer it may, in general, be a synthetic or a naturally occurring polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, *e.g.* a homo- or hetero- polysaccharide. Specific optional substituents which may be present on the above-mentioned synthetic polymers include one or more hydroxy, methyl or methoxy groups. Specific examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

An antibody, of the present invention may be conjugated to a molecule that modulates or alters serum half-life. An antibody, of the invention may specifically bind to albumin, for example in order to modulate the serum half-life. In one embodiment, an antibody of the invention will also include a paratope specific for albumin. In another
5 embodiment, an antibody of the invention may include a peptide linker which is an albumin binding peptide. Examples of albumin binding peptides are included in WO2015/197772 and WO2007/106120 the entirety of which are incorporated by reference.

In another embodiment, an antibody, of the invention is not conjugated to an
10 effector molecule. In one embodiment, an antibody, of the invention is not an antibody drug conjugate. In one embodiment, an antibody, of the invention is not conjugated to a toxin, such as conjugated to a toxin via a linker. In another embodiment, an antibody, of the invention is not conjugated to a radioisotope. In another embodiment, an antibody of the invention is not conjugated to an agent for imaging.

15 In one preferred embodiment, it is the ability of an antibody of the present invention to specifically bind CD45 that brings about cell death and not the ability of a conjugated effector molecule.

Cell death and killing

20

In one especially preferred embodiment, an antibody of the present invention is able to induce cell death in a target cell expressing CD45. Types of cell death that may be induced to kill target cells include intrinsic apoptosis, extrinsic apoptosis, mitochondrial permeability transition (MPT)-driven necrosis, necroptosis, ferroptosis, pyroptosis,
25 parthanatos, entotic cell death, NETotic cell death, lysosome-dependent cell death, autophagy-dependent cell death, immunogenic cell death, cellular senescence, and mitotic catastrophe. In one embodiment, an antibody of the present invention is used to kill target cells.

In one embodiment, the target cell will be a cell expressing CD45, and in
30 particular on the surface of the cell. In one preferred embodiment, an antibody of the present invention, may induce cell death in at least T cells. In another preferred embodiment, an antibody of the present invention, may induce cell death in at least B cells. In another preferred embodiment, an antibody of the invention, may be able to induce cell death in B and T cells. In one preferred embodiment an antibody of the

present invention, is able to induce cell death in haematopoietic stem cells. In one embodiment, an antibody of the present invention, does not induce cell death in all immune cells, for example, not granulocytes, macrophages and monocytes. In one embodiment, an antibody of the invention, induces cell death in all immune cells apart from granulocytes, macrophages and monocytes. In one embodiment, the effect of inducing cell death in haematopoietic stem cells is effectively that all haematopoietic cells can be replaced. In one embodiment, an antibody of the present invention is used to kill the above-mentioned target cells by inducing cell death.

In one embodiment, an antibody of the present invention, may have different selectivity for different CD45-expressing cells. In a preferred embodiment, an antibody of the invention, may be able to induce cell death of T cells more efficiently than cell death of B cells.

In another particularly preferred embodiment, an antibody of the present invention induces cell death but does not bring about significant cytokine release. In another preferred embodiment an antibody of the invention, induces cell death but does not display Fc effector functions, for example because the antibody lacks an Fc region or has an Fc region with silencing modifications.

Cytokines

In one particularly preferred embodiment, an antibody, of the present invention does not bring about significant cytokine release. In an especially preferred embodiment, an antibody of the present invention is able to induce cell death in a target cell, but does not bring about significant cytokine release. The reduction or absence of cytokine release may mean that a subject does not suffer unwanted cytokine driven inflammation. For instance, a treatment of the present invention may kill target cells in a subject without triggering inflammation and in particular without a so-called “cytokine storm” associated with some treatments.

In one embodiment, an antibody of the present invention does not significantly induce the release of one or more of Interferon-gamma, IL-6, TNF-alpha, IL-1Beta, MCP1 and IL-8. In one preferred embodiment, an antibody of the invention does not bring about significant release of any of those cytokines. In another embodiment, an antibody does not significantly induce the release of one or more of CCL2, IL-1RA, IL-6, and IL-8. In another preferred embodiment, it does not significantly induce release of any

of those cytokines. In one embodiment, such levels will be the case for one or more of Interferon-gamma, IL-6, TNF-alpha, IL-1Beta, MCP1 and IL-8. In another embodiment, such levels will be the case for one or more of CCL2, IL-1RA, IL-6, and IL-8. In another embodiment such levels will be seen for at least one of CCL2, IL-1RA, IL-6, IL-8, IL-10, and IL-11. In another embodiment, such levels will be the case for at least one of CCL2, IL-1RA, IL-6, and IL-8.

Cytokine release may be measured using any suitable assay. For example, the ability of an antibody of the invention to bring about cytokine release may be determined by culturing cells *in vitro* with the antibody and measuring cytokine release. In one embodiment, whole blood is incubated with the antibody and then cytokine levels measured, for example any of those cytokines mentioned above. In another embodiment, white blood cells isolated from a whole blood sample may be incubated with the antibody of the present invention and the level of cytokine(s) measured. Alternatively, it may be that the level of cytokine(s) is measured in a sample from a subject administered an antibody of the present invention, in particular cytokine level(s) may be measured in a serum sample from a subject.

In one embodiment, not “significantly inducing” cytokine release means that an antibody of the invention does not induce cytokine release more than five, four, three, or two-fold of that seen with a negative control, for example compared to a negative control of *in vitro* treatment with PBS alone. In some embodiments, the level of cytokine release will be compared to a positive control, for example *in vitro* treatment with Campath. In one embodiment, an antibody of the present invention will trigger not more than 50%, 40%, 30%, 20%, 10% or less compared to that seen with treatment with Campath. In one embodiment, the level of cytokine release seen with an antibody of the present invention will be under one tenth of that seen with Campath. In one embodiment, the level of cytokine release following incubation with Campath will be at least double, triple, four times, five times, ten times or more that seen following incubation with an antibody of the present invention. In one embodiment, following incubation of whole blood for 24 hours the level seen with Campath will be those levels compared to an antibody of the present invention.

In another embodiment the comparator for defining not significantly induced will be another antibody. For example, where an antibody of the present invention comprises a modification designed to reduce cytokine release, the comparator will be the equivalent antibody, but without such a modification. In another embodiment, where the antibody

either has an Fc region modification intended to reduce cytokine release or no Fc region, the comparison performed is with the equivalent antibody that lacks such a modification or which has an Fc region.

In another embodiment, the comparison for not significantly releasing cytokines will be performed *in vivo*. For example, when an antibody of the present invention is given to a subject it will show any of the levels of cytokine release discussed above compared to the comparators discussed above. In another embodiment, not significantly inducing cytokine release may be in terms of the level of cytokine or cytokines compared to before administration of an antibody of the present invention. It may be, for example, that the level of a cytokine rises no more than ten-fold, fivefold, or less following administration of an antibody of the present invention. The measurement may be performed, for instance, immediately before or at the same time as administration of the antibody and, for example, one day, one week, or two weeks or more after administration. In one embodiment, the measurement is performed one day to one week after the administration. In another embodiment, an antibody of the present invention does not significantly induce cytokine release in the sense that the subject treated does not experience adverse effects associated with unwanted cytokine release, for example the subject does not experience fever, low blood pressure, or irregular or rapid heartbeat.

Functional Assays

In one embodiment, a functional assay may be employed to determine if an antibody of the present invention has a particular property, for instance such as any of those mentioned herein. Hence, functional assays may be used in evaluating an antibody of the present invention. A “functional assay,” as used herein, is an assay that can be used to determine one or more desired properties or activities of the antibody or molecules of the present invention.

In one particularly preferred embodiment, a functional assay measures the ability to bind to CD45. In one preferred embodiment, the ability to bind to human CD45 may be measured. In another embodiment, the ability to bind to cynomolgus CD45 may be measured. In one preferred embodiment, the ability to bind to both human CD45 and cynomolgus monkey CD45 may be measured. Such binding may be measured, for instance on CD45 protein. Alternatively, it may be measured in respect of CD45 expressed on the surface of target cells expressing CD45. Preferred measurement

techniques include those described in the Examples of the present application, such as SPR or flow cytometry. Further preferred approaches that may be employed include those set out herein in respect of cross-blocking, but without a cross-blocking antibody. Such assays may be in respect of binding of a monospecific, monovalent antibody.

- 5 Alternatively, they may be in respect of any of the overall antibody formats and specific antibodies set out herein.

Further suitable functional assays may be binding assays, cell death (such as apoptosis) assays, antibody-dependent cellular cytotoxicity (ADCC) assays, complement-dependent cytotoxicity (CDC) assays, inhibition of cell growth or proliferation (cytostatic effect) assays, cell-killing (cytotoxic effect) assays, cell-signalling assays, cytokine
10 production assays, antibody production and isotype switching, and cellular differentiation assays. In one embodiment, an assay may measure the degree of cell depletion, for example for a specific cell type, via employing an antibody of the present invention. In one preferred embodiment, an assay may measure the ability of an antibody of the
15 invention to induce cell death in target cells expressing CD45. In a further preferred embodiment, a functional assay may measure the ability of an antibody of the present invention to induce cytokine release. In one preferred embodiment, a functional assay may be used to determine if an antibody of the present invention may be used to kill cells but not significantly induce cytokines.

20 Preferred functional assays include those set out in the Examples. For instance, the PBMC cell killing assays described in the Examples may be employed. A Jurkat cell killing assay as set out in the Examples of the present application may be employed. In one preferred embodiment, the target cells are human cells expressing CD45. In another embodiment, the target cells are cynomolgus monkey cells expressing CD45.

25 The functional assays may be repeated a number of times as necessary to enhance the reliability of the results. Various statistical tests known to the skilled person can be employed to identify statistically significant results and thus identify antibodies with biological functions. In one embodiment, multiple antibodies are tested in parallel or essentially simultaneously. Simultaneously as employed herein refers to the where the
30 samples/molecules/complexes are analysed in the same analysis, for example in the same "run". In one embodiment simultaneously refers to concomitant analysis where the signal output is analysed by the instrument at essentially the same time. This signal may require deconvolution to interpret the results obtained. Advantageously, testing multiple biparatopic protein complexes allows for more efficient screening of a large number of

antibodies and the identification of new and interesting relationships. Clearly, different variable regions to the target antigens of interesting CD45 can give access to subtle nuances in biological function.

5 In one embodiment, where an antibody of the present invention comprises more than one specificity for CD45, a functional assay may be used to compare the properties of that antibody with, for example, an antibody having the same valency but just one of the specificities of an antibody of the present invention. In one embodiment, such assays may be used to show that an antibody of the present invention with at least two different specificities for CD45 is superior to the comparator antibody. Hence, in one preferred 10 embodiment, the efficacy of antibodies of the present invention comprising at least two different specificities for CD45, in particular such antibodies according to the present invention, can be compared to individual “comparator” antibodies, in particular “comparator” antibodies, comprising just one of the specificities against CD45 from an antibody of the present invention. For example, where the assay is performed to study 15 cross-linking, or the effect of cross-linking, of CD45, an antibody having the same valency, but just one specificity, may be employed as a comparator. In one embodiment, an antibody of the present invention it may be compared with an antibody comprising the same one of the paratopes from the antibody of the invention at all of the antigen-binding sites of the antibody. In one embodiment, an antibody of the invention may be compared 20 with one of the same valency and format as the antibody of the invention, but where the same one of the paratopes from the antibody of the invention is present at all of the antigen-binding sites. In one embodiment, a bivalent antibody comprising the two different paratopes specific for different epitopes of CD45 may be compared with each of the two possible bivalent antibodies comprising just one of those paratopes. In one 25 embodiment, such comparisons are performed with one comparator antibody for each different specificity, in particular for each different paratope, of the antibody of the invention specific for CD45. In one embodiment, an antibody of the invention will show better results than against one such comparator antibody. In another embodiment, it will show better results than all of the comparator antibodies for each specificity, in particular 30 paratope, of the antibody specific for CD45.

In another embodiment where the antibodies of the present invention comprise at least two different specificities for CD45, monospecific antibodies are first assessed and the chosen candidates then used in the generation of an antibody of the invention with at least two different specificities against CD45. In one embodiment, multiple antibodies, in

are tested by using a multiplex as defined above and subjecting the same to one or more functional assays.

5 The term “biological function” as used herein refers to an activity that is natural to or the purpose of the biological entity being tested, for example a natural activity of a cell, protein or similar. Ideally, the presence of the function can be tested using an *in vitro* functional assay, including assays utilizing living mammalian cells. Natural function as employed herein includes aberrant function, such as functions associated with cancers.

10 In one embodiment, an antibody of the invention will be able to cross-link CD45 to a greater extent than a comparator antibody, in particular than a comparator antibody, such as those discussed above. For instance, the ability of an antibody of the invention to form CD45 multimers of antibody:CD45 ECD may be studied when the two are mixed, such as in equal amounts. A multimer may be a structure with at least two antibody:CD45 ECD units. One suitable technique is mass photometry, with the
15 antibody, mixed with an equal concentration of CD45 ECD, such as that of SEQ ID No: 128 and mass photometry performed on the test sample. Controls with the antibody and CD45 ECD alone may be performed. An antibody of the present invention may give rise to more multimers than a comparator antibody. An antibody of the present invention may give rise to a greater amount of multimers with two, three, four, or more antibody:CD45
20 ECD units than the comparator. It may do so for all of the possible comparators for each of the specificities (in particular paratopes) specific for CD45. A further suitable technique for such comparison is analytical ultracentrifugation (AUC). Again, the comparison performed may also be between a mixture of antibodies compared with each individual type of antibodies in the mixture on their own.

25 In another embodiment, the comparison may be in terms of the ability of an antibody of the present invention to induce cell death. For example, an antibody of the invention may induce more target cells expressing CD45 to undergo cell killing than a comparator, for instance than a comparator antibody. It may induce a higher amount of cell killing when measured using T cells. For example, T cells isolated from PBMC may
30 be used. Any antibody of the invention may induce a higher level of cell killing in CD4+ T cells. It may do so in CD8+ T cells. It may do so in CD4+ memory T cells. It may do so in CD4+ naïve T cells. In another embodiment, the total cell count in whole blood may be measured after incubation with an antibody of the invention and compared to the

results seen for a comparator. In one embodiment, a total cell count may be measured and compared for antibody of the invention with a control antibody.

5 In one embodiment *in vivo* assays, such as animal models, including mouse tumour models, models of auto-immune disease, virus-infected or bacteria-infected rodent or primate models, and the like, may be employed to test antibodies of the present invention. In another embodiment, the degree of depletion of a particular cell type may be measured, for example *in vivo*. In one embodiment, an antibody of the invention will bring about a greater level of depletion than a comparator in an animal model of a disorder and in a preferred embodiment in an animal model of cancer.

10 In one embodiment an antibody molecule according to the present invention has a novel or synergistic function. The term "synergistic function" as used herein refers to biological activity that is not observed or higher than observed when comparator(s) are employed instead. Therefore, "synergistic" includes novel biological function. In one embodiment, an antibody of the present invention comprising at least two specificities for
15 CD45 is synergistic in that it is more effective than an antibody, comprising either of the specificities against CD45 individually, such as the comparators discussed above. In one preferred embodiment, such synergy is shown in relation to cross-linking of CD45. In one embodiment, a mixture of antibodies displays synergy compared to any of the individual antibodies making up the mixture on their own.

20 In one embodiment, novel biological function as employed herein refers to function which is not apparent or absent until the two or more synergistic entities [protein A and protein B] are brought together or a previously unidentified function. Higher as employed herein refers to an increase in activity including an increase from zero i.e. some activity in the antibody or molecules where comparator has/have no activity in the
25 relevant functional assay, also referred to herein as new activity or novel biological function. Higher as employed herein also includes a greater than additive function in the antibody in a relevant functional assay in comparison to the individual paratopes, for example 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300% or more increase in a relevant activity.

30 In one embodiment the novel synergistic function is a higher inhibitory activity.

In one particularly preferred embodiment of the invention, the synergy is in relation to cell depletion of a target cell type expressing CD45. In one embodiment, synergy is in relation to cell killing.

Suitable binding domains for use in the present invention can also be identified by testing one or more binding domain pairs in a functional assay. For example, antibodies, for example an antibody, comprising at least a binding site specific to the antigen CD45 may be tested in one or more functional assays.

5 In one embodiment, the ability of an antibody to kill CD45 expressing cancer cell lines may be assayed. The methods for assessing the ability of an antibody to bring about killing of such cell lines employed in the Examples of the present application may be used to study the ability of a given antibody to kill cells. In one embodiment, a variant antibody of the present invention will have the same or greater ability to kill cancer cells
10 in such assays as one of the specific antibodies set out herein. In one embodiment, they will have at least 50%, 75%, 80%, 90%, 100% or more of the activity of one the specific antibodies set out herein to kill one of the cancer cell lines mentioned above in such an assay. In one embodiment, an antibody of the present invention will kill at least 25%, 40%, 50%, 60% or 75% of cancer cells in such an assay. In another embodiment, an
15 antibody of the present invention will kill 100% of the cancer cells in such an assay.

 In one embodiment, an antibody of the present invention will have similar activities to those antibodies for which results are shown in Figures 17 and 18.

 For instance, in one embodiment, a biparatopic antibody of the invention will have an EC50 value in a human T lymphocyte depletion assay of 0.01 to 0.30 nM. In one
20 embodiment, the EC50 value will be 0.02 to 0.200 nM. In one embodiment, it will have an Emax% value of 50 to 100%. In one preferred embodiment it will have an Emax% value of 60 to 90%. In one embodiment, a biparatopic may have a KD value for human CD45 measured by SPR Biocore of 5 to 20 nM, such as 5 to 15nM. In one embodiment, a biparatopic may have a KD value for cynomolgus monkey CD45 measured by SPR
25 Biocore of 0.10 to 0.50 nM. In one embodiment, a biparatopic of the present invention may have a EC50 value for binding to human CD45 on cells of 0.5 nM to 10 nM, such as 0.5 to 5 nM. In one embodiment, a biparatopic of the present invention may have a EC50 value for binding to cynomolgus monkey CD45 on cells of 0.5 nM to 10 nM, such as 1 to 10 nM. In a preferred embodiment, the techniques used in the Examples are used to
30 perform such measurements. In an even more preferred embodiment, the techniques used to generate the results shown in Figure 18 are used.

Pathological conditions, medical uses, and cell depletion

The present invention provides an antibody of the invention for use in a method of treatment of the human or animal body. An antibody of the present invention may be employed in any context where targeting CD45 may be of therapeutic benefit, in particular where killing such cells may be of therapeutic benefit. An antibody of the present invention may also be used in diagnosis or detection of CD45. The present invention further provides a pharmaceutical composition of the invention for such use. The present invention also provides nucleic acid molecule(s) and vector(s) of the present invention for such uses.

Hence, the antibodies of the present invention may be employed therapeutically. In one embodiment, rather than an antibody of the present invention being administered nucleic acid molecule(s) or vector(s) of the present invention may be administered to bring about expression of the antibody inside the target cell. In another embodiment, a pharmaceutical composition of the present invention is the preferred therapeutic administered. Whilst antibodies, are set out as the preferred therapeutic below, pharmaceutical composition, nucleic acid molecule(s), and vector(s) of the present invention may also be employed in any of the embodiments set out. In a preferred embodiment though antibodies or a pharmaceutical composition comprising them are the preferred therapeutic.

In one particularly preferred embodiment, the present invention may be employed to deplete target cells expressing CD45. In a particularly preferred embodiment, the present invention is used to deplete a disease-causing cell type expressing CD45. In particular, the present invention may be used to deplete target cells expressing CD45 on the surface of the cells. In a particularly preferred embodiment, the antibody employed is one that has at least two different specificities for CD45, i.e. is biparatopic for CD45.

