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(54) **Title:** CD30 ANTIGEN-BINDING MOLECULES

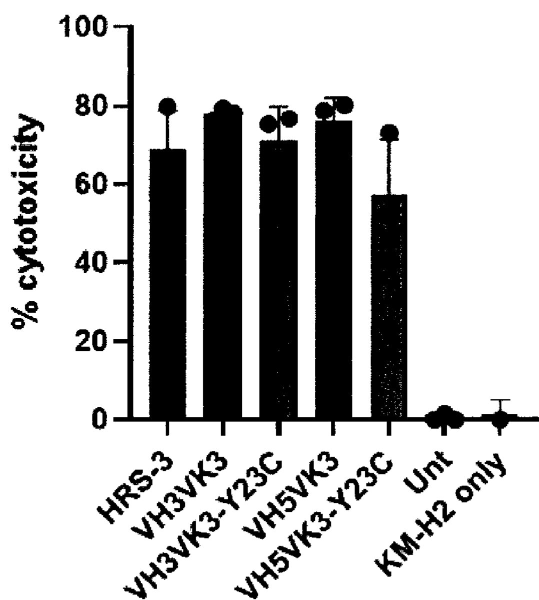

Figure 4B

(57) **Abstract:** The present disclosure provides antigen-binding molecules that bind to CD30, having novel biophysical and/or functional properties as compared to antigen-binding molecules disclosed in the prior art. Also provided is a chimeric antigen receptor (CAR) comprising the novel CD30-specific antigen-binding molecules. Also provided is a nucleic acid or plurality of nucleic acids encoding said antigen-binding molecule or CAR, an expression vector or a plurality of expression vectors comprising said nucleic acids, methods of making such molecules, and the use of such molecules in a method of medical treatment or prophylaxis.

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CD30 Antigen-Binding Molecules

Technical Field

The present disclosure relates to the fields of molecular biology, more specifically antibody technology. The present disclosure also relates to methods of medical treatment and prophylaxis.

Background

CD30 is an attractive immunotherapeutic target for several haematologic malignancies due to its restricted expression on activated lymphocytes but high expression on malignant cells such as the Hodgkin/Reed-Sternberg cells in classical Hodgkin lymphoma (HL) (1,2). Antibodies against CD30 have previously been generated but yielded disappointing therapeutic results (3). The development of a novel antibody-drug conjugate, brentuximab vedotin (BV), consisting of an anti-CD30 monoclonal antibody and the anti-tubulin drug monomethyl auristatin E yielded improved clinical responses in relapsed and refractory HL (4). However, durable responses were limited and furthermore, a major concern of BV treatment is the associated adverse effects such as neuropathy that has been observed in a majority of patients treated (5, 6).

The development of chimeric antigen receptor (CAR) T cell therapy has significantly changed the treatment of patients suffering from certain B cell leukemia and lymphoma (7), providing effective and durable clinical responses by utilizing engineered T cells to eliminate target cells expressing a non-major histocompatibility complex (MHC) restricted antigen. CARs are synthetic receptors that comprise of 4 main domains: the antigen specific domain, commonly a single chain variable fragment of an antibody (scFv), the spacer domain that connects the scFv to the third or transmembrane domain of the receptor, and finally the signalling domains (8). Design and engineering of the CAR receptor is critical for the efficacy and safety of the CAR T cell strategy. Individual domains have to be optimized for different antigens and carefully evaluated for the ideal properties (9).

The murine anti-CD30 antibody, HRS3 has been employed in existing CD30-specific CAR constructs. Murine antibody sequences are potentially immunogenic and may induce immune responses against CAR T cells bearing murine scFvs on administration to human subjects. Cellular immune responses and antibodies generated may not only neutralise but actively deplete the infused CAR T cells, leading to poor efficacy and persistence.

Summary

The present disclosure provides an antigen-binding molecule, optionally isolated, which binds to CD30.

In some aspects and embodiments, the antigen-binding molecule comprises a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:45, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:49;

wherein the VH region does not consist of the amino acid sequence of SEQ ID NO:1, and wherein the VH region does not consist of the amino acid sequence of SEQ ID NO:9.

In some embodiments, the antigen-binding molecule comprises:

5 a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:52, 17, 21, 24, 26 or 28; and

a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:54, 30, 35, 38, 41, 43, 182, 183, 184, 185 or 186.

10 In some embodiments, the antigen-binding molecule comprises:

(i) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:52, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:54; or

(ii) a VH region having an amino acid sequence having at least 70% amino acid sequence 15 identity to SEQ ID NO:17, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:30; or

(iii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:21, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:35; or

20 (iv) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:24, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:38; or

(v) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:26, and a VL region having an amino acid sequence having at least 70% amino 25 acid sequence identity to SEQ ID NO:41; or

(vi) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:28, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:43; or

(vii) a VH region having an amino acid sequence having at least 70% amino acid sequence 30 identity to SEQ ID NO:17, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:182; or

(viii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:21, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:183; or

35 (ix) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:24, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:184; or

(x) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:26, and a VL region having an amino acid sequence having at least 70% amino 40 acid sequence identity to SEQ ID NO:185; or

(xi) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:28, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:186.