In one preferred embodiment where an antibody of the present invention is employed, the induction of cell death in the target cell via the antibody of the present invention may mean that it is unnecessary for an antibody of the invention to display one or more Fc region effector functions that an antibody would normally display. In one particularly preferred embodiment, an antibody of the invention is therefore able to induce cell death in a target cell, but do not have an active Fc region. In a particularly preferred embodiment, the antibody induces cell death, but do not induce significant cytokine release.

In a particularly preferred embodiment depletion of cells by the present invention is followed by the transfer of cells or a tissue to the subject. In a further particularly preferred embodiment, the transferred cells or tissues replace those that have been depleted using the invention. Treatment as discussed herein therefore includes, rather
5 than targeting the actual mechanism of the disorder, replacing wholly or partially, a cell type involved in the disorder or one whose killing, in particular replacement, can simply have therapeutic benefit. In one embodiment, the invention therefore provides a method of depleting cells comprising employing the invention. In another embodiment, a method of the invention may comprise both depleting cells and the subsequent transfer of cells or
10 tissue. Cell depletion may be used in a number of therapeutic contexts, effectively to kill target cells.

In a preferred instance, an antibody of the invention is used to kill immune cells. As used herein, the term “immune cell” is intended to include, but is not limited to, a cell that is of hematopoietic origin and that plays a role in the immune response. In one
15 embodiment the invention is employed to deplete T cells in a subject. In one embodiment the invention is employed to deplete B cells in a subject. In another embodiment, the invention is employed to deplete both. In another embodiment, the invention is used to deplete T cells, but not macrophages. In another embodiment, the invention is used to deplete B cells, but not macrophages. In another embodiment, the invention is used to
20 deplete B and T cells, but does not result in the depletion of macrophages. In one embodiment, the present invention is used to deplete haematopoietic stem cells (HSCs). In another embodiment, the invention is employed to deplete haematopoietic stem cells. In one preferred embodiment, HSCs are depleted via the invention in a subject prior to the transfer of HSC to repopulate the immune system of the subject. In another embodiment,
25 the invention depletes a particular cell type, but does not deplete haematopoietic stem cells. In another embodiment, the invention is used to kill the above-mentioned cell types. Hence, in any of the embodiments mentioned herein for cell depletion, the invention may be employed to kill the stated cells.

In one embodiment, the subject treated via the invention is one with an
30 autoimmune disease, a blood disease, a metabolic disorder, cancer, or an immunodeficiency. The ability to treat conditions through first depleting and then replacing cells means that the antibodies of the invention are particularly useful in treating cancers. In a particularly preferred embodiment, the disorder to be treated is therefore a cancer. In one embodiment, the invention is therefore employed to deplete

cancer cells, for instance cancer cells originating from immune system cells. In one preferred embodiment, the invention provides a method of treating a cancer comprising administering the invention is employed to deplete cancer cells expressing CD45. The method may further comprise transplantation of cells to the subject. In one embodiment, 5 the transferred cells replace the depleted cells. In one embodiment, the transferred cells are haematopoietic stem cells.

In one particularly preferred embodiment the disorder to be treated is a blood cancer. In one preferred embodiment, the cancer is one involving the bone marrow and in particular one involving cells of the haematopoietic system.

10 In one preferred embodiment, the cancer may be a leukaemia. In one embodiment, the cancer may be a T-cell leukaemia. In one embodiment, the cancer may be a B-cell leukaemia.

In one embodiment of the invention the blood cancer to be treated may be a lymphoma.

15 In one embodiment, the blood cell cancer to be treated is a myeloma

In another embodiment, the subject to be treated has an autoimmune disorder.

In one embodiment, the condition to be treated is one known to involve abnormal CD45 expression. In one particularly preferred embodiment, the treatment depletes a cell type expressing CD45 that plays a role in a disorder in the subject.

20 In one embodiment, the invention is used to deplete cells in advance of a cell transplant, hence a method of the invention may include, in some embodiments, a depletion step employing a therapeutic, in particular antibody, of the present invention followed by a step of transferring cells to the subject, for instance to help replace the depleted cells. In one embodiment, such a transfer may be of allogenic cells. In another 25 embodiment, such a transfer may be of autologous cells. In one embodiment, the transferred cells may be cells expressing a chimeric antigen receptor (CAR). In some embodiments, the subject is in need of chimeric antigen receptor T-cell (CART) therapy. For instance, such therapy may form part of a method of the present invention.

30 In another preferred embodiment, the invention provides a method of promoting the engraftment of a cell population in a subject, where the method further comprises employing an antibody of the invention to deplete cells prior to the engraftment of a cell population. The present invention therefore provides a method of promoting engraftment of transferred cells comprising depleting cells expressing CD45 in a subject via administering an antibody of the invention and then transferring the cells of interest. In

one embodiment, the present invention provides a method for promoting the engraftment of stem cells and in particular hematopoietic stem cells. In one embodiment, hematopoietic stem cells are administered to a subject defective or deficient in one or more cell types of the hematopoietic lineage in order to re-constitute, or partially re-constitute, the defective or deficient population of cells *in vivo*. In one embodiment, the invention is used to treat a stem cell deficiency, for instance where the invention is used to deplete target cells and replace them with transplanted cells, where the transplanted cells address the stem cell deficiency. In one embodiment, the reintroduced cells have been genetically modified. In one embodiment, cells from the subject have been removed and genetically modified then returned to the subject after the invention has been used to kill target cells, for instance the unmodified cells of that type still present in the subject. In one preferred embodiment, the transferred cells that have been genetically modified are haematopoietic stem cells.

In one preferred embodiment, the depleted cells and the transferred cells are, or comprise, the same cell type. In one preferred embodiment, the depleted cells are haematopoietic cells and in particular haematopoietic stem cells. In one embodiment, the present invention is employed to deplete cells prior to a bone marrow transplant. In another embodiment, the present invention is employed instead of irradiation to deplete cells. In another embodiment, it is employed in addition to irradiation to deplete cells.

In another embodiment, the present invention provides a method of helping reducing the chances of rejection of transplanted cells, the method comprising administering a therapeutic of the invention to deplete cells prior to the transfer of the cells. In another embodiment, the invention may be employed to promote the acceptance of transplanted immune cells in a subject by depleting target cells expressing CD45 prior to the transfer of the immune cells. Target cells may be any of those discussed herein. In one embodiment, the cells transplanted or transferred to a subject are stem cells.

Any of the ways discussed herein to eliminate cells expressing CD45 may be employed in cell depletion or killing. In one particularly preferred embodiment of the invention though, the present invention may be used to bring about cell death of CD45 expressing cells and hence depletion of such cells.

In one embodiment, as part of the cell transfer the subject may be given bone marrow as a way of transferring cells. In another embodiment, the subject may have been given cord blood, or cells isolated from cord blood, as a way of transferring cells. In another embodiment, the transplanted cells may have come from differentiated stem

cells, for instance where stem cells have been differentiation *in vitro* and then transplanted.

In one embodiment where the antibodies of the invention are used to deplete or kill cells, a further cell depleting or killing agent may also be used as well. In a preferred
5 embodiment, the antibody of the invention is the only cell depleting agent administered to the subject. In one embodiment, the level of depletion of the target cell is enough to be effective, for instance about at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 99% of the target cells. For example, on one embodiment, at least 50% of the target cells are depleted. In another embodiment, at least 75% of the target cells are depleted. In
10 another embodiment, at least about 90% of the target cells are depleted. In another embodiment, at least about 95% of the target cells are depleted.

As discussed above, in a particularly preferred embodiment, the antibodies of the present invention may be used to bring about cell killing of a CD45 expressing cell. Any suitable method may be used for assessing cell viability and hence cell killing.

15 In another embodiment, the present invention may be used in respect of graft-versus-host disease (GVHD), in particular in respect of treating a cell population, tissue, or organ with an antibody to deplete cells.

In another embodiment, the present invention provides a method of treatment comprising first performing such *ex vivo* treatment and then performing the
20 transplantation. In another embodiment, the present invention is used to deplete or kill cells in a subject prior to the transplantation, so that there are fewer host cells able to attack the transplanted material as a way of reducing the chance of GVHD. Hence, the present invention also provides a method for treating or preventing GVHD comprising administering an antibody of the present invention to deplete cells in a population of
25 cells, tissue, or an organ prior to transplanting the population of cells, tissue, or an organ. The method may further comprise the transplantation itself. The depleted cells and transplanted cells, tissue, or organ, may be any of those mentioned herein. In one preferred embodiment, the transplanted cells are haematopoietic stem cells. In one preferred embodiment the depleted cells are T cells. In another preferred embodiment,
30 the ability of the present invention to treat or prevent GVHD is employed in heart, lung, kidney, or liver transplants.

In another embodiment, the invention provides a method of depleting and/or killing cells in a population of cells, tissue, or organ *in vitro* prior to their transplantation by applying the antibodies of the invention, rather than treating the recipient. Hence, the

present invention also provides a method of removing target cells from a population of cells, tissue, or organ *in vitro* prior to transplantation comprising treating the population of cells, tissue, or organ prior to transplantation and then performing the transplantation.

In one embodiment, the present invention may be used to deplete immune cells in
5 organs or tissues, particularly where conventional therapies are unable to access readily or will lead to exaggerated inflammation as part of their inherent mechanism. In one embodiment, the present invention is used to deplete cells in an enclosed organ, for instance in the brain, spinal cord, eye or testes. In one embodiment, the invention may be employed to deplete CD45⁺ cells in immune privileged organs. The ability of the
10 antibodies of the invention to deplete CD45⁺ cells without employing Fc mediated functions may help avoid unwanted side-effects and damage. In one embodiment, the invention may be used to deplete cells immunosilently and without the need for antibody effector mechanisms. That may have the advantage of minimising, or at least reducing, unwanted damage, for instance as enclosed organs can contain delicate and often non-
15 dividing tissue cells that can be destroyed by infiltrating leukocytes. When the current invention is applied directly to the organ, for example the brain, spinal cord, eye or testes, it may result in the elimination of CD45 positive cells without inducing further damage or inflammation to the tissue or with reduced further damage. In one embodiment, the target cells in the enclosed organ are selected from lymphocytes, B cells, and T cells. In
20 one embodiment, the target cells in the enclosed organ are, or comprise, CD4⁺ T cells. In another embodiment, the target cells are, or comprise, CD8⁺ T cells.

In a further preferred embodiment, the condition the invention is applied to is one characterised by infiltrating CD8⁺ T cells.

25 **Pharmaceutical compositions**

In one aspect a pharmaceutical composition is provided comprising: (a) an antibody, a nucleic acid molecule or molecules, or a vector or vectors of the present invention; and (b) a pharmaceutically acceptable carrier or diluent. In an especially
30 preferred embodiment, it comprises an antibody or antibodies of the present invention. Various different components can be included in the composition, including pharmaceutically acceptable carriers, excipients and/or diluents. The composition may, optionally, comprise further molecules capable of altering the characteristics of the molecule(s) of the invention thereby, for example, reducing, stabilizing, delaying,

modulating and/or activating the function of the molecule(s). The composition may be in solid, or liquid form and may be, *inter alia*, be in the form of a powder, a tablet, a solution or an aerosol.

The present invention also provides a pharmaceutical or diagnostic composition comprising a molecule of the present invention in combination with one or more of a pharmaceutically acceptable excipient, diluent or carrier. Accordingly, provided is the use of an antibody of the invention for use in the treatment of, and for the manufacture of a medicament for the treatment of, a pathological condition or disorder. In one embodiment where the therapeutic of the invention is administered to a subject who is also being given a second therapeutic agent, the two may be given, for example, simultaneously, sequentially or separately. In one embodiment, the two are given in the same pharmaceutical composition. In another embodiment, the two are given in separate pharmaceutical compositions. In one embodiment, the present invention provides an antibody, of the invention for use in a method where the subject is also being treated with a second therapeutic agent. In another embodiment, the present invention provides the second therapeutic agent for use in a method where the subject is being treated with an antibody of the present invention. The nucleic acid molecule(s) and vector(s) of the present invention may also be administered in such combinations.

A composition of the present invention will usually be supplied as a sterile, pharmaceutical composition. A pharmaceutical composition of the present invention may additionally comprise a pharmaceutically-acceptable adjuvant. In another embodiment, no such adjuvant is present in a composition of the present invention. The present invention also provides a process for preparation of a pharmaceutical or diagnostic composition comprising adding and mixing the antibody of the present invention together with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

The term “pharmaceutically acceptable excipient” as used herein refers to a pharmaceutically acceptable formulation carrier, solution or additive to enhance the desired characteristics of the compositions of the present invention. Excipients are well known in the art and include buffers (e.g., citrate buffer, phosphate buffer, acetate buffer and bicarbonate buffer), amino acids, urea, alcohols, ascorbic acid, phospholipids, proteins (e.g., serum albumin), EDTA, sodium chloride, liposomes, mannitol, sorbitol, and glycerol. Solutions or suspensions can be encapsulated in liposomes or

biodegradable microspheres. The formulation will generally be provided in a substantially sterile form employing sterile manufacture processes.

This may include production and sterilization by filtration of the buffered solvent solution used for the formulation, aseptic suspension of the antibody in the sterile
5 buffered solvent solution, and dispensing of the formulation into sterile receptacles by methods familiar to those of ordinary skill in the art.

The pharmaceutically acceptable carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as proteins,
10 polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates. Pharmaceutically
15 acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragées, capsules, liquids, gels, syrups, slurries and suspensions, for ingestion by the patient.

A thorough discussion of pharmaceutically acceptable carriers is available in
20 Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. For any antibody the therapeutically effective amount can be estimated initially either in cell culture assays or
25 in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

Compositions may be administered individually to a patient or may be
30 administered in combination (e.g. simultaneously, sequentially or separately) with other agents, drugs or hormones. The dose at which the present invention is administered depends on the nature of the condition to be treated, the extent of the inflammation present and on whether the antibodies being used prophylactically or to treat an existing condition.

The present invention also provides a process for preparation of a pharmaceutical or diagnostic composition comprising adding and mixing an antibody, together with one or more of a pharmaceutically acceptable excipient, diluent or carrier.

5 The antibody, nucleic acid molecule, or vector may be the sole active ingredient in the pharmaceutical or diagnostic composition or may be accompanied by other active ingredients including antibody ingredients or non-antibody ingredients such as steroids or other drug molecules.

10 The pharmaceutical compositions suitably comprise a therapeutically effective amount of the antibody of the invention. The term “therapeutically effective amount” as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic or preventative effect. A “therapeutically effective amount” may be the amount required to bring about the desired level of cell depletion. For any antibody the therapeutically effective amount can be estimated initially either in cell culture assays or in animal
15 models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

20 The precise therapeutically effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. Generally, a therapeutically effective amount will be from 0.01 mg/kg to 50 mg/kg, for example 0.1 mg/kg to 20 mg/kg per day. In one embodiment, the amount in a given dose is at least
25 enough to bring about a particular function.

Pharmaceutical compositions may be conveniently presented in unit dose forms containing a predetermined amount of an active agent of the invention per dose. A pharmaceutical composition of the present invention may be provided in a receptacle that provides means for administration to a subject. A pharmaceutical composition of the
30 present invention may be provided in a prefilled syringe. The present invention therefore provides such a loaded syringe. It also provides an auto-injector loaded with a pharmaceutical composition of the present invention.

Compositions may be administered individually to a patient or may be administered in combination (*e.g.* simultaneously, sequentially or separately) with other agents, drugs or hormones.

5 Agents as employed herein refers to an entity which when administered has a physiological affect. Drug as employed herein refers to a chemical entity which at a therapeutic dose has an appropriate physiological affect.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, in one or more embodiments the compositions are adapted for administration to human subjects.

10 In one embodiment, the antibody of the present invention may be used to functionally alter the activity of the antigen or antigens of interest and in particular to modulate CD45. For example, the invention may neutralize, antagonize or agonise the activity of said antigen or antigens, directly or indirectly.

The present invention also extends to a kit, comprising an antibody of the invention. In one embodiment a kit comprising any of the antibodies of the invention is provided, optionally with instructions for administration.

In yet another embodiment, the kit further comprises one or more reagents for performing one or more functional assays.

15 In one embodiment, molecules of the present invention including an antibody of the invention is provided for use as a laboratory reagent.

Further Aspects

25 In a further aspect, there is provided a nucleotide sequence or sequences, for example a DNA sequence or sequences encoding an antibody molecule of the present invention as described herein. In one embodiment, the nucleotide sequence is collectively present on more than one polynucleotide but collectively together they are able to encode an antibody of the present invention.

30 The invention herein also extends to a vector comprising a nucleotide sequence as defined above. The term “vector” as used herein refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. An example of a vector is a “plasmid,” which is a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of

autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell, where they are subsequently replicated along with the host genome. In the present specification, the terms “plasmid” and “vector” may be used interchangeably as a plasmid is the most commonly used form of vector. General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to “Current Protocols in Molecular Biology”, 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

The term vector herein also includes, for example, particles comprising the vector, for example LNP (Lipid Nanoparticle) particles and in particular LNP-mRNA particles. It also includes viral particles used for transferring a vector of the present invention.

The term “selectable marker” as used herein refers to a protein whose expression allows one to identify cells that have been transformed or transfected with a vector containing the marker gene. A wide range of selection markers are known in the art. For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. The selectable marker can also be a visually identifiable marker such as a fluorescent marker for example. Examples of fluorescent markers include rhodamine, FITC, TRITC, Alexa Fluors and various conjugates thereof.

In one embodiment, the invention provides a vector encoding an antibody of the invention. In another embodiment, the invention provides vectors which collectively encode an antibody of the invention.

Also provided is a host cell comprising one or more cloning or expression vectors comprising one or more DNA sequences encoding an antibody of the present invention. Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used or eukaryotic, for example mammalian, host cell expression systems may also be used. Suitable mammalian host cells include CHO, myeloma or hybridoma cells. A host cell comprising a nucleic acid molecule or vector of the present invention is also provided.

The present invention also provides a process for the production of a molecule according to the present invention or a component thereof comprising culturing a host cell containing a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the molecule of the present invention, and
5 isolating the molecule.

A method for producing an antibody which comprises a heterodimeric tether may further comprise mixing the two parts of the antibody and allowing the binding partners of the heterodimeric tether to associate. The method may further comprise purification, for example to remove any species apart from the desired heterodimers.

10 The antibodies of the present invention may be used in diagnosis/detection kits. In one embodiment, antibodies of the present invention are fixed on a solid surface. The solid surface may for example be a chip, or an ELISA plate.

The antibodies, of the present invention may be for example conjugated to a fluorescent marker which facilitates the detection of bound antibody-antigen complexes.
15 They can be used for immunofluorescence microscopy. Alternatively, the antibody, may also be used for western blotting or ELISA.

In one embodiment, there is provided a process for purifying an antibody of the present invention or a component thereof. In one embodiment, there is provided a process for purifying an antibody according to the present invention or a component thereof
20 comprising the steps: performing anion exchange chromatography in non-binding mode such that the impurities are retained on the column and the antibody is maintained in the unbound fraction. The step may, for example be performed at a pH about 6-8. The process may further comprise an initial capture step employing cation exchange chromatography, performed for example at a pH of about 4 to 5. The process may further
25 comprise of additional chromatography step(s) to ensure product and process related impurities are appropriately resolved from the product stream. The purification process may also comprise of one or more ultra-filtration steps, such as a concentration and diafiltration step.

“Purified form” as used supra is intended to refer to at least 90% purity, such as
30 91, 92, 93, 94, 95, 96, 97, 98, 99% w/w or purer.

In the context of this specification "comprising" is to be interpreted as "including". Aspects of the invention comprising certain elements are also intended to extend to alternative embodiments "consisting" or "consisting essentially" of the relevant elements. For example, a CDR that is described as “comprising” a sequence of a

specified SEQ ID NO may “consist” of the sequence, i.e. contain only that sequence and no further sequence.

Positively recited embodiments may be employed herein as a basis for a disclaimer.

5 Where the singular is referred to herein, the plural is also encompassed unless otherwise stated or apparent. In particular, the singular forms “a,” “an,” “the” and the like include plural referents unless the context clearly dictates otherwise.

All references referred to herein are specifically incorporated by reference.

10 The sub-headings herein are employed to assist in structuring the specification and are not intended to be used to construct the meaning of technical terms herein.

Sequences of the invention are provided herein below.

In the context of this specification “comprising” is to be interpreted as “including”.

Aspects of the invention comprising certain elements are also intended to extend to alternative embodiments “consisting” or “consisting essentially” of the relevant elements.

15 Positively recited embodiments may be also employed herein as a basis for a disclaimer.

All references referred to herein are specifically incorporated by reference in their entirety. They are further incorporated in respect of the specific context they are cited.

EXAMPLES

20

Example 1: Assessment of antibody binding to human CD45 via Biacore

The present Example sets out the methodology used in other Examples for measuring binding affinity via Biacore.

25 The kinetics of humanised IgG grafts or biparatopic IgG molecules binding to human CD45 were assessed by surface plasmon resonance on a Biacore T200 or 8K+ apparatus (Cytiva).

30 A goat anti-human IgG Fc specific F(ab')₂ (Jackson ImmunoResearch) was immobilised on a CM5 Sensor Chip (Cytiva) via amine coupling chemistry to a level of typically 5000 to 7000RU. Each analysis cycle consisted of capture of the anti-CD45 IgG molecules to the anti-Fc surface followed by injection of human CD45 (prepared in-house) at 25°C and at a flow rate of 30 or 50µl/min. At the end of each cycle the surface was regenerated at a flowrate of 10µL/min using a 60s injection of 50mM HCl followed by a 30s injection of 5mM NaOH and a final 60s injection of 50mM HCl. Human CD45

was injected in HBS-EP+ running buffer (Cytiva) over the captured sample and a reference flow cell at concentrations of 800nM to 3.3nM (5 x 3-fold serial dilutions) or 800 to 3.13nM (4 x 4-fold serial dilutions) in HBS-EP+ running buffer (Cytiva). The binding response of the reference flow cell was subtracted from that of the active flow cell, and buffer blank injections were included to subtract instrument noise and drift. Kinetic parameters were determined using a 1:1 kinetic binding model using either Biacore T200 Evaluation software (version 3.0) or Biacore Insight Evaluation (version 4.0.8.20368).

10 **Example 2: Generation and characterisation of CD45 antibodies**

Introduction:

The present Example describes the initial generation and characterization of antibodies specific for CD45.

Immunisations:

15 Rabbits were immunized with a mixture of rabbit fibroblast cells expressing either human CD45RO, a truncated form of CD45 (SEQ ID 136), or the equivalent cynomolgus monkey CD45RO (SEQ ID NO: 137). Following 3 to 5 shots, animals were sacrificed and PBMC, spleen, bone marrow and lymph nodes harvested. Sera was monitored for binding to human and cyno CD45RO protein by ELISA.

20 *Antibody discovery:*

B cell cultures were prepared using a method similar to that described by Zubler R. H., Erard F., Lees R. K., *et al.* Mutant EL-4 Thymoma Cells Polyclonally Activate Murine and Human B Cells via Direct Cell Interaction. J. Immunol. 1985, 134, (6), 3662–3668. Briefly, spleen or PBMC-derived B cells from immunized rabbits were
25 cultured at a density of approximately 2000-5000 cells per well in bar-coded 96-well tissue culture plates with 200 ul/well RPMI 1640 medium (Gibco BRL) supplemented with 10% FCS (PAA laboratories ltd), 2% HEPES (Sigma Aldrich), 1% L-Glutamine (Gibco BRL), 1% penicillin/streptomycin solution (Gibco BRL), 0.1% β -mercaptoethanol (Gibco BRL), 3% activated splenocyte culture supernatant and gamma-
30 irradiated mutant EL4 murine thymoma cells (5×10^4 /well) for seven days at 37° C in an atmosphere of 5% CO₂.

Primary screening:

B-cell cultures were set up and 34 supernatants were first screened for their ability to bind human and cyno CD45RO using a bead-based Mirrorball FMAT assay. This was a homogeneous assay using biotinylated human and cyno

5 CD45RO coated onto streptavidin beads and a goat anti-rabbit Fcγ fragment-specific FITC conjugate as a reveal agent. Following primary screening, positive supernatants were consolidated on 96-well bar-coded master plates whilst B-cells in cell culture plates were frozen at -80°C. To determine cell binding and species cross-reactivity, master plate supernatants were then screened for binding to HEK293 cells transfected with human and

10 cyno CD45RO.

Fluorescent foci method:

To allow recovery of antibody variable region genes from antigen-specific B cells in a well that contained a heterogeneous B cell population, a deconvolution step was performed. This was achieved using the fluorescent foci method (Clargo *et al.*, 2014.

15 Mabs 2014 Jan. 1: 6(1) 143-159; EP1570267B1). Briefly, Immunoglobulin-secreting B cells from a positive well were mixed with streptavidin beads (New England Biolabs) coated with biotinylated human CD45RO and a 1:1200 final dilution of a goat anti-rabbit Fcγ fragment-specific FITC conjugate (Jackson). After static incubation at 37° C for 1 hour, antigen-specific B cells could be identified due to the presence of a fluorescent halo

20 surrounding that B cell. A number of these individual B cell clones were then picked and deposited into PCR tubes. The fluorescent foci method was also used to identify antigen-specific B cells from a heterogeneous population of B cells directly from the bone marrow of immunized rabbits.

Antibody V-region Discovery:

25 Antibody variable region genes were recovered from single cells by reverse transcription (RT)-PCR. cDNA was synthesized from individual B cells using SuperScript IV VILO Master Mix (Life Technologies) in the presence of 0.5 % Nonidet P-40 (Roche). Using heavy and light chain variable region-specific primers two rounds of PCR were performed, with the nested secondary PCR incorporating restriction sites at the

30 3' and 5' ends allowing cloning of the variable region into Fab X and Fab Y mammalian expression vectors allowing the expression of Fab-X/Fab-Y (e.g. as described in WO 2015/181282 and WO 2017/093402).

Fab X and Fab Y constructs for the expression vectors were co-transfected into Expi293 cells using Expifectamine (Life Technologies) and recombinant antibody expressed in tissue culture flasks in a volume of 30 ml. After 5-7 days expression, supernatants were harvested. To confirm the specificity of the recombinant antibodies, 5 supernatants were tested in a homogeneous fluorescence-based binding assay on HEK293 cells transfected with human and cyno CD45RO.

Assessment of antibody binding and jurkat cell killing

The methods described in the other Examples were used for assessing antibody binding to CD45 and the ability to kill Jurkat cells.

10 *Results:*

Screening a number of clones for KD values for human and cyno CD45RO binding as measured by surface plasmon resonance (SPR) and Jurkat cell killing as a monospecific antibody identified two clones for further study and development. Antibody 17415 and antibody 17552 bound both human and cyno CD45. The rabbit 15 17415 antibody achieved potent killing of Jurkat cells expressing CD45. Biparatopic antibodies were subsequently developed based on using 17415 derived sequences as the “killing arm” of the biparatopic antibody, whilst 17552 derived sequences were developed as the “non-killing” or “helper” arm of the biparatopic antibodies. Both 17415 and 17552 sequences were humanised as described further below.

20 **Example 3: Comparison of the ability of 17415 and 4133 antibodies to kill human CD45 expressing cells**

Introduction:

The ability of the 17415 antibody to kill CD45 expressing target cells was 25 compared to that of the 4133 antibody described in International application No. PCT/EP2021/078516 (published as WO 2022/079199 A1).

PBMC depletion assay

Human PBMC derived from blood leukocyte platelet-apheresis cones (NHSBT 30 Oxford) were banked as frozen aliquots. Prior to an assay being performed, 1 vial of frozen cells, each containing 5×10^7 cells in 1 ml, were thawed in a 37°C water bath and

then added to 50 ml complete media (RPMI 1640 + 2 mM GlutaMAX + 1% Pen/Strep, all supplied by Invitrogen, + 10 % Fetal Bovine Serum (FBS), Sigma Aldrich). Cells were spun (300 g, 5 min, at RT) and re-suspended in 50 ml complete media to wash and spun again. Cells were resuspended in 10 ml complete media and then counted using a
5 ChemoMetec NucleoCounter NC-3000. 1×10^5 cells per well in 50 μ l were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 07-200-95). PBMCs from UCB-Cone 1014 were used in this assay.

Stocks at 400 nM of purified VR17415, VR4133, and 17552 IgG1 antibodies and Isotype control IgG1 (5604) in complete media were prepared in a Greiner 96-well non-
10 binding microplate. The reagents were serially diluted in complete media 1 in 5, seven times (two for Isotype control) to produce an 8-point dose curve. 50 μ l of each dilution (final well concentrations 200-0.0026 nM) was added to the cells and incubated for 4 hours at 37°C, 5% CO₂. Following the incubation, the plate was spun at 300 g, 5 minutes, Room Temperature, the buffer was aspirated with a BioTek ELx405 microplate
15 washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then re-spun and buffer was aspirated to leave the cells in 20 μ l residual media. Cells were analysed live using the Intellicyt iQue 3. Lymphocytes were gated using FSC vs. SSC plot and live cell counts were extracted as metrics and graphical representations generated using
20 Graphpad Prism version 8.1 (Graphpad). Asymmetric (4 parameter) curve fitting was applied to derive EC₅₀ and E_{max} values.

Results:

The percentage reduction of lymphocytes in a PBMC population treated with
25 CD45 IgG1 antibodies is shown in Figure 1. VR17415 has an EC₅₀ of 0.71 and VR4133 has an EC₅₀ of 44.13, showing a >60-fold difference between the EC₅₀s of the antibodies. VR17552 IgG1 was unable to deplete lymphocytes.

Example 4: Humanisation of antibody 17415

30 Rabbit antibody 17415 obtained and assessed using the methods set out in Example 2 was humanised by grafting the CDRs from the rabbit V-region onto human germline antibody V-region frameworks. In order to recover the activity of the antibody, a number of framework residues from the rabbit V-region were also retained in the humanised sequence. These residues were selected using the protocol outlined by Adair

et al. (1991) (Humanised antibodies - WO91/09967, incorporated by reference both in its entirety and specifically in relation to the protocol for selecting residues for retention during humanisation). Alignments of the rabbit antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences are shown in Figures 2 and 3, together with the designed humanised sequences. The CDRs grafted from the donor to the acceptor sequences are as defined by Kabat (Kabat *et al.* (*supra*)), with the exception of CDR-H1 where the combined Chothia/Kabat definition is used (see Adair *et al.*, 1991 Humanised antibodies, WO91/09967).

Human V-region IGKV1-9 and IGKJ4-1 J-region (IMGT, <http://www.imgt.org/>) were initially chosen as the acceptor for antibody 17415 light chain CDRs. Donor residues from the rabbit antibody light chain framework were retained at positions 2 (Valine, V2), 3 (Valine, V3) and 63 (Lysine, K63) (Figure 2). CDRL3 of antibody 17415 contains an unpaired/free Cysteine residue at position 90 (C90): free cysteine residues can be subject to post-translational modification, such as cysteinylolation, and may contribute to covalent aggregation and poor stability. The Cysteine amino acid side chain has both polar and hydrophobic characteristics, thus in humanized graft variants gL2 to gL5, residue C90 was removed by mutation to amino acids with either polar (Serine (C90S), Glutamine (Q90V)) or hydrophobic (Alanine (C90A), Valine (C90V)) side chains (Figure 2).

Human V-region IGHV3-72 and IGHJ4-1 J-region (IMGT, <http://www.imgt.org/>) were chosen as the acceptor for the heavy chain CDRs of antibody 17415. In common with many rabbit antibodies, framework 3 of the 17415 rabbit VH region lacks one residue (78, with reference to SEQ ID NO: 17 gH1) in the loop between beta sheet strands D and E: in the initial humanized graft variants the gap was filled with the corresponding residue (Lysine 78, K78) from the selected human acceptor sequence (Figure 3). Donor residues from the rabbit antibody heavy chain framework were retained at positions 23 (Threonine, T23), 49 (Isoleucine, I49), 74 (Lysine, K74), 76 (Serine, S76), 79 (Threonine, T79), 81 (Valine, V81), 99 (Glutamic acid, E99) and 100 (Leucine, L100). In some humanized graft variants (gH2 to gH4), a disulphide bond formed between Cysteine residues at positions 36 (C36) and 51 (C51) was removed by mutating both residues to either Serine (S36 and S51), Alanine (A36 and A51) or Valine (V36 and V51) (Figure 3).

Genes encoding variant heavy and light chain humanised V-region sequences were designed and constructed by an automated synthesis approach by ATUM (Newark,

CA). For transient expression in mammalian cells, the humanized light chain V-region genes were cloned into the UCB light chain expression vector pMhCK, which contains DNA encoding the human Kappa chain constant region (Km3 allotype). The humanized heavy chain V-region genes were cloned into the UCB human gamma-1 heavy chain expression vector pMhg1 L234A L235A, which contains DNA encoding the human gamma-1 heavy chain constant region (G1m17,1 allotype) with L234A and L235A mutations to reduce binding to Fc gamma receptors. The rabbit V-region genes of antibody 17415 were also cloned into human antibody expression vectors: the light chain V-region was cloned into a modified version of the human Kappa vector comprising the S171C mutation, to re-create the additional disulphide bond found in rabbit VK light chains. Co-transfection of the resulting heavy and light chain vectors into CHOS-XE suspension cells gave expression of the humanized and chimeric recombinant antibodies in the human IgG1 LALA format. The antibodies were assessed for their activity in an *in vitro* T lymphocyte depletion assay, as well as their binding properties. All of the humanised antibodies had both reduced potency (EC50) and efficacy (Emax) compared to the chimeric parental antibody 17415 (Table 3 below).

Table: 3

Antibody 17415 variant				Lymphocyte depletion assay	
Light chain graft	Heavy chain graft	Light chain mutation	Heavy chain Mutations	EC50 (nM) (n=1)	Emax (%) (n=1)
cL	cH	-	-	0.5	84.2
gL1	gH1	-	-	4.48	43.4
gL2	gH1	C90S	-	11.58	53.9
gL3	gH1	C90A	-	23	65.8
gL4	gH1	C90V	-	10.38	53.4
gL5	gH1	C90Q	-	38.45	55.2
gL1	gH2	-	C36S, C51S	4.92	58.7
gL1	gH3	-	C36A, C51A	6.32	53
gL1	gH4	-	C36V, C51V	6.5	40.1

20

To elucidate whether the humanised heavy or light chain was responsible for the loss of functional activity, the humanised light and heavy chain grafts gL1 and gH1 were expressed in combination with the respective chimeric antibody chain (cL and cH) and the antibodies screened for activity in the T lymphocyte depletion assay, and for their

binding affinity for human CD45 by SPR using the methods set out in subsequent Examples with the results presented in Table 4 below. The humanised heavy chain graft paired with the chimeric light chain (cLgH1) demonstrated reduced potency and efficacy in T cell depletion and a reduced binding affinity for CD45. In contrast, the combination of the humanised light chain paired with the chimeric antibody heavy chain (gL1cH), retained binding affinity for CD45 and potency in the T cell depletion assay, but had reduced efficacy compared to the parental antibody 17415.

Combining humanized heavy chain variants gH2 to gH4 with the chimeric light chain revealed that removing the disulphide bond formed between C36 and C51 by mutation of the Cysteine residues to Serine (cLgH2) resulted in an unexpected increase of binding affinity for CD45 and increased potency and efficacy in the T lymphocyte depletion assay compared to cLgH1. In contrast, replacing the Cysteine residues with Valine (cLgH4) resulted in a further loss of binding affinity and function in the T cell depletion assay (compared to cLgH1), whilst replacing the residues with Alanine (cLgH3) had little impact on binding or T cell depletion activity (Table B). Combining humanized light chain graft variants gL2 to gL5 with the chimeric heavy chain demonstrated that removal of the free Cysteine residue by mutation to Serine (C90S, gL2cH) maintained binding affinity, but resulted in some loss of functional activity in the T lymphocyte depletion assay, with reduced potency and efficacy compared to gL1cH. Mutation of C90 to either Alanine (gL3cH), Valine (gL4cH) or Glutamine (gL5cH) resulted in a loss of binding affinity for CD45 and a decrease in T cell depletion activity (Table 4).

Table 4

25

Antibody 17415 variant				Lymphocyte depletion assay				
Light chain graft	Heavy chain graft	Light chain mutation	Heavy chain Mutations	EC50 (nM) (n=1)	Emax (%) (n=1)	k_a (M ⁻¹ s ⁻¹)	k_d (s ⁻¹)	Affinity (KD) nM
cL	cH	-	-	1.17	82.1	4.42E+04	4.29E-03	97.2
gL1	cH	-	-	1.667	36.5	4.98E+04	4.57E-03	91.7
gL2	cH	C90S	-	14.2	52.2	4.66E+04	4.50E-03	96.6
gL3	cH	C90A	-	8.36	32.1	4.79E+04	8.21E-03	171.3
gL4	cH	C90V	-	4.34	42.4	4.75E+04	5.58E-03	117.5
gL5	cH	C90Q	-	4.12	27.1	4.85E+04	1.17E-02	241.8

cL	gH1	-	-	5.61	63.9	4.48E+04	1.83E-02	407.3
cL	gH2	-	C36S, C51S	4.22	70.1	3.83E+04	1.27E-02	332.5
cL	gH3	-	C36A, C51A	4.07	65.1	3.08E+04	1.43E-02	465.3
cL	gH4	-	C36V, C51V	7.66	59.4	4.77E+04	2.81E-02	588.7

Attempting to recover functional activity, the humanised heavy and light chains were further engineered as described herein. In heavy chain grafts gH5 and gH6, residue 78 (Lysine, K78) was deleted to reinstate the gap in framework 3 and recapitulate the architecture of the rabbit loop between beta sheet strands D and E: graft gH6 also included the C36S and C51S mutations to remove the disulphide bond between CDRH1 and CDRH2. In light chain grafts gL6 and gL7, additional donor residues from the rabbit light chain framework were introduced at positions 10 (Serine, S10), 42 (Glutamine, Q42), 83 (Alanine, A83), 106 (Glutamic acid, E106) and 108 (Valine, V108): graft gL7 also included the mutation C90S to remove the free cysteine residue in CDRL3. Additional humanised light chains were designed using human V-region IGKV4-1 plus IGKJ4 J-region (IMGT, <http://www.imgt.org/>) as the acceptor framework (Figure 2). Donor residues from the rabbit antibody light chain framework were retained at five or more positions from the group comprising residues 2 (Valine, V2), 4 (Leucine, L4), 12 (Serine, S12), 19 (Valine, V19), 60 (Serine, S60), 63 (Lysine, K63), 70 (Glutamic acid, E70), 83 (Alanine, A83), 85 (Threonine, T85), 106 (Glutamic acid, E106) and 108 (Valine, V108): grafts gL15 and gL16 also included the mutation C90S to remove the free cysteine residue in CDRL3. The humanised heavy and light chains were expressed as hIgG1 LALA antibodies in different graft combinations, and the resulting antibodies tested for functional activity in the T lymphocyte depletion assay and for their binding affinity for CD45 for SPR (Table 5 below).