In some embodiments, the VL region comprises a cysteine residue at the position corresponding to position 23 of SEQ ID NO:9.

The present disclosure also provides an antigen-binding molecule, optionally isolated, which binds to CD30, wherein the antigen-binding molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:2

HC-CDR2 having the amino acid sequence of SEQ ID NO:3

HC-CDR3 having the amino acid sequence of SEQ ID NO:4; and

(ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:10

LC-CDR2 having the amino acid sequence of SEQ ID NO:11

LC-CDR3 having the amino acid sequence of SEQ ID NO:12; and

wherein the VL region comprises a cysteine residue at the position corresponding to position 23 of SEQ ID NO:9.

In some embodiments, the antigen-binding molecule comprises:

a VH region having an amino acid sequence having at least 70% amino acid sequence identity to any one of SEQ ID NOs:1, 52, 17, 21, 24, 26 or 28; and

a VL region having an amino acid sequence having at least 70% amino acid sequence identity to any one of SEQ ID NOs:181, 54, 182, 183, 184, 185 or 186.

In some embodiments, the antigen-binding molecule comprises:

(i) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:1, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:181; or

(ii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:52, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:54; or

(iii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:17, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:182; or

(iv) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:21, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:183; or

(v) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:24, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:184; or

(vi) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:26, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:185; or

(vii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:28, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:186.

In some embodiments, the antigen-binding molecule is or comprises a single chain variable fragment (scFv) comprising the VH region and the VL region.

In some embodiments, the antigen-binding molecule is a multispecific antigen-binding molecule, and wherein the antigen-binding molecule further comprises an antigen-binding domain that binds to an antigen other than CD30.

The present disclosure also provides a chimeric antigen receptor (CAR) comprising an antigen-binding molecule according to the present disclosure. In some embodiments, the CAR comprises, or consists of, an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250 or 251.

The present disclosure also provides a nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an antigen-binding molecule or CAR according to the present disclosure.

The present disclosure also provides an expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to the present disclosure.

The present disclosure also provides a cell comprising an antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, or expression vector or plurality of expression vectors according to the present disclosure. In some embodiments, the cell is an immune cell. In some embodiments, the immune cell is a T cell. In some embodiments, the cell is a virus-specific T cell. In some embodiments, the cell is Epstein Barr Virus (EBV)-specific T cell.

The present disclosure also provides a method comprising culturing a cell according to the present disclosure under conditions suitable for expression of an antigen-binding molecule or CAR by the cell.

The present disclosure also provides a composition comprising an antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors or cell according to the present disclosure, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

The present disclosure also provides an antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition according to the present disclosure, for use in a method of medical treatment or prophylaxis.

The present disclosure also provides an antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition according to the present disclosure, for use in the treatment or prevention of a cancer.

In some embodiments, the cancer is selected from the group consisting of: a CD30-positive cancer, an EBV-associated cancer, a hematological cancer, a myeloid hematologic malignancy, a hematopoietic malignancy a lymphoblastic hematologic malignancy, myelodysplastic syndrome, leukemia, T cell leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, lymphoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, B cell non-Hodgkin's lymphoma, diffuse large B cell lymphoma, primary mediastinal B cell lymphoma, EBV-associated lymphoma, EBV-positive B cell lymphoma, EBV-positive diffuse large B cell lymphoma, EBV-positive lymphoma associated with X-linked lymphoproliferative disorder, EBV-positive lymphoma associated with HIV infection/AIDS, oral hairy leukoplakia, Burkitt's lymphoma, post-transplant lymphoproliferative disease, central nervous system lymphoma, anaplastic large cell lymphoma, T cell lymphoma, ALK-positive anaplastic T cell lymphoma, ALK-negative anaplastic T cell lymphoma, peripheral T cell lymphoma, cutaneous T cell lymphoma, NK-T cell lymphoma, extra-nodal NK-T cell lymphoma, thymoma, multiple myeloma, a solid cancer, epithelial cell cancer, gastric cancer, gastric carcinoma, gastric adenocarcinoma, gastrointestinal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, head and neck cancer, head and neck squamous cell carcinoma, oral cavity cancer, oropharyngeal cancer, oropharyngeal carcinoma, oral cancer, laryngeal cancer, nasopharyngeal carcinoma, oesophageal cancer, colorectal cancer, colorectal carcinoma, colon cancer, colon carcinoma, cervical carcinoma, prostate cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, lung adenocarcinoma, squamous lung cell carcinoma, bladder cancer, urothelial carcinoma, skin cancer, melanoma, advanced melanoma, renal cell cancer, renal cell carcinoma, ovarian cancer, ovarian carcinoma, mesothelioma, breast cancer, brain cancer, glioblastoma, prostate cancer, pancreatic cancer, mastocytosis, advanced systemic mastocytosis, germ cell tumor or testicular embryonal carcinoma.