Table 5

Antibody 17415 variant				T lymphocyte depletion assay				
Light chain graft	Heavy chain graft	Light chain mutns	Heavy chain mutns	EC50 (nM)	E _{max} (%)	k _a (M ⁻¹ s ⁻¹)	k _d (s ⁻¹)	Affinity (KD) nM
cL	cH	-	-	0.41 (n=3) Range (0.32-0.47)	85.3 (n=3) Range (80.4-90.7)	4.15E+04	4.34E-03	104.7

gL1	gH1	-	-	10.84 (n=2) Range (8.69- 12.99)	60.45 (n=2) Range (56.9- 64.0)	5.99E+04	2.51E-02	418.5
gL1	gH5	-	-	19.17 (n=2) Range (12.48- 25.86)	58.75 (n=2) Range (55.5- 62.0)	5.18E+04	1.45E-02	279.9
gL6	gH5	-	-	1.99 (n=2) Range (0.85- 3.13)	88.45 (n=2) Range (82.8- 94.1)	5.56E+04	1.61E-02	290.1
gL7	gH5	C90S	-	2.4 (n=2) Range (1.90- 2.89)	88.15 (n=2) Range (83.8- 92.5)	4.62E+04	1.57E-02	339.7
gL1	gH6	-	C36S, C51S	-	-	3.63E+04	6.50E-03	179.2
gL6	gH6	-	C36S, C51S	1.76 (n=2) Range (1.63- 1.89)	76.15 (n=2) Range (73.4- 78.9)	3.46E+04	7.74E-03	223.8
gL7	gH6	C90S	C36S, C51S	0.81 (n=3) Range (0.56- 1.18)	96.7 (n=3) Range (76.9- 107.8)	3.85E+04	8.78E-03	228
gL13	gH5	-		2.62 (n=2) Range (1.55- 3.68)	76.0 (n=2) Range (61.3- 90.7)	4.74E+04	1.42E-02	299.6
gL14	gH5	-	-	6.4 (n=1)	97.2 (n=1)	4.90E+04	1.35E-02	274.5
gL15	gH5	C90S		3.61 (n=2) Range (1.97- 5.24)	83.8 (n=2) Range (76.3- 91.3)	4.81E+04	1.33E-02	276.9
gL16	gH5	C90S	-	4.05 (n=2) Range (3.98- 4.12)	86.45 (n=2) Range (83.2- 89.7)	5.10E+04	1.27E-02	248.7
gL13	gH6	-		1.21 (n=2) Range (0.37- 2.05)	80.0 (n=2) Range (72.2- 87.7)	3.61E+04	6.98E-03	193.2
gL14	gH6	-	C36S, C51S	3.46 (n=2) Range	72.6 (n=2) Range	3.63E+04	6.04E-03	166.3

				(2.45-4.47)	(64.8-80.4)			
gL15	gH6	C90S		0.53 (n=3) Range (0.26-0.89)	83.5 (n=3) Range (77.1-95.6)	3.90E+04	6.28E-03	161.1
gL16	gH6	C90S	C36S, C51S	1.10 (n=3) Range (0.61-1.41)	85.5 (n=3) Range (62.2-104.1)	3.82E+04	4.91E-03	128.7

Deletion of residue 78 in framework 3 of the humanised heavy chain graft gH5 increased binding affinity for CD45 (Table 5, gL1gH1 (418.5nM) compared to gL1gH5 (279.9nM)) but resulted in a reduction of potency and efficacy in the T cell depletion assay. Consistent with the unexpected increase in binding affinity observed upon removal of the disulphide bond in humanized heavy chain graft gH2, the binding affinity for CD45 was further increased in graft gH6 (gL1gH6 179.2nM).

The T cell depletion activity of humanized 17415 antibodies comprising grafts gH5 and gH6 were restored by increasing the donor residue content in light chain grafts gL6 and gL7 (gL1gH5 EC50 19.17nM, Emax 58.75% compared to gL6gH5 EC50 1.99nM, Emax 88.45% or gL7gH5 EC50 2.4nM, Emax 88.15%, and gL6gH6 EC50 1.76nM, Emax 76.15% or gL7gH6 EC50 0.63nM, Emax 106%). Similarly, grafting the light chain CDRs onto the alternative human acceptor framework IGKV4-1 plus JK4 J-region (grafts gL13 to gL16) afforded increased potency and efficacy of T cell depletion when paired with heavy chain grafts gH5 and gH6 (relative to gL1gH5 and gL1gH6), whilst maintaining affinity for CD45 (Table C).

A further description of some of the experiments forming part of the humanisation of the original 17415 antibody is provided in Example 6 below.

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Example 5: Humanisation of antibody 17552

Rabbit antibody 17552 obtained and assessed using the methods set out in Example 2 was humanised by grafting the CDRs from the rabbit V-region onto human germline antibody V-region frameworks. In order to recover the activity of the antibody, a number of framework residues from the rabbit V-region were also retained in the humanised sequence. These residues were selected using the protocol outlined by Adair *et al.* (1991) (Humanised antibodies. WO91/09967 - supra). Alignments of the rabbit

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antibody (donor) V-region sequences with the human germline (acceptor) V-region sequences are shown in Figure 4, together with the designed humanised sequences. The CDRs grafted from the donor to the acceptor sequences are as defined by Kabat (Kabat *et al.*, 1987, *supra*), with the exception of CDR-H1 where the combined Chothia/Kabat definition is used (see Adair *et al.*, 1991 Humanised antibodies. WO91/09967).

Human V-region IGKV1-8 and IGKJ4-1 J-region (IMGT, <http://www.imgt.org/>) were chosen as the acceptor for antibody 17552 light chain CDRs. The light chain framework residues in the humanized graft variants are all from the human germline gene, with the exception of one or more residues from the group comprising residues 2, 3 and 63 (with reference to SEQ ID NO: 3 gL1), where the donor residues Leucine (L2), Valine (V3) and Glutamic acid (E63) were retained, respectively, (Figure 4). A free Cysteine residue at position 77 (C77) in Framework 3 was mutated to Serine (C77S). Human V-region IGHV4-4 and IGHJ4-1 J-region (IMGT, <http://www.imgt.org/>) were chosen as the acceptor for the heavy chain CDRs of antibody 17552. In common with many rabbit antibodies, the VH gene of antibody 17552 is shorter than the selected human acceptor. When aligned with the human acceptor sequence, framework 1 of the VH region of antibody 17552 lacks the N-terminal residue, which is retained in the humanized antibody (Figure 4). Framework 3 of the 17552 rabbit VH region also lacks two residues (75 and 76, with reference to SEQ ID NO: 17, gH1) in the loop between beta sheet strands D and E: in the humanized graft variants the gap is filled with the corresponding residues (Lysine 75, K75; Asparagine 76, N76) from the selected human acceptor sequence (Figure 4). The heavy chain framework residues in the humanized graft variants are all from the human germline gene, with the exception of residues 23, 47, 67, 71, 73, 78 and 96 (with reference to SEQ ID NO: 17, gH1), where the donor residues Threonine (T23), 47 Tyrosine (Y47), Phenylalanine (F67), Lysine (K71), Serine (S73), Valine (V78) and Threonine (T96) were retained, respectively. In some humanized graft variants, a potential Aspartic acid isomerization site in CDRH3 was modified by either replacing the Aspartic acid residue at position 101 with Glutamic acid (D101E), or by replacing the Glycine residue at position 102 with Serine (G102S) or Alanine (G102A).

Genes encoding variant heavy and light chain humanised V-region sequences were designed and constructed by an automated synthesis approach by ATUM (Newark, CA). For transient expression in mammalian cells, the humanized light chain V-region genes were cloned into the UCB light chain expression vector pMhCK, which contains

DNA encoding the human Kappa chain constant region (Km3 allotype). The humanized heavy chain V-region genes were cloned into the UCB human gamma-1 heavy chain expression vector pMhg1 L234A L235A, which contains DNA encoding the human gamma-1 heavy chain constant region (G1m17,1 allotype) with L234A and L235A mutations to reduce binding to Fc gamma receptors. The rabbit V-region genes of antibody 17552 were also cloned into human antibody expression vectors: the light chain V-region was cloned into a modified version of the human Kappa vector comprising the S171C mutation, to re-create the additional disulphide bond found in rabbit VK light chains. Co-transfection of the resulting heavy and light chain vectors into CHOS-XE suspension cells gave expression of the humanized and chimeric recombinant antibodies in the human IgG1 LALA format. The antibodies were assessed for their binding affinity for CD45 by SPR Table 6.

The humanized 17552 gL1gH1 antibody retained binding affinity for CD45 relative to the chimeric parental rabbit antibody (gL1gH1, 7.9nM compared to cLcH, 5.6nM). Modification of the potential Aspartic acid isomerisation site by mutation of residue Glycine 102 to Alanine (G102A) in humanized heavy chain graft gH4 retained affinity compared to gL1gH1 (gL1gH4, 6.5nM), whilst mutations D101E (gH2) and G102S (gH3) both resulted in a reduction of binding affinity (gL1gH2, 72.3nM and gL1gH3, 20.7nM).

Table 6

Antibody 17415 variant					
Light chain graft	Heavy chain graft	Heavy chain Mutation	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	Affinity (KD) nM
cL	cH	-	1.21E+05	6.83E-04	5.6
gL1	gH1	-	1.10E+05	8.75E-04	7.9
gL1	gH2	D101E	1.78E+05	1.29E-02	72.3
gL1	gH3	G102S	8.90E+04	1.84E-03	20.7
gL1	gH4	G102A	9.31E+04	6.06E-04	6.5
gL2	gH1	-	8.55E+04	9.04E-04	10.6

Example 6: Human PBMC cell depletion assay using supernatants of monospecific antibodies with different permutations of 17415 light and heavy chain graft variants

5 *Introductions:*

The present Example provides further description of the experiments involved in the humanisation of the original rabbit 17415 antibody also described in Example 3 above.

10 *Materials & Methods - PBMC depletion assay:*

Human PBMC derived from blood leukocyte platelet-apheresis cones (NHSBT Oxford) were banked as frozen aliquots. Prior to an assay being performed, 1 vial of frozen cells, each containing 5×10^7 cells in 1 ml, were thawed in a 37°C water bath and then added to 50 ml complete media (RPMI 1640 + 2 mM GlutaMAX + 1% Pen/Strep, all supplied by Invitrogen, + 10 % Fetal Bovine Serum (FBS), Sigma Aldrich). Cells
15 were spun (300 g, 5 min, at RT) and re-suspended in 50 ml complete media to wash and spun again. Cells were resuspended in 10 ml complete media and then counted using a ChemoMetec NucleoCounter NC-3000. 1×10^5 cells per well in 50µl were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate
20 (cat no. 07-200-95). PBMCs from 7 donors were used in this assay, UCB Cones 032, 034, 036, 037, 045, 858 and 1031.

Stocks at 400 nM of each humanised 17415 IgG1 LALA graft supernatant in complete media were prepared in a Greiner 96-well non-binding microplate. The reagents were serially diluted in complete media 1 in 5, seven times to produce an 8-
25 point dose curve. 50µl of each dilution (final well concentrations 200-0.0026nM) was added to the cells and incubated for 4 hrs at 37°C, 5% CO₂. Following the incubation, the plate was spun at 300 g, 5 min, RT, the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then
30 re-spun and buffer was aspirated to leave the cells in 20µl residual media. For lymphocyte analysis, 20µl of LIVE/DEAD™ Fixable Near-IR Dead Cell Stain (Invitrogen, 1:5000 dilution) was added to the wells and incubated for 15 minutes at 4°C. For CD3 staining, cells were stained with 20µl antibody solution containing anti-Human CD3 FITC (BD Biosciences, cat no. 561806, 1:50 dilution) and LIVE/DEAD™ Fixable

Near-IR Dead Cell Stain (Invitrogen, 1:5000 dilution). Following the incubation, the plate was spun at 300 g, 5 min, RT, the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0. 1 % NaN₃+ 2 mM EDTA, Sigma Aldrich) to wash and then
5 re-spun and buffer was aspirated to leave the cells in 20µl residual media. Cells were analysed live using the Intellicyt iQue Screener PLUS and iQUE 3. Live cell counts were extracted as metrics and graphical representations generated using Graphpad Prism version 8. 1 (Graphpad). Asymmetric (4 parameter) curve fitting was applied to derive EC50 and Emax values.

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Results:

Percentage reduction of lymphocytes in PBMC population treated with 17415 IgG1 LALA heavy chain H1 and 5 Light chain grafts (L1-5) is shown in Figure 5(A). Percentage reduction of lymphocytes in a PBMC population treated with 17415 light
15 chain L1 and 4 heavy chain grafts (H1-4) is shown in Figure 5(B). Data from one donor, Cone 1031 is shown. Humanisation alterations in both the heavy and light chain have reduced the EC50 and Emax of 17415 IgG1 LALA antibody compared to the chimeric (cLcH).

To identify the humanisation mutations that reduces functional killing of the
20 17415 IgG1 antibody, each light chain graft (L1-5) was paired with the chimeric heavy chain (cH) and percentage reduction of lymphocytes in a PBMC population is shown in Figure 6(A). All of the humanised light chain grafts showed a reduced EC50 and Emax of 17415 IgG1 LALA antibody compared to the chimeric (cLcH). Each heavy chain graft (H1-4) was paired with the chimeric light chain (cL) and percentage reduction of
25 lymphocytes in a PBMC population is shown in Figure 6(B). Data from one donor, Cone 858 is shown. The humanised heavy chain grafts show less impact on the EC50 and Emax of 17415 IgG1 LALA antibody compared to the chimeric (cLcH), indicating the humanised light chains contribute more to the loss of functional killing.

To increase functional killing of humanised 17415 IgG1 LALA, further heavy
30 chains (H5 and H6) were tested with further light chains (L6, 7, 13, 14, 15, 16). Percentage reduction of T cells in PBMC population treated with these humanised grafts is shown in Figure 7. Humanised 17415 grafts with heavy chain H5 is shown in Figure 7(A) and humanised 17415 grafts with heavy chain H6 is shown in Figure 7(B). Data from Cone 036 is shown as representative of data from 3 donors. Humanised 17415

grafts containing heavy chain H6 show Emax and EC50 values similar to the chimeric (cLcH) 17415 compared to H5. Of the grafts containing H6, L7, L15 and L16 light chain grafts show the best functional and humanisation properties.

5 Example 7: Human Jurkat cell depletion assay using supernatants of monospecific antibodies with different permutations of 17415 light and heavy chain graft variants

Jurkat depletion assay

Prior to an assay being performed, 1 vial of Jurkat (acute T-cell leukaemia cell lines) was thawed in a 37°C water bath and then added to 20 ml complete media (RPMI 1640 + 2 mM 25 GlutaMAX + 1 % Pen/Strep, all supplied by Invitrogen, + 10 % Fetal Bovine Serum (FBS), Sigma Aldrich). Cells were spun (300 g, 5 min, at RT) and re-suspended in 20 ml complete media to wash and spun again. Cells were resuspended in 10 ml complete media and were counted using a ChemoMetec NucleoCounter NC-3000. 1x10⁵ cells in 50µl complete media were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 07-200-95).

Stocks at 400 nM of each humanised 17415 IgG1 LALA graft supernatant in complete media were prepared in a Greiner 96-well non-binding microplate. The reagents were serially diluted in complete media 1 in 5, seven times to produce an 8-point dose curve. 50µl of each dilution (final well concentrations 200-0.0026nM) was added to the cells and incubated for 4 hrs at 37°C, 5% CO₂. Following the incubation, the plate was spun at 300 g, 5 min, RT, the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then re-spun and buffer was aspirated to leave the cells in 20µl residual media. 10µL of Sytox Blue (Invitrogen, 1:500 dilution) was added to the wells and incubated for 10 mins, RT. Cells were analysed live using the Intellicyt iQue Screener PLUS and iQUE 3. Live cell counts were 25 extracted as metrics and graphical representations generated using Graphpad Prism version 8.1 (Graphpad). Asymmetric (4 parameter) curve fitting was applied to derive EC50 and Emax values.

Results:

The results obtained for the different permutations of 17415 light and heavy chain graft variants are shown in Figure 8, parts (A) to (D).

Example 8: Human PBMC cell depletion assay using purified monospecific antibodies with 17415 light chain graft variants 7, 15 and 16

5 *Materials & Methods - PBMC depletion assay*

Human PBMC derived from blood leukocyte platelet-apheresis cones (NHSBT Oxford) were banked as frozen aliquots. Prior to an assay being performed, 1 vial of frozen cells, each containing 5×10^7 cells in 1 ml, were thawed in a 37°C water bath and then added to 50 ml complete media (RPMI 1640 + 2 mM GlutaMAX + 1% Pen/Strep, 10 all supplied by Invitrogen, + 10 % Fetal Bovine Serum (FBS), Sigma Aldrich). Cells were spun (300 g, 5 min, at RT) and re-suspended in 50 ml complete media to wash and spun again. Cells were resuspended in 10 ml complete media and then counted using a ChemoMetec NucleoCounter NC-3000. 1×10^5 cells per well in 50µl were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate 15 (cat no. 07-200-95). PBMCs from 3 donors, UCB-Cones 1033, 1001 and 1000 were used in this assay.

Stocks at 500 nM of each purified humanised 17415 IgG1 LALA graft, 17552 IgG1 LALA graft, 17552 IgG1, and Isotype control IgG1 (5604) in complete media were prepared in a Greiner 96-well non-binding microplate. The reagents were serially diluted 20 in complete media 1 in 5, seven times to produce an 8-point dose curve, except 17552 IgG1 and 5604 IgG1 where one concentration was used (final well concentration 250nM). 50µl of each dilution (final well concentrations 250-0.0032nM) was added to the cells and incubated for 4 hrs at 37°C, 5% CO₂. Following the incubation, the plate was spun at 300 g, 5 min, RT, the buffer was aspirated with a BioTek ELx405 microplate 25 washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then re-spun and buffer was aspirated to leave the cells in 20µl residual media. For CD3 staining, cells were stained with 20µl antibody solution containing anti-Human CD3 FITC (BD Biosciences, cat no. 561806, 1:50 dilution) and LIVE/DEAD™ Fixable Near-IR Dead 30 Cell Stain (Invitrogen, 1:5000 dilution) for 30mins, 4°C. Following the incubation, the plate was spun at 300 g, 5 min, RT, the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then re-spun. Cells were washed again, buffer was aspirated to leave the cells in 20µl residual

media. Cells were analysed live using the Intellicyt iQue 3. Live cell counts were extracted as metrics and graphical representations generated using Graphpad Prism version 8. 1 (Graphpad). Asymmetric (4 parameter) curve fitting was applied to derive EC50 and Emax values.

5

Results:

The percentage reduction of T cells in a PBMC population treated with humanised 17415 IgG1 LALA grafts containing 17415 light chain modifications 7, 15 and 16 and with humanised 17552 IgG1 LALA grafts containing the 17552 heavy chain modification 1 and 4 are shown in in Figure 9. Data from Cone 1000 is shown as representative of data from 3 donors. The grafts all showed reduction of T cells with a Emax range of 63.4-83.3% and EC50 range of 0.21-0.85nM. 17552 IgG1 LALA grafts do not deplete T-cells.

15 **Example 9: Jurkat cell depletion assay using purified monospecific antibodies with 17415 light chain graft variants 7, 15 and 16**

Jurkat depletion assay:

Prior to an assay being performed, 1 vial of Jurkat (acute T-cell leukaemia cell lines) was thawed in a 37°C water bath and then added to 20 ml complete media (RPMI 1640 + 2 mM 25 GlutaMAX + 1 % Pen/Strep, all supplied by Invitrogen, + 10 % Fetal Bovine Serum (FBS), Sigma Aldrich). Cells were spun (300 g, 5 min, at RT) and re-suspended in 20 ml complete media to wash and spun again. Cells were resuspended in 10 ml complete media and were counted using a ChemoMetec NucleoCounter NC-3000. 1x10⁵ cells in 50µl complete media were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 07-200-95).

Stocks at 500 nM of each humanised 17415 IgG1 LALA graft, 17552 IgG1, and Isotype control IgG1 (5604) in complete media were prepared in a Greiner 96-well non-binding microplate. The reagents were serially diluted in complete media 1 in 5, seven times to produce an 8-point dose curve, except 17552 IgG1 and 5604 IgG1 where one concentration was used (final well concentration 250nM). 50µl of each dilution (final well concentrations 250-0.0032nM) was added to the cells and incubated for 4 hrs at 37°C, 5% CO₂. Following the incubation, the plate was spun at 300 g, 5 min, RT, the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-

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suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then re-spun and buffer was aspirated to leave the cells in 20 µl residual media. 10 µL of Sytox Blue (Invitrogen, 1:500 dilution) was added to the wells and incubated for 10 mins, RT. Cells were analysed live using the
5 Intellicyt iQue 3. Live cell counts were extracted as metrics and graphical representations generated using Graphpad Prism version 8.1 (Graphpad). Asymmetric (4 parameter) curve fitting was applied to derive EC₅₀ and Emax values.

Results:

10 The percentage reduction of Jurkat cells in a PBMC population treated with humanised 17415 IgG1 LALA grafts containing 17415 light chain modifications 7, 15 and 16 are shown in in Figure 10. The 17415 grafts all showed reduction of Jurkat cells with an Emax range of 87.9-89.4% and EC₅₀ range of 0.08-0.59 nM. 17552 IgG1 did not deplete Jurkat cells.

15

Example 10: Assessment of binding of monospecific CD45 antibodies to ExpiHEK cells expressing human CD45

Materials & Methods:

20 Expi293F™ (Gibco) cells were transfected with human CD45 RO ECD mRNA using Lipofectamine™ RNAiMAX transfection reagent (RNAiMAX, Invitrogen). Expi293F cells were prepared at 5 × 10⁵ cells / mL in Expi293™ Expression Medium (Gibco) before transfection. A total of 2 µg of mRNA was diluted in 100 µL of Opti-MEM™ I Reduced Serum Medium (Opti-MEM, Invitrogen) and mixed with 3 µL of
25 RNAiMAX pre-diluted in 100 µl Opti-MEM. After 10 - 20 min incubation at room temperature, 50 µL of mixture were added to the cells in a final volume of 0.5 mL medium in each well of 24-well plate (Corning). Cells were incubated at 37°C, 5% CO₂ for 18 – 24 hours to allow protein expression. Cells were collected from the 24 well plate then diluted in 10ml FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 %
30 NaN₃ + 2 mM EDTA, Sigma Aldrich) and spun (200xg, 6 mins). Cells were resuspended in 10ml FACS buffer and then counted using a ChemoMetec NucleoCounter NC-3000. 20,000 cells per well in 25 µl were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 07-200-95).

Stocks at 500 nM of each purified humanised 17415 IgG1 LALA grafts and control molecules in FACS buffer were prepared in a Greiner 96-well non-binding microplate. The reagents were serially diluted in FACS buffer 1 in 5, seven times to produce an 8-point dose curve. 25µl of each dilution (final well concentrations 250-
 5 0.0032nM) was added to the cells and incubated on a shaker for 1 hr at 4°C. Cells were stained with 25µl secondary antibody solution containing Anti-human IgG AF488 Fab fragment goat anti-human IgG Fcγ, (Jackson ImmunoResearch, cat no.109-547-008, 1:200 dilution) and LIVE/DEAD™ Fixable Near-IR Dead Cell Stain (Invitrogen, 1:5000 dilution) for 1hr, 4°C. Following the incubation, the plate was spun at 300 g, 5 min, RT,
 10 the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0. 1 % NaN₃+ 2 mM EDTA, Sigma Aldrich) to wash and then re-spun. Cells were washed again, and buffer was aspirated to leave the cells in 20µl residual media. Cells were analysed live
 15 graphical representations generated using Graphpad Prism version 8. 1 (Graphpad).

Protein sequences:

>Hu_CD45_RO ECD (SEQ ID NO: 136)
 20 MTMYLWLKLLAFGFAFLDTEVFVTGQSPTPSPTDAYLNASETTTLSPSGSAVIST
 TTIATTPSKPTCDEKYANITVDYLYNKETKLFTAKLNVNENVECGNNTCTNNEV
 HNLTECKNASVSISHNSCTAPDKTLILDVPPGVEKFQLHDCTQVEKADTTICLKW
 KNIETFTCDTQNITYRFQCGNMIFDNKEIKLENLEPEHEYKCDSEILYNNHKFTNA
 SKIIKTDGSPGEPQIIFCRSEAAHQGVITWNPPQRSFHNFTLCYIKETEKDCLNLD
 25 KNLIKYDLQNLKPYTKYVLSLHAYIIAKVQRNGSAAMCHFTTKSAPPSQVWNM
 TVSMTSDNSMHVKCRPPRDRNGPHERYHLEVEAGNTLVRNESHKNCDFRVKDL
 QYSTDYTFKAYFHNGDYPGEPFILHHSTSYNSKALIAFLAFLIIVTSIALLVVLYKI
 YDL
 30

Results:

The mean fluorescent intensities (MFI, Geomean) of binding of humanised 17415 IgG1 LALA grafts containing 17415 light chain modifications 7, 15 and 16 to human
 35 CD45 expressing HEK cells are shown in Figure 11 alongside an isotype control IgG1 (5604).

Example 11: Production of biparatopic format antibodies*Introduction:*

- 5 Various biparatopic antibodies were generated and then assessed in the subsequent Examples. The present Example therefore describes a possible approach for generating the biparatopic antibodies and their initial assessment.

Parental antibody expression:

- 10 For the Knob in Hole (KiH) heterodimerisation technology, Knob mutation (T366W) and hole mutations (T366S L368A Y407V) were introduced to respective heavy chain constant domains to promote biparatopic antibody formation. As a first step the parental antibodies were expressed. Genes encoding the respective light and heavy chain V-
- 15 The DNA was amplified using a QIAGEN Plasmid Plus Giga Kit (cat. no. 12991) as per the supplier's instructions and finally transfected into CHO-SXE cells using ExpiCHO™ Expression system Kit (A29133). Supernatants were harvested after 7 days.

Protein exchange method:

- 20 Supernatants of CD45-specific IgG1 were purified using 2x5 mL MabSelect Sure columns (Cytiva) attached to an AKTA Pure purification system (GE Healthcare Life Sciences). Once the column was equilibrated into PBS, clarified culture supernatant was loaded onto the column at a flow rate of 10 ml/min. Post loading, the column was washed with 4CVs of PBS and bound product eluted from the column with 0.1 M sodium citrate
- 25 pH3.4 The eluate was neutralised with 1/5th of the elution volume with 2 M Tris-HCl, pH8.5.
- Once eluted and neutralised, the concentration of the post affinity material was determined and heavy chain 1 to heavy chain 2 were combined. Cysteamine (Sigma-M9768) was added to the mixture to give a final concentration of 5 mM (from 500 mM
- 30 stock in PBS) and incubated at room temperature for 4 h. The material was then concentrated to approximately 4 mL using an Amicon® Ultra-15 Centrifugal Filter Units (Merck-UFC903024) and loaded onto a HiLoad 26/600 Superdex 200 pg column (Cytiva) and eluted with PBS. Desired fractions were pooled and subjected to endotoxin

removal using a Proteus NoEndoHC column (Protein Ark) and finally sterile filtered using a Steriflip™ vacuum filter unit (Merck).

Characterisation and quality control:

- 5 Protein concentration was calculated after determining A280 absorbance readings using the Lunatic system (Unchained labs). Percentage monomer was determined by loading 2 µg protein onto an ACQUITY BEH200 column attached to a Waters ACQUITY UPLC system. HIC analysis was performed using a Dionex ProPac HIC-10 column attached to an Agilent 1200 binary HPLC with a fluorescence detector. SDS-PAGE was performed
- 10 using 4 to 20%, Tris-Glycine gels run at 180 V for 45 min. Endotoxin was determined using Endosafe nexgen-MCS system (Charles River). Mass identification of constructs was determined by treating samples with NuPAGE™ Sample Reducing Agent (10X) (Invitrogen- NP0004) and/or Rapid™ PNGase F (Neb- P0710s) as per suppliers' instructions. Samples were then loaded onto a XEVO G2 QToF (Waters) equipped with a
- 15 BioResolveT RP mAb Polyphenyl, 450 Å, 2.7 µm column.

Results:

Results for specific experiments on biparatopic antibodies generated are described in the subsequent Examples.

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Example 12: Assessment of binding of biparatopic CD45 antibodies to ExpiHEK cells expressing human and cynomolgus CD45

Introduction:

- 25 Various bispecific humanised 17415-17552 IgG1 LALA grafts containing 17415 light chain modifications were assessed for their ability to bind to human or cyno CD45 RO ECD expressed by human Expi293F™ (Gibco) cells was assessed as discussed further below. The ability to bind both cyno and human is useful as the former means that the antibodies can be studied in cyno monkeys prior to human trials.

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Materials & Methods - Cell Binding Method

The same cell binding assay described above in relation to monospecific CD45 antibodies was used to assess binding to CD45 on the surface of Expi293F™ (Gibco)

cells transfected with human or cyno CD45 RO ECD mRNA using Lipofectamine™ RNAiMAX transfection reagent (RNAiMAX, Invitrogen).

Stocks at 500 nM of each purified humanised 17415-17552 IgG1 LALA and control molecule grafts in FACS buffer were prepared as described.

5 *Protein sequences:*

>Hu_CD45_RO ECD (SEQ ID NO: 136)
 MTMYLWLKLLAFGFAFLDTEVFVTGQSPTPSPTDAYLNASETTTLSPSGSAVIST
 TTIATTPSKPTCDEKYANITVDYLYNKETKLFTAKLNVNENVECGNNTCTNNEV
 10 HNLTECKNASVSISHNSCTAPDKTLILDVPPGVEKFQLHDCTQVEKADTTICLKW
 KNIETFTCDTQNITYRFQCGNMIFDNKEIKLENLEPEHEYKCDSEILYNNHKFTNA
 SKIIKTDFGSPGEPQIIFCRSEAAHQGVITWNPPQRSFHNFTLCYIKETEKDCLNLD
 KNLIKYDLQNLKPYTKYVLSLHAYIIAKVQRNGSAAMCHFTTKSAPPSQVWNM
 TVSMTSDNSMHVKCRPPRDRNGPHERYHLEVEAGNTLVRNESHKNCDFRVKDL
 15 QYSTDYTFKAYFHNGDYPGEPFILHHSTSYNSKALIAFLAFLIIVTSIALLVVLYKI
 YDL

>Cyno_CD45_RO ECD (SEQ ID NO: 137)
 MTMCLWLKLLAFVFAFLDTEVFVTGQGSTLSPTVSYLNASETTTPSPSGSTVISTP
 20 TIATTTSKPTCAEKYATIPVDYLYNNKTKLFTAKLNVNENVECTNNNHTHNICTN
 NEVLNLPECKEMNVFVSHNSCTDRHKELKLDVPPEVEKFQLDDCTPDVEANTTI
 CLKWKIITFACDKSKITYRFQCGNKTYNKEGIYLENLEPEYEEKCDSEILYNNH
 KYINITKLIKTDGIPGQPQNVCRRHEDAHQGVITWNPPQRSFHNFTLCYVKNPA
 KKCLILDKHLTTYHLQNLKPYTNYSLHAYIIAKVQRNGTAATCNFTTESAPPS
 25 QVQNMIVSTSDNSMHVKCEVPRDVNGPTGLYHLEVEAGNTLVRNLSQSKCDF
 VNNLQYSTYYNLKAYYHNGKYSGEVPILRESTSYNSKALIAFLAFLIIVTSIALLV
 VLYKIYDL

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Results

The mean fluorescent intensities (MFI, Geomean) of binding of humanised 17415-17552 IgG1 LALA grafts containing 17415 light chain modifications 7 (A), 15 (B) and 16 (C) to human CD45 expressing HEK cells are shown in Figure 12 alongside relevant control molecules. The MFI of binding of humanised 17415-17552 grafts containing 17415 light chain modifications 7 (A), 15 (B) and 16 (C) to cyno CD45 expressing HEK cells are shown in Figure 13 alongside relevant control molecules. Each light chain was paired with 17552 heavy chain grafts H1 and H4. The 17415-17552 grafts show Emax MFIs ranging from 4×10^6 - 6.8×10^6 for human CD45 binding and 10×10^6 - 11.3×10^6 for cyno CD45 binding, indicating all biparatopic antibodies grafts bind human and cyno CD45 expressed on cells well.

Example 13: Human T cell depletion assay using biparatopic antibodies with 17415 light chain graft variants 7, 15 and 16

Materials & Methods - PBMC depletion assay

5 The human PBMC depletion assay employed was the same as that used in earlier Examples, but the assays were performed using stocks at 500 nM each of purified humanised 17415-17552 IgG1 LALA grafts and control antibodies. PBMCs from 3 donors, UCB-Cones 1001, 1000 and 947 were used in this assay.

10 *Results:*

 The percentage reduction of T cells in a PBMC population treated with humanised 17415-17552 IgG1 LALA grafts containing 17415 light chain modifications 7 (A), 15 (B) and 16 (C) are shown in Figure 14 alongside relevant control molecules. Data from Cone 1000 is shown as representative of data from 3 donors. Each light chain was
15 paired with 17552 heavy chain grafts H1 and H4. The grafts all showed reduction of T cells with a Emax range of 60.2-88.2% and EC50 range of 0.02-0.21nM thereby demonstrating successful cell depletion. The biparatopic graft which most effectively depleted T cells was 17415gL7gH6-17552gL1gH4 IgG1 LALA.

20 **Example 14: Jurkat cell depletion assay using biparatopic antibodies with 17415 light chain graft variants 7, 15 and 16**

Introduction

 Jurkat cells were again used as a model for cell depletion of cancer cells via
25 targeting CD45.

Materials & Methods:

 The method performed was as described in earlier Examples. Stocks at 500 nM of each humanised 17415-17552 IgG1 LALA graft and control antibodies in complete
30 media were prepared as described in other Examples.

Results:

 The percentage reduction of Jurkat cells treated with humanised 17415-17552 IgG1 LALA grafts containing 17415 light chain modifications 7 (A), 15 (B) and 16 (C)

are shown in Figure 15 alongside relevant control molecules. Each light chain was paired with 17552 heavy chain grafts H1 and H4. The grafts all showed reduction of Jurkat cells with an Emax range of 83-95.7% and EC50 range of 0.14-033nM. The biparatopic which most effectively depleted Jurkat cells was 17415gL15gH6-17552gL1gH1 IgG1 LALA.

5

Example 15: Cell depletion assays on cyno PBMCs

Introduction

The present Example describes the methodology for assessing ability of both monospecific and biparatopic CD45 antibodies to deplete cyno monkey cells.

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Materials & Methods - Cyno T cell depletion assay

Cynomolgus PBMC were acquired from Primacyt as frozen aliquots. Prior to an assay being performed, 1 vial of frozen cells, each containing 2×10^7 cells in 1 ml, were thawed in a 37°C water bath and then added to 50 ml complete media (RPMI 1640 + 2 mM GlutaMAX + 1% Pen/Strep, all supplied by Invitrogen, + 10 % Fetal Bovine Serum (FBS), Sigma Aldrich). Cells were spun (300 g, 5 min, at RT) and re-suspended in 50 ml complete media to wash and spun again. Cells were resuspended in 10 ml complete media and then counted using a ChemoMetec NucleoCounter NC-3000. 1×10^5 cells per well in 50µl were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 07-200-95).

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Stocks at 500 nM of each purified 17415 IgG1, 17552 IgG1, 17415-17552 IgG1, 4133-6294 IgG1 and Isotype IgG1 antibodies in complete media were prepared in a Greiner 96-well non-binding microplate. The reagents were serially diluted in complete media 1 in 5, seven times to produce an 8-point dose curve. 50µl of each dilution (final well concentrations 250-0.0032nM) was added to the cells and incubated for 24 hrs at 37°C, 5% CO₂. Following the incubation, the plate was spun at 300 g, 5 min, RT, the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then re-spun and buffer was aspirated to leave the cells in 20µl residual media. For CD3 staining, cells were stained with 20µl antibody solution containing anti-Human/NHP CD3 BV605 (BD Biosciences, cat no. 562994, 1:50 dilution) and LIVE/DEAD™ Fixable Near-IR Dead Cell Stain (Invitrogen, 1:5000 dilution) for 30mins, 4°C. Following the incubation, the plate was spun at 300 g, 5 min,

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RT, the buffer was aspirated with a BioTek ELx405 microplate washer, and the cells were re-suspended in FACS buffer (PBS + 1 % bovine serum albumin (BSA) + 0.1 % NaN₃ + 2 mM EDTA, Sigma Aldrich) to wash and then re-spun. Cells were washed again, buffer was aspirated to leave the cells in 20µl residual media. Cells were analysed live using the Intellicyt iQue 3. Live cell counts were extracted as metrics and graphical representations generated using Graphpad Prism version 8.1 (Graphpad). Asymmetric (4 parameter) curve fitting was applied to derive EC₅₀ and Emax values.

Results:

The percentage reduction of T cells in a cyno PBMC population treated with 17415-17552 IgG1, 17552 IgG1, 17415 IgG1, 4133-6294 IgG1 and Isotype IgG1 are shown in Figure 16. Of the antibodies for which results are shown in the Figure, only the biparatopic 17415-17552 IgG1 depletes cyno T cells to a high level (Emax 75%, EC₅₀ 1.5nM).

Example 16: Comparison of the ability of 17415-17552 and other known biparatopic antibodies to kill human CD45 expressing cells

Introduction:

The present Example investigates the ability of the 17415-17552 antibody to kill CD45 expressing target cells, compared to the 4133-6294 antibody described in International application No. PCT/EP2021/078516 (published as WO 2022/079199 A1) and an IgG1 format of the YTH24.5-YTH54.12 antibody. The variable region (V-region) sequences for anti-CD45 antibody YTH24.5-YTH54.12 were taken from International application No. PCT/GB2021/052458 (published as WO 2022/064191 A1). None of these CD45 biparatopic antibodies were conjugated to cytotoxic agents.

PBMC depletion assay:

The human PBMC depletion assay employed was the same as that used in earlier Examples, but the assays were performed using stocks at 500 nM each of 4133-6294 IgG1, 17415-17552 IgG1, YTH24.5-YTH54.12 IgG1 and 5604 IgG1. PBMCs from 4 donors, UCB-Cones 1001, 1000, 1033 and 947 were used in this assay to compare 4133-6294 IgG1, 17415-17552 IgG1 and PBMCs from 1 donor, UCB-Cone 924, was used to compare 17415-17552 IgG1 and YTH24.5-YTH54.12 IgG1.

Jurkat depletion assay:

The method performed was as described in earlier Examples. Stocks at 500 nM each of 4133-6294 IgG1, 17415-17552 IgG1, YTH24.5-YTH54.12 IgG1 and 5604 IgG1.

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Results:

The percentage reduction of T cells in a PBMC population treated with 4133-6294 IgG1 or 17415-17552 IgG1 are shown in Figure 17 alongside the isotype (5604 IgG1) control. Both 4133-6294 IgG1 and 17415-17552 IgG1 showed reduction of T cells. 4133-6294 IgG1 had an Emax range of 80.7-89.9% and EC50 range of 0.17-0.53 nM, and 17415-17552 IgG1 had an Emax range of 74.4-83.5% and EC50 range of 0.08-0.4 nM.

The percentage reduction of T cells in a PBMC population treated with 17415-17552 IgG1 or YTH24.5-YTH54.12 IgG1 are shown in Figure 18 alongside the isotype (5604 IgG1) control. Only 17415-17552 IgG1 showed reduction of T cells. 17415-17552 IgG1 had an Emax of 88.8% and EC50 of 0.05 nM. The Emax and EC50 of YTH24.5-YTH54.12 IgG1 was not determinable.

The percentage reduction of Jurkat cells treated with 4133-6294 IgG1 or 17415-17552 IgG1 are shown in Figure 19 alongside the isotype (5604 IgG1) control. Both 4133-6294 IgG1 and 17415-17552 IgG1 showed reduction of Jurkat cells. 4133-6294 IgG1 had an Emax of 91.9% and EC50 of 0.47 nM, and 17415-17552 IgG1 had an Emax of 92.7% and EC50 range of 0.06 nM.

The percentage reduction of Jurkat cells treated with 17415-17552 IgG1 or YTH24.5-YTH54.12 IgG1 are shown in Figure 21 alongside the isotype (5604 IgG1) control. Only, 17415-17552 IgG1 showed reduction of Jurkat cells. 17415-17552 IgG1 had an Emax of 90.9% and EC50 of 0.05 nM. The Emax and EC50 of YTH24.5-YTH54.12 IgG1 was not determinable.

In both PBMCs and Jurkat cells, the EC50 of the biparatopic 17415-17552 IgG1 antibody was lower compared to the EC50 of the biparatopic 4133-6294 IgG1 antibody. The biparatopic YTH24.5-YTH54.12 IgG1 was unable to induce T cell or Jurkat cell killing.

30

Conclusion:

Overall, this data shows that the claimed biparatopic CD45 antibodies have an increased potency compared to previously known biparatopic CD45 antibodies.

5 **Example 17: Cytokine release in whole blood at 24 hours measured by Meso Scale Discovery (MSD) assay**

Introduction:

10 The present Example investigates the ability of CD45 antibodies to induce cytokine release in whole blood. Cytokine release could drive unwanted inflammation in treated subjects.

Material & Methods:

15 Human whole blood (Lithium heparin tubes) was collected from two donors at UCB Pharma Slough, UK according to approved ethical sample collection protocol.

20 In a Greiner 96-well non-binding microplate, stocks of purified 17415gL15gH6-17552gL1gH4 IgG1 LALA, 17415gL15gH6 IgG1 LALA, YTH24.5-YTH54.12 IgG1 and 5604 IgG1 LALA antibodies were prepared in PBS at 8000 nM. Reagents were then serially diluted in PBS 1 in 5, seven times to produce an 8-point dose curve. Campath was diluted in PBS at to produce a 200 µg/ml stock.

25 12.5 µl of the reagent dilution was transferred into Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 3799) and 237.5 µl of whole blood added to each well. Final well concentrations of the antibodies were 400- 0.00512 nM. Campath (diluted from 10 mg/ml stock to 0.2 mg/ml in PBS, lot number CHV0387) was used as a positive control at a final concentration of 10 µg/ml. Plates were sealed with a gas permeable adhesive seal and plate lids were replaced. Plates were then incubated for 24 hours at 37°C and 5% humidified CO₂ in an undisturbed location.

30 Following the 24-hour incubation, plates were spun at 1000 g for 10 mins and 50 µl of plasma was transferred to a separate plate and stored at -80°C until cytokine quantification analysis.

Measurement of cytokines was carried out using the V-PLEX Human Proinflammatory Panel I (which includes interferon (IFN)-γ, interleukin (IL)-6, tumor necrosis factor (TNF)-α, cat no K15052D, Meso Scale Discovery) according to manufacturer's instructions. Briefly, the plasma samples were defrosted at RT and diluted

1 in 2 with Diluent 2 (Cat. no R51BB-3, Meso Scale Discovery). The standard curve calibrator was prepared using 500 µl of Diluent 2 (Cat. no R51BB-3, Meso Scale Discovery). The Proinflammatory Panel I plates were washed with PBS (supplemented with 0.05% Tween-20) using a BioTek ELx405 microplate washer, and 50 µl of sample or standard curve calibrator was added to each well. The plates were sealed with an adhesive seal and incubated for 2 hours on a plate shaker (750 rpm) at RT. The plates were washed as before, and 25 µl of detection antibody was added to each well. The plates were incubated for a further 2 hours on a plate shaker at RT. The plates were washed as before, and 150 µl of read buffer (diluted 1 in 2 in dH₂O) was added to each well. The plates were then analysed on a SECTOR Imager 6000 (Meso Scale Discovery).

Results:

The levels of inflammatory cytokines detected are shown in Figure 20 (A) IFN- γ , (B) IL-6 and (C) TNF- α . Significantly, little or no induction of inflammatory cytokines by 17415gL15gH6-17552gL1gH4 and 17415gL15gH6 IgG1 LALA was observed with the levels matching those in PBS and 5604 IgG1 LALA-treated wells.

Conclusion:

Overall, this data shows that the claimed antibodies induce cell death without inducing a significant increase in cytokine release. The absence of cytokine reduces the likelihood of unwanted cytokine driven inflammation in treated subjects.

Example 18: Depletion assay on leukaemic T-cell lines and leukaemic B-cell lines

Introduction:

The present Example investigates the ability of the claimed CD45 antibodies to deplete immune cells of different leukaemic cell lines.

Materials & Methods – Cell line depletion assay:

Prior to an assay being performed, 1 vial each of (a) leukaemic T-cell line (SUDHL1, SUPT11 and Peers), and (b) leukaemic B-cell line (DOHH2 and Ramos) was thawed in a 37°C water bath and then added to 5 ml complete media (RPMI 1640, Corning, + 20% Fetal Bovine Serum (FBS), Gibco, for Ramos, DOHH2 and SUDHL1; RPMI 1640 + 10% FBS for SUPT11 and Peers) for culturing. For the depletion assay, cells were spun

(200 g, 5 mins, at RT) and re-suspended in 5 ml complete media to wash and spun again. Cells were resuspended in 5 ml complete media and were counted using a Biorad TC20TM automated cell counter. 1×10^5 cells in 90µl complete media were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 3879).

Stocks at 2 µM of purified 17415gL15gH6-17552gL1gH4 IgG1 LALA, 17415gL15gH6 IgG1 LALA and Isotype control IgG1 LALA (5604) in complete media were prepared in a Sarstedt 96-well microplate (cat no. 83.3924.005). The antibodies were serially diluted 1 in 3.16 in complete media, nine times to produce a 10-point dose curve. 10µl of each dilution (final well concentrations 200-0.002nM) was added to the cells and incubated for 2 hours at 37°C, 5% CO₂. Following the incubation, 150µl PBS was added to each well, the plate was spun at 400 g, 5 min, RT, the supernatant was discarded by inverting the plate and blotting on tissue paper. The cells were re-suspended in 150µl PBS, the plate was re-spun and the supernatant discarded. For viability staining, cells were stained with 50µl solution containing LIVE/DEAD™ Fixable Near-IR Dead Cell Stain (Invitrogen, 1:1000 dilution) for 30 mins, 4°C. Following the incubation, 150µl FACS buffer (PBS + 1 % bovine serum albumin (BSA), Fisher BioReagents) was added to each well, the plate was spun at 400 g, 5 mins, RT, and the supernatant was discarded. The cells were washed once more with FACS buffer and then re-suspended in 150µl FACS Lysing Solution (BD Biosciences, cat no. 349202). For flow cytometric analysis, 100µl/well volume was acquired on the Attune NxT flow cytometer (Invitrogen). Live, single cell counts were extracted as metrics and graphical representations generated using Graphpad Prism version 9.0 (Graphpad). Asymmetric (4 parameter) curve fitting was applied to derive EC50 and Emax values.

25

Results:

The percentage reduction of Peers, SUPT11, and SUDHL1 T-cells treated with 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA are shown in Figures 22A, 22B and 22C respectively, alongside the isotype control (5604 IgG1 LALA). The percentage reduction of Ramos and DOHH2 B-cells treated with 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA are shown in Figures 23A, and 23B respectively, alongside the isotype control (5604 IgG1 LALA).

Of the tested cell lines, the monospecific antibody 17415gL15gH6 IgG1 LALA showed a marked reduction of certain T-cell lines (Peers and SUPT11) and a certain B-cell

line (Ramos) compared to the control. The Emax range was 28.4-66.8% and the EC50 range of 0.45-18.58nM.

The biparatopic antibody 17415gL15gH6-17552gL1gH4 IgG1 LALA showed a marked reduction of in all tested T-cell and B-cell lines (Peers, SUPT11, SUDHL1, Ramos and DOHH2) compared to the control. The Emax range was 29.3-85.7% and the EC50 range of 0.03-0.30nM.

Conclusion:

This data demonstrates the ability of both the monospecific killing arms, as well as the biparatopic antibodies to successfully deplete T-cell and B-cell lines, with biparatopic antibodies showing an increased potency and increased efficacy compared to monospecific killing arms.

Example 19: Cell depletion of PBMCs derived from healthy and leukemic patients

Introduction:

The present Example investigates the ability of CD45 antibodies to deplete immune cells in healthy and diseased subjects.

Materials & Methods - PBMC depletion assay:

Blood samples were collected from healthy volunteers (University of Leicester) and from T-/ B-cell leukaemia patients (Leicester Royal hospital) to isolate human PBMC using a Lymphoprep (StemCell) density gradient. PBMC were resuspended in 10 ml complete media (RPMI 1640, Corning, + 10% Fetal Bovine Serum (FBS) + 2 mM GlutaMAX, both supplied by Gibco, + 1% Pen/Strep, Fisher, 0.004ul/ml Beta-mercaptoethanol (BME) (Sigma) and counted using a Biorad TC20TM automated cell counter. 1×10^5 cells per well in 90µl media were then added to each well of a Corning Costar 96-well, cell culture treated, U-shaped-bottom microplate (cat no. 3879). PBMCs from 8 donors, 6 healthy volunteers (330CD, 334ES, 335AC, 336BB, 365DS, 370EE), 1 T-cell leukaemia patient (4386POS, Sézary syndrome) and 1 B-cell leukaemia patient (4650ADG, Mantle cell lymphoma), were used in this assay.

Stocks at 2 µM of purified 17415gL15gH6-17552gL1gH4 IgG1 LALA, 17415gL15gH6 IgG1 LALA and Isotype control IgG1 LALA (5604) in complete media were prepared in a Sarstedt 96-well microplate (cat no. 83.3924.005). The reagents were

serially diluted 1 in 3.16 in complete media, ten times to produce an 11-point dose curve. 10µl of each dilution (final well concentrations 200-0.002nM) was added to the cells and incubated for 22 hours at 37°C, 5% CO₂. Following the incubation, 150µl PBS was added to each well, the plate was spun at 400 g, 5 mins, RT, the supernatant was discarded by

5 inverting the plate and blotting on tissue paper. The cells were re-suspended in 150µl PBS, the plate was re-spun and supernatant was discarded. For viability staining, cells were stained with 50µl of a solution containing LIVE/DEAD™ Fixable Violet Dead Cell Stain (Invitrogen, 1:1000 dilution) for 30mins, 4°C. Following the incubation, 150µl FACS buffer (PBS + 1 % bovine serum albumin (BSA), Fisher BioReagents) was added to each

10 well, the plate was spun at 400 g, 5 mins, RT, and the supernatant was discarded. The cells were washed one more in FACS buffer, and the supernatant was discarded. For extracellular marker staining, cells were stained with 50µl of an antibody solution containing anti-human CD16 BV480 (BD Biosciences, cat no. 566171, 1:100 dilution), CD19 Alexafluor 700 (BD Biosciences, cat no. 557921, 1:200 dilution), CD3 Alexafluor

15 594 (Biolegend, cat no. 300446, 1:250 dilution), CD4 PerCP Cy5.5 (Biolegend, cat no. 300530, 1:100 dilution), CD8 APC Cy7 (Biolegend, cat no. 301016, 1:400 dilution), HLA-DR PECy7 (Thermofisher, 25-9956-42, 1:200 dilution), CD56 APC (Biolegend, 318310, 1:50 dilution) for 30 mins, 4°C. Following the incubation, 150µl FACS buffer was added to each well, the plate was spun at 400 g, 5 mins, RT, and the supernatant was discarded.

20 The cells were washed once more with FACS buffer and then re-suspended in 150µl FACS Lysing Solution (BD Biosciences, cat no. 349202). For flow cytometric analysis, 100µl/well volume was acquired on the Attune NxT flow cytometer (Invitrogen). Live, single cell counts for each leukocyte population were extracted as metrics and graphical representations generated using Graphpad Prism version 9.0 (Graphpad). Asymmetric (4

25 parameter) curve fitting was applied to derive EC50 and Emax values.

Results:

PBMC depletion assay

30 The percentage reduction of T cells in a PBMC population derived from healthy volunteers (A) and a patient with T-cell leukaemia (B) treated with 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA are shown in Figure 24 alongside the isotype control (5604 IgG1 LALA). The percentage reduction of B cells in a PBMC population derived from healthy volunteers (A) and a patient with B-cell

leukaemia (B) treated with 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA are shown in Figure 25 alongside the isotype control (5604 IgG1 LALA). Data from healthy donors 336BB and 330CD are shown as representative data from 6 healthy donors.

5 Both 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA showed a reduction of T-cells for healthy volunteers, with an Emax range of 74.2-96.7% and EC50 range of 0.05-8.94nM. Both 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA showed a similar ability to reduce T-cells in a patient with T-cell leukaemia (4368POS), with an Emax range of 74.2-97% and EC50
10 range of 0.03-8.94nM. Although, the biparatopic antibody (17415gL15gH6-17552gL1gH4 IgG1 LALA) depleted T-cells in healthy and diseased subjects more effectively than the monospecific antibody (17415gL15gH6 IgG1 LALA), the T-cell reduction achieved using the monospecific antibody (17415gL15gH6 IgG1 LALA) was still significant compared to the control.

15 Both 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA showed a reduction of B-cells for healthy volunteers with an Emax range of 36.5-80.2% and EC50 range of 0.57-3.1nM. Both 17415gL15gH6-17552gL1gH4 IgG1 LALA and 17415gL15gH6 IgG1 LALA showed a similar ability to reduce B-cells in a patient with B-cell leukaemia (4650ADG), with an Emax range of 71.2-79% and EC50 range
20 0.11-0.84nM. A similar trend was seen in B-cell depletion, in that, although the biparatopic antibody (17415gL15gH6-17552gL1gH4 IgG1 LALA) depleted B-cells in healthy and diseased subjects more effectively than the monospecific antibody (17415gL15gH6 IgG1 LALA), the B-cell reduction achieved using the monospecific (17415gL15gH6 IgG1 LALA) was still significant compared to the control.

25

Conclusion:

This data demonstrates the successful depletion of CD45 expressing target cells in both healthy and diseased subjects. It further demonstrates the therapeutic potential of the both the monospecific and biparatopic antibodies in different populations of CD45
30 expressing target cells.

NUMBERED EMBODIMENTS

The following represent additional numbered embodiments of the invention but do not presently represent claims:

5

1. An antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light and heavy chain variable regions:

- (a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 33, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 35 and 39 to 42; and
- (b) a heavy chain variable region comprising a CDR1 comprising a sequence selected from any one of SEQ ID NOs: 46, 52, 53 and 54, a CDR2 comprising a sequence selected from any one of SEQ ID NOs: 47, 55, 56, and 57, and a CDR3 comprising the sequence of SEQ ID NO: 48.

15

2. The antibody or antigen-binding fragment of 1, wherein the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of any one of: SEQ ID NOs: 33, 34, and 35; SEQ ID NOs: 33, 34, and 39; SEQ ID NOs: 33, 34, and 40; SEQ ID NOs: 33, 34, and 41; and SEQ ID NOs: 33, 34, and 42.

20

3. The antibody or antigen-binding fragment of 1 or 2, wherein the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 of any one of:

25

SEQ ID NOs: 46, 47 and 48;
SEQ ID NOs: 52, 55 and 48;
SEQ ID NOs: 53, 56 and 48; and
SEQ ID NOs: 54, 57, and 48.

30

4. The antibody or antigen-binding fragment of any one of 1 to 3, wherein:

(i) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 35 and the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:

- (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or
- (ii) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 39 and the heavy chain variable region of
- 5 (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of: (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or
- (iii) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 40 and the heavy chain variable region of (b)
- 10 comprises the combination of CDR1, CDR2, and CDR3 selected from any one of: (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or
- (iv) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 41 and the heavy chain variable region of (b)
- 15 comprises the combination of CDR1, CDR2, and CDR3 selected from any one of: (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or
- (v) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 42 and the heavy chain variable region of (b)
- 20 comprises the combination of CDR1, CDR2, and CDR3 selected from any one of: (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48.
5. The antibody or antigen-binding fragment of any one of 1 to 4, wherein the antibody or antigen-binding fragment comprises a light chain having the CDR set of
- 25 LCDR1, LCDR2, and LCDR3 comprising respectively the sequences of SEQ ID NOs: 33, 34, and 39 and for the heavy chain variable region the CDR set of HCDR1, HCDR2, and HCDR3 comprising respectively the sequences of SEQ ID NOs: 52, 55, and 48.
6. The antibody or antigen-binding fragment of any one of 1 to 5, wherein the light and heavy chain variable regions are humanised.
- 30 7. The antibody or antigen-binding fragment of 6, wherein the light chain variable region of (a) is humanised using an IGKV4-1 or IGKV1-9 framework as an acceptor sequence for the framework regions, but optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more

corresponding residues of donor framework sequence, preferably wherein the acceptor framework sequence is derived from IGKV4-1.

8. The antibody or antigen-binding fragment of any one of 1 to 7, wherein the light chain variable region of (a) comprises a sequence selected from one of
- 5 SEQ ID NOs: 11 to 14 and SEQ ID NOs: 3 to 9, preferably from one SEQ ID NOs: 11 to 14.
9. The antibody or antigen-binding fragment of any one of 6 to 8, wherein the heavy chain variable region of (b) is humanised using an IGHV3-72 framework as an acceptor sequence for the framework regions, but optionally wherein one or more amino acid
- 10 residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence.
10. The antibody or antigen-binding fragment of any one 1 to 9, wherein the heavy chain variable region of (b) comprises a sequence selected from any one of SEQ ID NOs: 17 to 22.
- 15 11. The antibody or antigen-binding fragment of any one of 1 to 10, wherein the at least one antigen-binding site comprises a light chain variable region sequence selected from any one SEQ ID NOs: 11 to 14 and SEQ ID NOs: 3 to 9 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22, preferably wherein the light chain variable region is selected from any one of SEQ ID NOs: 11 to 14.
- 20 12. The antibody or antigen-binding fragment of any one of 1 to 11, wherein the at least one antigen-binding site comprises:
- (i) a light chain variable region having the sequence of SEQ ID NO: 9 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
- (ii) a light chain variable region having the sequence of SEQ ID NO: 13 and a
- 25 heavy chain variable region having the sequence of SEQ ID NO: 22; or
- (iii) a light chain variable region having the sequence of SEQ ID NO: 14 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
- (iv) a light chain variable region sequence of SEQ ID NO: 11 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- 30 (v) a light chain variable region sequence of SEQ ID NO: 12 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- (vi) a light chain variable region sequence of SEQ ID NO: 13 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or

- (vii) a light chain variable region sequence of SEQ ID NO: 14 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (viii) a light chain variable region sequence of SEQ ID NO: 3 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22;
 - 5 (ix) a light chain variable region sequence of SEQ ID NO: 4 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (x) a light chain variable region sequence of SEQ ID NO: 5 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (xi) a light chain variable region sequence of SEQ ID NO: 6 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - 10 (xii) a light chain variable region sequence of SEQ ID NO: 7 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (xiii) a light chain variable region sequence of SEQ ID NO: 8 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - 15 (xiv) a light chain variable region sequence of SEQ ID NO: 9 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22.
13. The antibody or antigen-binding fragment of 12, wherein the at least one antigen-binding site comprises:
- (i) a light chain variable region having the sequence of SEQ ID NO: 9 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
 - 20 (ii) a light chain variable region having the sequence of SEQ ID NO: 13 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
 - (iii) a light chain variable region having the sequence of SEQ ID NO: 14 and a heavy chain variable region having the sequence of SEQ ID NO: 2.
- 25 14. An antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light and heavy chain variable regions:
- (a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96; and
 - 30 (b) a heavy chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 102 to 105.

15. The antibody or antigen-binding fragment of 14, wherein:
- (i) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102; or
- (ii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 103; or
- (iii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 104; or
- (iv) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102 105.
16. The antibody or antigen-binding fragment of 15, wherein:
- (i) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102; or
- (ii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b)

comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 105.

17. The antibody or antigen-binding fragment of 15 or 16, wherein the light and heavy chain variable regions are humanised.

5 18. The antibody or antigen-binding fragment of 17, wherein the light chain variable region of (a) are humanised using an IGKV1-8 framework as an acceptor sequence for the framework regions, optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence.

10 19. The antibody or antigen-binding fragment of any one of 15 to 18, wherein the light chain variable region of (a) comprises a sequence selected from SEQ ID NOs: 25 and 26.

20. The antibody or antigen-binding fragment of any one of 15 to 19, wherein the heavy chain variable region of (b) is humanised using an IGHV4-4 framework as an
15 acceptor sequence for the framework regions, optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence.

21. The antibody or antigen-binding fragment of any one of 15 to 20, wherein the heavy chain variable region of (b) comprises a sequence selected from any one of SEQ
20 ID NOs: 29 to 32.

22. The antibody or antigen-binding fragment of any one of 15 to 21, comprising a light chain variable region sequence selected from SEQ ID NOs: 25 and 26 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 29 to 32.

23. The antibody or antigen-binding fragment of 22 wherein the antibody comprises:
25 (i) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 29; or

(ii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 32.

(iii) a light chain variable region sequence of SEQ ID NO: 25 and a heavy
30 chain variable region sequence of SEQ ID NO: 30; or

(iv) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 31; or

(v) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 32; or

- (vi) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 29; or
- (vii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 30; or
- 5 (viii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 31.
24. The antibody or antigen-binding fragment of 23, wherein the antibody comprises:
- (i) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 29; or
- 10 (ii) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 32.
25. The antibody or antigen-binding fragment of any one of 1 to 24 wherein the antibody or antigen binding fragment thereof is monospecific for CD45.
26. The antibody or antigen-binding fragment of any one of 1 to 24, wherein the
- 15 antibody or antigen binding fragment thereof is biparatopic for CD45.
27. The antibody or antigen-binding fragment of 26, wherein the antibody or antigen-binding fragment comprises a first variable domain specific for CD45 as defined in any one of 1 to 14 and a second variable domain specific for CD45 as defined in any one of 15 to 24.
- 20 28. The antibody or antigen-binding fragment of 27, which is biparatopic for CD45 and has the CDR sets from one of the following pairs of specificities:
- (a) 17415gL7gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the
- 25 CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- (b) 17415gL7gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39
- 30 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or

- (c) 17415gL15gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of
5 SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- (d) 17415gL15gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the
10 CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or
- (e) 17415gL16gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the
15 CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- (f) 17415gL16gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the
20 CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or
- (g) 17415gL7gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the
25 CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID
30 Nos:100, 101, and 102); or
- (h) 17415gL7gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the
CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of

SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or

- (i) 17415gL15gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- (j) 17415gL15gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or
- (k) 17415gL16gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17416gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- (l) 17415gL16gH6 x 17552gL1gH4 biparatopic; (the CDRs for the 17416gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105).

29. The antibody or antigen-binding fragment of 28, which is biparatopic for CD45 wherein the antibody or antigen-binding fragment thereof has the light and heavy chain variable regions pairs for each specificity of one of the following:

- (a) 17415gL7gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);

- (b) 17415gL7gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- 5 (c) 17415gL15gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- (d) 17415gL15gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- 10 (e) 17415gL16gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- (f) 17415gL16gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- 20 (g) 17415gL7gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- 25 (h) 17415gL7gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- (i) 17415gL15gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- 30 (j) 17415gL15gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of

- SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- (k) 17415gL16gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of
- 5 SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- (l) 17415gL16gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of
- 10 SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32).
30. The antibody or antigen-binding fragment of any one of 1 to 29, wherein a constant region of the antibody or antigen-binding fragment thereof comprises a modification or modifications to reduce or eliminate binding to an Fc receptor.
31. The antibody or antigen-binding fragment of 26, wherein the constant region of
- 15 the antibody comprises the heavy chain constant region modifications 234A and 235A by EU numbering.
32. The antibody or antigen-binding fragment of 31, wherein the constant region is an IgG1 constant regions and the modifications are a LALA double mutation of the constant region.
- 20 33. The antibody or antigen-binding fragment of 31, wherein the constant region is an IgG4 constant regions and the modifications are a FALA double mutation of the constant region.
34. The antibody or antigen-binding fragment of any one of 1 to 33, wherein the antibody or antigen-binding fragment thereof is able to bind specifically both human
- 25 CD45 and cynomolgus monkey CD45.
35. A nucleic acid molecule or molecules encoding an antibody or antigen-binding fragment thereof as defined in any one of 1 to 35.
36. A vector or vectors encoding an antibody as defined in any one of 1 to 34 or comprising a nucleic acid molecule or molecules according to 35.
- 30 37. A pharmaceutical composition comprising:
- (a) an antibody according to any one of 1 to 34, a nucleic acid molecule or molecules according to 35, or a vector or vectors according to 36; and
- (b) a pharmaceutically acceptable carrier or diluent.
38. A pharmaceutical composition according to 37 for use in a method of therapy.

39. A pharmaceutical composition of 38 for use in a method of killing or depleting CD45-expressing cells in a subject.
40. A pharmaceutical composition of 38 for use in a method of treating a blood cancer, for example leukaemia, lymphoma or multiple myeloma.
- 5 41. A pharmaceutical composition of 38 for use in a method of treating an autoimmune disease, for example multiple sclerosis or scleroderma.
42. A pharmaceutical composition for use in the method of any one of 38 to 41, wherein the method further comprises transferring cells to the subject after the cell depletion.
- 10 43. A method of killing or depleting CD45-expressing cells in a subject, the method comprising administering a pharmaceutical composition according to 37 to the subject.
44. A method of 43, wherein the method is for treating a blood cancer, for example leukaemia, lymphoma or multiple myeloma.
45. A method of 43, wherein the method is for treating an autoimmune disease, for
15 example multiple sclerosis or scleroderma.
46. A method of any one of 43 to 45, wherein the method further comprises transferring cells to the subject after the cell killing or depletion.
47. Use of an antibody or antigen-binding fragment thereof according to any one of 1 to 34, a nucleic acid molecule or molecules according to claim 35 or a vector or vectors
20 according to 36 in the manufacture of a medicament for killing or depleting CD45-expressing cells in a subject.
48. The use of 47 wherein the medicament is for treating a blood cancer, for example leukaemia, lymphoma or multiple myeloma.
49. The use of 43 wherein the medicament is for treating an autoimmune disease, for
25 example multiple sclerosis or scleroderma.
50. The use of any one of 47 to 49, wherein the medicament is for use in a method that further comprises transferring cells to the subject after the cell killing or depletion.
51. An ex vivo method of depleting or killing target cells expressing CD45 in a population of cells, tissue, or organ comprising contacting said cells tissue or organ with
30 an antibody or antigen-binding fragment according to any one of 1 to 34.
52. An antibody according to any one of 1 to 34 use in a method of treating or preventing graft versus host disease (GVHD) in a subject, the method comprising

(a) contacting ex vivo a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of claims 1 to 34 to kill target cells expressing CD45; and

5 (b) transplanting the treated population of cells, tissue, or organ to said subject.

53. A method of treating or preventing graft versus host disease (GVHD) comprising:

(a) contacting a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of 1 to 34 to kill target cells expressing CD45 ex vivo; and

10 (b) transplanting the treated population of cells, tissue, or organ to a subject in need of such a transplantation.

54. Use of an antibody according to any one of 1 to 34 in the manufacture of a medicament for treating or preventing graft versus host disease (GVHD) in a method comprising:

15 (a) contacting a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of 1 to 34 to kill target cells expressing CD45 ex vivo; and

(b) transplanting the treated population of cells, tissue, or organ to a subject in need of such a transplantation.

20

FURTHER NUMBERED EMBODIMENTS

The following represent further numbered embodiments of the invention but do not presently represent claims:

25 [1] An antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light and heavy chain variable regions:

(a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 33, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR3 comprising
30 a sequence selected from any one of SEQ ID NOs: 35 and 39 to 42; and

(b) a heavy chain variable region comprising a CDR1 comprising a sequence selected from any one of SEQ ID NOs: 46, 52, 53 and 54, a CDR2 comprising a sequence selected from any one of SEQ ID NOs: 47, 55, 56, and 57, and a CDR3 comprising the sequence of SEQ ID NO: 48.

- [2] The antibody or antigen-binding fragment of [1], wherein the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of any one of:
 SEQ ID NOs: 33, 34, and 35;
 SEQ ID NOs: 33, 34, and 39;
 5 SEQ ID NOs: 33, 34, and 40;
 SEQ ID NOs: 33, 34, and 41; and
 SEQ ID NOs: 33, 34, and 42.
- [3] The antibody or antigen-binding fragment of [1] or [2], wherein the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 of any one
 10 of:
 SEQ ID NOs: 46, 47 and 48;
 SEQ ID NOs: 52, 55 and 48;
 SEQ ID NOs: 53, 56 and 48; and
 SEQ ID NOs: 54, 57, and 48.
- 15 [4] The antibody or antigen-binding fragment of any one of [1] to [3], wherein:
 (i) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 35 and the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:
 (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs:
 20 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or
 (ii) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 39 and the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:
 (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs:
 25 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or
 (iii) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 40 and the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of: (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56
 30 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or
 (iv) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 41 and the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of: (aa)

SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or

- (v) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 42 and the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of: (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48.

- [5] The antibody or antigen-binding fragment of any one of [1] to [4], wherein the antibody or antigen-binding fragment comprises a light chain having the CDR set of LCDR1, LCDR2, and LCDR3 comprising respectively the sequences of SEQ ID NOs: 33, 34, and 39 and for the heavy chain variable region the CDR set of HCDR1, HCDR2, and HCDR3 comprising respectively the sequences of SEQ ID NOs: 52, 55, and 48.

[6] The antibody or antigen-binding fragment of any one of [1] to [5], wherein the light and heavy chain variable regions are humanised.

- [7] The antibody or antigen-binding fragment of [6], wherein the light chain variable region of (a) is humanised using an IGKV4-1 or IGKV1-9 framework as an acceptor sequence for the framework regions, but optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence, preferably wherein the acceptor framework sequence is derived from IGKV4-1.

[8] The antibody or antigen-binding fragment of any one of [1] to [7], wherein the light chain variable region of (a) comprises a sequence selected from one of SEQ ID NOs: 11 to 14 and SEQ ID NOs: 3 to 9, preferably from one SEQ ID NOs: 11 to 14.

- [9] The antibody or antigen-binding fragment of any one of [6] to [8], wherein the heavy chain variable region of (b) is humanised using an IGHV3-72 framework as an acceptor sequence for the framework regions, but optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence.

- [10] The antibody or antigen-binding fragment of any one of [1] to [9], wherein the heavy chain variable region of (b) comprises a sequence selected from any one of SEQ ID NOs: 17 to 22.

[11] The antibody or antigen-binding fragment of any one of [1] to [10], wherein the at least one antigen-binding site comprises a light chain variable region sequence selected

from any one SEQ ID NOs: 11 to 14 and SEQ ID NOs: 3 to 9 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22, preferably wherein the light chain variable region is selected from any one of SEQ ID NOs: 11 to 14.

- [12] The antibody or antigen-binding fragment of any one of [1] to [11], wherein the at least one antigen-binding site comprises:
- (i) a light chain variable region having the sequence of SEQ ID NO: 9 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
 - (ii) a light chain variable region having the sequence of SEQ ID NO: 13 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
 - (iii) a light chain variable region having the sequence of SEQ ID NO: 14 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
 - (iv) a light chain variable region sequence of SEQ ID NO: 11 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (v) a light chain variable region sequence of SEQ ID NO: 12 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (vi) a light chain variable region sequence of SEQ ID NO: 13 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (vii) a light chain variable region sequence of SEQ ID NO: 14 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (viii) a light chain variable region sequence of SEQ ID NO: 3 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22;
 - (ix) a light chain variable region sequence of SEQ ID NO: 4 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (x) a light chain variable region sequence of SEQ ID NO: 5 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (xi) a light chain variable region sequence of SEQ ID NO: 6 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (xii) a light chain variable region sequence of SEQ ID NO: 7 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (xiii) a light chain variable region sequence of SEQ ID NO: 8 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
 - (xiv) a light chain variable region sequence of SEQ ID NO: 9 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22.

[13] The antibody or antigen-binding fragment of [12], wherein the at least one antigen-binding site comprises:

- (i) a light chain variable region having the sequence of SEQ ID NO: 9 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
- 5 (ii) a light chain variable region having the sequence of SEQ ID NO: 13 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
- (iii) a light chain variable region having the sequence of SEQ ID NO: 14 and a heavy chain variable region having the sequence of SEQ ID NO: 2.

[14] The antibody or antigen-binding fragment of any one of [1] to [11], wherein the
10 antibody comprises a heavy chain having the sequence of SEQ ID NO: 140 or 147 and a light chain having the sequence of SEQ ID NO: 141, preferably wherein the antibody comprises a heavy chain having the sequence of SEQ ID NO: 147.

[15] An antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light and heavy chain variable
15 regions:

- (a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96; and
- (b) a heavy chain variable region comprising a CDR1 comprising a sequence of SEQ
20 ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 102 to 105.

[16] The antibody or antigen-binding fragment of [15], wherein:

- (i) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a
25 CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102; or
- (ii) the light chain variable region of (a) comprises a CDR1 comprising a
30 sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 103;

(iii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 104;
or

(iv) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102
105.

[17] The antibody or antigen-binding fragment of [16], wherein:

(i) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102;
or

(ii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 105.

[18] The antibody or antigen-binding fragment of [16] or [17], wherein the light and heavy chain variable regions are humanised.

[19] The antibody or antigen-binding fragment of [20] wherein the light chain variable region of (a) are humanised using an IGKV1-8 framework as an acceptor sequence for the framework regions, optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence.

[20] The antibody or antigen-binding fragment of any one of [16] to [19], wherein the light chain variable region of (a) comprises a sequence selected from SEQ ID NOs: 25 and 26.

[21] The antibody or antigen-binding fragment of any one of [16] to [20], wherein the heavy chain variable region of (b) is humanised using an IGHV4-4 framework as an acceptor sequence for the framework regions, optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more
5 corresponding residues of donor framework sequence.

[22] The antibody or antigen-binding fragment of any one of [16] to [21], wherein the heavy chain variable region of (b) comprises a sequence selected from any one of SEQ ID NOs: 29 to 32.

[23] The antibody or antigen-binding fragment of any one of [16] to [22], comprising
10 a light chain variable region sequence selected from SEQ ID NOs: 25 and 26 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 29 to 32.

[24] The antibody or antigen-binding fragment of [23] wherein the antibody comprises:

(i) a light chain variable region sequence of SEQ ID NO: 25 and a heavy
15 chain variable region sequence of SEQ ID NO: 29; or

(ii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 32.

(iii) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 30; or

(iv) a light chain variable region sequence of SEQ ID NO: 25 and a heavy
20 chain variable region sequence of SEQ ID NO: 31; or

(v) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 32; or

(vi) a light chain variable region sequence of SEQ ID NO: 26 and a heavy
25 chain variable region sequence of SEQ ID NO: 29; or

(vii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 30; or

(viii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 31.

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[25] The antibody or antigen-binding fragment of [24], wherein the antibody comprises:

(i) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 29; or

(ii) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 32.

[26] The antibody or antigen-binding fragment of any one of [15] to [23], wherein the antibody comprises a heavy chain having the sequence of SEQ ID 142 and a light chain
5 having the sequence of SEQ ID NO: 143.

[27] The antibody or antigen-binding fragment of any one of [1] to [26] wherein the antibody or antigen binding fragment thereof is monospecific for CD45.

[28] The antibody or antigen-binding fragment of any one of [1] to [26], wherein the antibody or antigen binding fragment thereof is biparatopic for CD45.

10 [29] The antibody or antigen-binding fragment of [28], wherein the antibody or antigen-binding fragment comprises a first variable domain specific for CD45 as defined in any one of [1] to [14] and a second variable domain specific for CD45 as defined in any one of [15] to [25].

[30] The antibody or antigen-binding fragment of [29], which is biparatopic for CD45
15 and has the CDR sets from one of the following pairs of specificities:

(a) 17415gL7gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of
20 SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or

(b) 17415gL7gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of
25 SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or

(c) 17415gL15gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of
30 SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or

- (d) 17415gL15gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of
5 SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or
- (e) 17415gL16gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the
10 CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- (f) 17415gL16gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39
15 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or
- (g) 17415gL7gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL7gH6
20 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- (h) 17415gL7gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL7gH6
25 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID
30 Nos:100, 101, and 105); or
- (i) 17415gL15gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of

SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or

(j) 17415gL15gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or

(k) 17415gL16gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17416gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or

(l) 17415gL16gH6 x 17552gL1gH4 biparatopic; (the CDRs for the 17416gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105).

[31] The antibody or antigen-binding fragment of [30], which is biparatopic for CD45 wherein the antibody or antigen-binding fragment thereof has the light and heavy chain variable regions pairs for each specificity of one of the following:

(a) 17415gL7gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the

17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);

(b) 17415gL7gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the

17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);

(c) 17415gL15gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of

- SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- (d) 17415gL15gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of
- 5 SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- (e) 17415gL16gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the
- 10 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- (f) 17415gL16gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- 15 (g) 17415gL7gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- (h) 17415gL7gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain
- 20 variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- (i) 17415gL15gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of
- 25 SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
- (j) 17415gL15gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the
- 30 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
- (k) 17415gL16gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);

- (l) 17415gL16gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32).
- 5 [32] The antibody or antigen-binding fragment of any one of [1] to [31], wherein a constant region of the antibody or antigen-binding fragment thereof comprises a modification or modifications to reduce or eliminate binding to an Fc receptor.
- [33] The antibody or antigen-binding fragment of any one of [1] to [32], wherein the constant region of the antibody comprises the heavy chain constant region modifications
- 10 234A and 235A by EU numbering.
- [34] The antibody or antigen-binding fragment of [33], wherein the constant region is an IgG1 constant regions and the modifications are a LALA double mutation of the constant region.
- [35] The antibody or antigen-binding fragment of [33], wherein the constant region is
- 15 an IgG4 constant regions and the modifications are a FALA double mutation of the constant region.
- [36] The antibody or antigen-binding fragment of any one of [1] to [33], wherein the antibody is a biparatopic antibody comprising:
- (i) a heavy chain constant region comprising a first heavy chain constant region
- 20 sequence having the sequence of SEQ ID NO: 146 and a second heavy chain constant region sequence having the sequence of SEQ ID NO 148; and
- (ii) a light chain constant region sequence having the sequence of SEQ ID NO: 145.
- [37] The antibody of any one of [1] to [36], wherein the antibody is the VR17415gL15gH6 x VR17552gL1gH4 IgG1 LALA, wherein the heavy and light chain
- 25 sequences for the VR17415gL15gH6 portion of the biparatopic comprise those of SEQ ID NOs: 147 and 141, whilst the heavy and light chain sequences for the VR17552gL1gH4 portion of the biparatopic comprise those of SEQ ID NOs: 142 and 143.
- [38] The antibody or antigen-binding fragment of any one of [1] to [37], wherein the antibody or antigen-binding fragment thereof is able to bind specifically both human
- 30 CD45 and cynomolgus monkey CD45.
- [39] A monospecific antibody specific for CD45 comprising a heavy chain comprising the sequence of SEQ ID NO: 140 and a light chain comprising the sequence of SEQ ID NO: 141.

- [40] A biparatopic antibody comprising:
- (a) a heavy chain comprising the sequence of SEQ ID NO: 147 and a light chain comprising the sequence of SEQ ID NO: 141 giving a first specificity for CD45; and
- 5 (b) a heavy chain comprising the sequence of SEQ ID NO: 142 and a light chain comprising the sequence of SEQ ID NO: 143 giving a first specificity for CD45.
- [41] A nucleic acid molecule or molecules encoding an antibody or antigen-binding fragment thereof as defined in any one of the preceding claims.
- [42] A vector or vectors encoding an antibody as defined in any one of [1] to [40] or
- 10 comprising a nucleic acid molecule or molecules according to [41].
- [43] A pharmaceutical composition comprising:
- (a) an antibody according to any one of [1] to [40], a nucleic acid molecule or molecules according to [41], or a vector or vectors according to [42]; and
- (b) a pharmaceutically acceptable carrier or diluent.
- 15 [44] A pharmaceutical composition according to [43] for use in a method of therapy.
- [45] A pharmaceutical composition of [44] for use in a method of killing or depleting CD45-expressing cells in a subject.
- [46] A pharmaceutical composition of [43] for use in a method of treating a blood cancer, for example leukaemia, lymphoma or multiple myeloma.
- 20 [47] A pharmaceutical composition of [43] for use in a method of treating an autoimmune disease, for example multiple sclerosis or scleroderma.
- [48] A pharmaceutical composition for use in the method of any one of [44] to [47], herein the method further comprises transferring cells to the subject after the cell depletion.
- 25 [49] A method of killing or depleting CD45-expressing cells in a subject, the method comprising administering a pharmaceutical composition according to [43] to the subject.
- [50] A method of [49], wherein the method is for treating a blood cancer, for example leukaemia, lymphoma or multiple myeloma.
- [51] A method of [49], wherein the method is for treating an autoimmune disease, for
- 30 example multiple sclerosis or scleroderma.
- [52] A method of any one of [49] to [51], wherein the method further comprises transferring cells to the subject after the cell killing or depletion.
- [53] Use of an antibody or antigen-binding fragment thereof according to any one of [1] to [40], a nucleic acid molecule or molecules according to [41] or a vector or vectors

according to [42] in the manufacture of a medicament for killing or depleting CD45-expressing cells in a subject.

[54] The use of [53] wherein the medicament is for treating a blood cancer, for example leukaemia, lymphoma or multiple myeloma.

5 [55] The use of [53] wherein the medicament is for treating an autoimmune disease, for example multiple sclerosis or scleroderma.

[56] The use of any one of [53] to [55], wherein the medicament is for use in a method that further comprises transferring cells to the subject after the cell killing or depletion.

[57] An ex vivo method of depleting or killing target cells expressing CD45 in a
10 population of cells, tissue, or organ comprising contacting said cells tissue or organ with an antibody or antigen-binding fragment according to any one of [1] to [40].

[58] An antibody according to any one of [1] to [40] for use in a method of treating or preventing graft versus host disease (GVHD) in a subject, the method comprising

(a) contacting ex vivo a population of cells, tissue, or organ with an antibody
15 or antigen-binding fragment thereof according to any one of [1] to [40] to kill target cells expressing CD45; and

(b) transplanting the treated population of cells, tissue, or organ to said subject.

[59] A method of treating or preventing graft versus host disease (GVHD) comprising:

20 (a) contacting a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of [1] to [40] to kill target cells expressing CD45 ex vivo; and

(b) transplanting the treated population of cells, tissue, or organ to a subject in need of such a transplantation.

25 [60] Use of an antibody according to any one of [1] to [40] in the manufacture of a medicament for treating or preventing graft versus host disease (GVHD) in a method comprising:

(a) contacting a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of [1] to [38] to kill target cells
30 expressing CD45 ex vivo; and

(b) transplanting the treated population of cells, tissue, or organ to a subject in need of such a transplantation.

CLAIMS

1. An antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light and heavy chain variable
5 regions:
- (a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 33, a CDR2 comprising a sequence of SEQ ID NO: 34, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 35 and 39 to 42; and
- (b) a heavy chain variable region comprising a CDR1 comprising a sequence
10 selected from any one of SEQ ID NOs: 46, 52, 53 and 54, a CDR2 comprising a sequence selected from any one of SEQ ID NOs: 47, 55, 56, and 57, and a CDR3 comprising the sequence of SEQ ID NO: 48.
2. The antibody or antigen-binding fragment of claim 1, wherein:
- 15 (i) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of any one of:
- SEQ ID NOs: 33, 34, and 35;
SEQ ID NOs: 33, 34, and 39;
SEQ ID NOs: 33, 34, and 40;
20 SEQ ID NOs: 33, 34, and 41; and
SEQ ID NOs: 33, 34, and 42, and/or
- (ii) the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 of any one of:
- SEQ ID NOs: 46, 47 and 48;
25 SEQ ID NOs: 52, 55 and 48;
SEQ ID NOs: 53, 56 and 48; and
SEQ ID NOs: 54, 57, and 48.
3. The antibody or antigen-binding fragment of any one of the preceding claims,
30 wherein:
- (i) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 35 and the heavy chain variable region of (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:

(aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or

(ii) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 39 and the heavy chain variable region of
 5 (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:
 (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or

(iii) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 40 and the heavy chain variable region of
 10 (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:
 (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or

(iv) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 41 and the heavy chain variable region of
 15 (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:
 (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48; or

(v) the light chain variable region of (a) comprises the combination of CDR1, CDR2, and CDR3 of SEQ ID NOs: 33, 34, and 42 and the heavy chain variable region of
 20 (b) comprises the combination of CDR1, CDR2, and CDR3 selected from any one of:
 (aa) SEQ ID NOs: 46, 47 and 48; (bb) SEQ ID NOs: 52, 55 and 48; (cc) SEQ ID NOs: 53, 56 and 48; and (dd) SEQ ID NOs: 54, 57, and 48.

4. The antibody or antigen-binding fragment of any one of the preceding claims,
 25 wherein the antibody or antigen-binding fragment comprises a light chain having the CDR set of LCDR1, LCDR2, and LCDR3 comprising respectively the sequences of SEQ ID NOs: 33, 34, and 39 and for the heavy chain variable region the CDR set of HCDR1, HCDR2, and HCDR3 comprising respectively the sequences of SEQ ID NOs: 52, 55, and 48.

30

5. The antibody or antigen-binding fragment of any one of the preceding claims, wherein:

(i) the light and heavy chain variable regions are humanised, optionally wherein the light chain variable region of (a) is humanised using an IGKV4-1 or IGKV1-

9 framework as an acceptor sequence for the framework regions, but optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence, preferably wherein the acceptor framework sequence is derived from IGKV4-1;

5 (ii) the light chain variable region of (a) comprises a sequence selected from one of SEQ ID NOs: 11 to 14 and SEQ ID NOs: 3 to 9, preferably from one SEQ ID NOs: 11 to 14;

(iii) the heavy chain variable region of (b) is humanised using an IGHV3-72 framework as an acceptor sequence for the framework regions, but optionally wherein
10 one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence;

(iv) the heavy chain variable region of (b) comprises a sequence selected from any one of SEQ ID NOs: 17 to 22; and/or

(v) the at least one antigen-binding site comprises a light chain variable
15 region sequence selected from any one SEQ ID NOs: 11 to 14 and SEQ ID NOs: 3 to 9 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22, preferably wherein the light chain variable region is selected from any one of SEQ ID NOs: 11 to 14.

20 6. The antibody or antigen-binding fragment of any one of the preceding claims, wherein the at least one antigen-binding site comprises:

(i) a light chain variable region having the sequence of SEQ ID NO: 9 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or

(ii) a light chain variable region having the sequence of SEQ ID NO: 13 and a
25 heavy chain variable region having the sequence of SEQ ID NO: 22; or

(iii) a light chain variable region having the sequence of SEQ ID NO: 14 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or

(iv) a light chain variable region sequence of SEQ ID NO: 11 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or

30 (v) a light chain variable region sequence of SEQ ID NO: 12 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or

(vi) a light chain variable region sequence of SEQ ID NO: 13 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or

- (vii) a light chain variable region sequence of SEQ ID NO: 14 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- (viii) a light chain variable region sequence of SEQ ID NO: 3 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22;
- 5 (ix) a light chain variable region sequence of SEQ ID NO: 4 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- (x) a light chain variable region sequence of SEQ ID NO: 5 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- (xi) a light chain variable region sequence of SEQ ID NO: 6 and a heavy chain
10 variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- (xii) a light chain variable region sequence of SEQ ID NO: 7 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- (xiii) a light chain variable region sequence of SEQ ID NO: 8 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22; or
- 15 (xiv) a light chain variable region sequence of SEQ ID NO: 9 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 17 to 22
preferably wherein the at least one antigen-binding site comprises:
- (i) a light chain variable region having the sequence of SEQ ID NO: 9 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
- 20 (ii) a light chain variable region having the sequence of SEQ ID NO: 13 and a heavy chain variable region having the sequence of SEQ ID NO: 22; or
- (iii) a light chain variable region having the sequence of SEQ ID NO: 14 and a heavy chain variable region having the sequence of SEQ ID NO: 2.
- 25 7. The antibody or antigen-binding fragment of any one of claims 1 to 5, wherein the antibody comprises a heavy chain having the sequence of SEQ ID 140 or 147 and a light chain having the sequence of SEQ ID NO: 141, preferably wherein the antibody comprises a heavy chain having the sequence of SEQ ID 147.
- 30 8. An antibody or antigen-binding fragment thereof comprising at least one variable domain specific for CD45 comprising the following light and heavy chain variable regions:

(a) a light chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96; and

5 (b) a heavy chain variable region comprising a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence selected from any one of SEQ ID NOs: 102 to 105, optionally wherein:

(i) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a
10 CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102; or

(ii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a
15 CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 103;

(iii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a
20 CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 104; or

(iv) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a
25 CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102
30 105.

9. The antibody or antigen-binding fragment of claim 8, wherein:

(i) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a

CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 102; or

- 5 (ii) the light chain variable region of (a) comprises a CDR1 comprising a sequence of SEQ ID NO: 94, a CDR2 comprising a sequence of SEQ ID NO: 95, and a CDR3 comprising a sequence of SEQ ID NO: 96 and the heavy chain variable region of (b) comprises a CDR1 comprising a sequence of SEQ ID NO: 100, a CDR2 comprising a sequence of SEQ ID NO: 101, and a CDR3 comprising a sequence of SEQ ID NO: 105.

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10. The antibody or antigen-binding fragment of claim 8 or 9, wherein:

- (i) the light and heavy chain variable regions are humanised, optionally wherein the light chain variable region of (a) are humanised using an IGKV1-8 framework as an acceptor sequence for the framework regions, optionally wherein one or
15 more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence;

 (ii) the light chain variable region of (a) comprises a sequence selected from SEQ ID NOs: 25 and 26;

- (iii) the heavy chain variable region of (b) is humanised using an IGHV4-4
20 framework as an acceptor sequence for the framework regions, optionally wherein one or more amino acid residues of the acceptor framework sequence are replaced with one or more corresponding residues of donor framework sequence; and/or

 (iv) the heavy chain variable region of (b) comprises a sequence selected from any one of SEQ ID NOs: 29 to 32.

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11. The antibody or antigen-binding fragment of any one of claims 8 to 10, comprising a light chain variable region sequence selected from SEQ ID NOs: 25 and 26 and a heavy chain variable region sequence selected from any one of SEQ ID NOs: 29 to 32.

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12. The antibody or antigen-binding fragment of claim 11 wherein the antibody comprises:

- (i) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 29; or

- (ii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 32.
- (iii) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 30; or
- 5 (iv) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 31; or
- (v) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 32; or
- (vi) a light chain variable region sequence of SEQ ID NO: 26 and a heavy
- 10 chain variable region sequence of SEQ ID NO: 29; or
- (vii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 30; or
- (viii) a light chain variable region sequence of SEQ ID NO: 26 and a heavy chain variable region sequence of SEQ ID NO: 31,
- 15 preferably wherein the antibody comprises:
- (i) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 29; or
- (ii) a light chain variable region sequence of SEQ ID NO: 25 and a heavy chain variable region sequence of SEQ ID NO: 32.
- 20
13. The antibody or antigen-binding fragment of any one of claims 8 to 11, wherein the antibody comprises a heavy chain having the sequence of SEQ ID 142 and a light chain having the sequence of SEQ ID NO: 143.
- 25 14. The antibody or antigen-binding fragment of any one of the preceding claims wherein the antibody or antigen binding fragment thereof is:
- (i) monospecific for CD45; or
- (ii) the antibody or antigen binding fragment thereof is biparatopic for CD45.
- 30 15. The antibody or antigen-binding fragment of claim 14, wherein the antibody or antigen-binding fragment is biparatopic for CD45, wherein the antibody or antigen-binding fragment comprises a first variable domain specific for CD45 as defined in any one of claims 1 to 7 and a second variable domain specific for CD45 as defined in any one of claims 8 to 14.

16. The antibody or antigen-binding fragment of claim 15, which is biparatopic for CD45 and has the CDR sets from one of the following pairs of specificities:

- 5 (a) 17415gL7gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- 10 (b) 17415gL7gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or
- 15 (c) 17415gL15gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- 20 (d) 17415gL15gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or
- 25 (e) 17415gL16gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or
- 30

(f) 17415gL16gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or

(g) 17415gL7gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or

(h) 17415gL7gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL7gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or

(i) 17415gL15gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or

(j) 17415gL15gH6 x 17552gL1gH4 biparatopic (the CDRs for the 17415gL15gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105); or

(k) 17415gL16gH6 x 17552gL1gH1 biparatopic (the CDRs for the 17416gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH1 specificity being respectively LCDR1,

LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 102); or

- (l) 17415gL16gH6 x 17552gL1gH4 biparatopic; (the CDRs for the 17416gL16gH6 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 33, 34, and 39 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos: 52, 55, and 48, with the CDRs for the 17552 gL1gH4 specificity being respectively LCDR1, LCDR2, LCDR3 of SEQ ID NOs: 94, 95, and 96 and HCDR1, HCDR2 and HCDR3 of respectively SEQ ID Nos:100, 101, and 105).
- 10 17. The antibody or antigen-binding fragment of claim 16, which is biparatopic for CD45 wherein the antibody or antigen-binding fragment thereof has the light and heavy chain variable regions pairs for each specificity of one of the following:
 - (a) 17415gL7gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
 - (b) 17415gL7gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
 - (c) 17415gL15gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);
 - (d) 17415gL15gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);
 - (e) 17415gL16gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for

the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);

(f) 17415gL16gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32);

(g) 17415gL7gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);

(h) 17415gL7gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL7gH6 specificity having respectively the sequences of SEQ ID Nos 9 and 22 and the light and heavy chain variable regions for the 17552gL1gH4 specificity having respectively the sequences of SEQ ID Nos 25 and 32);

(i) 17415gL15gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);

(j) 17415gL15gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL15gH6 specificity having respectively the sequences of SEQ ID Nos 13 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32);

(k) 17415gL16gH6 x 17552gL1gH1 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 29);

(l) 17415gL16gH6 x 17552gL1gH4 biparatopic (with the light and heavy chain variable regions for the 17415gL16gH6 specificity having respectively the sequences of SEQ ID Nos 14 and 22 and the light and heavy chain variable regions for

the 17552gL1gH1 specificity having respectively the sequences of SEQ ID Nos 25 and 32).

18. The antibody or antigen-binding fragment of any one of the preceding claims,
5 wherein a constant region of the antibody or antigen-binding fragment thereof comprises a modification or modifications to reduce or eliminate binding to an Fc receptor.

19. The antibody or antigen-binding fragment of any one of the preceding claims,
wherein the constant region of the antibody comprises the heavy chain constant region
10 modifications 234A and 235A by EU numbering,
optionally wherein
(i) the constant region is an IgG1 constant regions and the modifications are a
LALA double mutation of the constant region; or
(ii) the constant region is an IgG4 constant regions and the modifications are a
15 FALA double mutation of the constant region.

20. The antibody or antigen-binding fragment of any one of claims 1 to 19, wherein
the antibody is a biparatopic antibody comprising:
(i) a heavy chain constant region comprising a first heavy chain constant region
20 sequence having the sequence of SEQ ID NO: 146 and a second heavy chain constant
region sequence having the sequence of SEQ ID NO 148; and
(ii) a light chain constant region sequence having the sequence of SEQ ID NO:
145.

21. The antibody of any one of the preceding claims, wherein:
(i) the antibody is the VR17415gL15gH6 x VR17552gL1gH4 IgG1 LALA,
wherein the heavy and light chain sequences for the VR17415gL15gH6 portion of the
biparatopic comprise those of SEQ ID NOs: 147 and 141, whilst the heavy and light
chain sequences for the VR17552gL1gH4 portion of the biparatopic comprise those of
30 SEQ ID NOs: 142 and 143; and/or
(ii) the antibody or antigen-binding fragment thereof is able to bind
specifically both human CD45 and cynomolgus monkey CD45.

22. A monospecific antibody specific for CD45 comprising a heavy chain comprising the sequence of SEQ ID NO: 140 and a light chain comprising the sequence of SEQ ID NO: 141.
- 5 23. A biparatopic antibody comprising:
- (a) a heavy chain comprising the sequence of SEQ ID NO: 147 and a light chain comprising the sequence of SEQ ID NO: 141 giving a first specificity for CD45; and
- (b) a heavy chain comprising the sequence of SEQ ID NO: 142 and a light chain comprising the sequence of SEQ ID NO: 143 giving a first specificity for CD45.
- 10 24. A nucleic acid molecule or molecules encoding an antibody or antigen-binding fragment thereof as defined in any one of the preceding claims.
- 15 25. A vector or vectors encoding an antibody as defined in any one of claims 1 to 23 or comprising a nucleic acid molecule or molecules according to claim 24.
26. A pharmaceutical composition comprising:
- (a) an antibody according to any one of claims 1 to 23, a nucleic acid molecule or molecules according to claim 24, or a vector or vectors according to claim 25; and
- 20 (b) a pharmaceutically acceptable carrier or diluent.
27. A pharmaceutical composition according to claim 26 for use in a method of
- 25 therapy.
28. A pharmaceutical composition of claim 27 for use in:
- (i) a method of killing or depleting CD45-expressing cells in a subject;
- (ii) a method of treating a blood cancer, for example leukaemia, lymphoma or multiple myeloma, treating an autoimmune disease, for example multiple sclerosis or scleroderma; and/or
- 30 (iii) wherein the method further comprises transferring cells to the subject after the cell depletion.

29. A method of killing or depleting CD45-expressing cells in a subject, the method comprising administering a pharmaceutical composition according to claim 26 to the subject.

5 30. A method of claim 29, wherein the method is:

(i) for treating a blood cancer, for example leukaemia, lymphoma, multiple myeloma, an autoimmune disease, for example multiple sclerosis or scleroderma;

(ii) further comprises transferring cells to the subject after the cell killing or depletion.

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31. Use of an antibody or antigen-binding fragment thereof according to any one of claims 1 to 23, a nucleic acid molecule or molecules according to claim 24 or a vector or vectors according to claim 25 in the manufacture of a medicament for killing or depleting CD45-expressing cells in a subject.

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32. The use of claim 31 wherein the medicament is:

(i) for treating a blood cancer, for example leukaemia, lymphoma, multiple myeloma, an autoimmune disease, for example multiple sclerosis or scleroderma; and/or

(ii) for use in a method that further comprises transferring cells to the subject
20 after the cell killing or depletion.

33. An *ex vivo* method of depleting or killing target cells expressing CD45 in a population of cells, tissue, or organ comprising contacting said cells tissue or organ with an antibody or antigen-binding fragment according to any one of claims 1 to 23.

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34. An antibody according to any one of claims 1 to 23 for use in a method of treating or preventing graft versus host disease (GVHD) in a subject, the method comprising

(a) contacting *ex vivo* a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of claims 1 to 23 to kill target
30 cells expressing CD45; and

(b) transplanting the treated population of cells, tissue, or organ to said subject.

35. A method of treating or preventing graft versus host disease (GVHD) comprising:

- (a) contacting a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of claims 1 to 23 to kill target cells expressing CD45 *ex vivo*; and
- (b) transplanting the treated population of cells, tissue, or organ to a subject in
5 need of such a transplantation.

36. Use of an antibody according to any one of claims 1 to 23 in the manufacture of a medicament for treating or preventing graft versus host disease (GVHD) in a method comprising:

- 10 (a) contacting a population of cells, tissue, or organ with an antibody or antigen-binding fragment thereof according to any one of claims 1 to 38 to kill target cells expressing CD45 *ex vivo*; and
- (b) transplanting the treated population of cells, tissue, or organ to a subject in need of such a transplantation.