The present disclosure also provides an antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition according to the present disclosure, for use in the treatment or prevention of a disease or condition characterised by an alloreactive immune response.

In some embodiments, the disease or condition characterised by an alloreactive immune response is selected from the group consisting of: a disease or condition associated with allotransplantation, graft versus host disease (GVHD) or graft rejection.

The present disclosure also provides the use of an antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition according to the present disclosure to deplete or increase killing of cells expressing CD30.

The present disclosure also provides an *in vitro* complex, optionally isolated, comprising an antigen-binding molecule or CAR according to the present disclosure bound to CD30.

The present disclosure also provides a method for detecting CD30 in a sample, comprising contacting a sample containing, or suspected to contain, CD30 with an antigen-binding molecule according to the present disclosure, and detecting the formation of a complex of the antigen-binding molecule with CD30.

The present disclosure also provides a method of selecting or stratifying a subject for treatment with a CD30-targeted agent, the method comprising contacting, *in vitro*, a sample from the subject with an antigen-binding molecule according to the present disclosure and detecting the formation of a complex of the antigen-binding molecule with CD30.

The present disclosure also provides the use of an antigen-binding molecule according to the present disclosure as an *in vitro* or *in vivo* diagnostic or prognostic agent.

Description

The present disclosure provides antigen-binding molecules that bind to CD30, having novel biophysical and/or functional properties as compared to antigen-binding molecules disclosed in the prior art.

In particular, the present disclosure provides CD30-specific antigen-binding molecules that are humanised versions of anti-CD30 antibody HRS3. The humanised anti-CD30 antibodies described herein are predicted to be less immunogenic in humans than HRS3, and will therefore have greater persistence and a reduced rate of clearance *in vivo* following administration to human subjects than HRS3. Unexpectedly, the humanised anti-CD30 antibodies described herein retain high-affinity binding to CD30 which is similar to the level of affinity of binding to CD30 displayed by HRS3, and also retain exquisite specificity for CD30. Furthermore, the humanised anti-CD30 antibodies described herein are surprisingly found to have greater stability than HRS3; in particular, the humanised anti-CD30 antibodies are found to be more thermostable than HRS3, having a higher melting temperature (T_m).

The present disclosure also provides CD30-specific antigen-binding molecules derived from HRS3 in which a cysteine residue is introduced into the VL at position 23 (numbered relative to SEQ ID NO:9). CD30-specific antigen-binding molecules comprising cysteine residue at position 23 are found to have greater stability, compared to their equivalent unmodified counterparts instead comprising a tyrosine residue at position 23.

The present disclosure also provides novel chimeric antigen receptor (CAR) constructs having a CD30-binding domain comprising the VH and VL domains of the novel CD30-specific antigen-binding molecules of the present disclosure. Unexpectedly, the T cells expressing the novel CAR constructs are shown to expand/proliferate similarly, to have a similar safety profile, and to display similar cytotoxicity to CD30-expressing cancer cells, as compared to equivalent CAR constructs instead having a CD30-binding domain comprising the VH and VL domains of HRS3.

CD30

CD30 (also known as TNFRSF8) is the protein identified by UniProt: P28908. CD30 is a single pass, type I transmembrane glycoprotein of the tumor necrosis factor receptor superfamily. CD30 structure and function is described *e.g.* in van der Weyden *et al.*, Blood Cancer Journal (2017) 7: e603 and Muta and Podack Immunol. Res. (2013) 57(1-3):151-8, both of which are hereby incorporated by reference in their entirety.

Alternative splicing of mRNA encoded by the human *TNFRSF8* gene yields three isoforms: isoform 1 (‘long’ isoform; UniProt: P28908-1, v1; SEQ ID NO:87), isoform 2 (‘cytoplasmic’, ‘short’ or ‘C30V’ isoform, UniProt: P28908-2; SEQ ID NO:88) in which the amino acid sequence corresponding to positions 1 to 463 of SEQ ID NO:87 are missing, and isoform 3 (UniProt: P28908-3; SEQ ID NO:89) in which the amino acid sequence corresponding to positions 1 to 111 and position 446 of SEQ ID NO:87 are missing. The N-terminal 18 amino acids of SEQ ID NO:87 form a signal peptide (SEQ ID NO:90), which is followed by a 367 amino acid extracellular domain (positions 19 to 385 of SEQ ID NO:87, shown in SEQ ID NO:91), a 21 amino acid transmembrane domain (positions 386 to 406 of SEQ ID NO:87, shown in SEQ ID NO:92), and a 189 amino acid cytoplasmic domain (positions 407 to 595 of SEQ ID NO:87, shown in SEQ ID NO:93).

In this specification ‘CD30’ refers to CD30 from any species and includes CD30 isoforms, fragments, variants or homologues from any species. As used herein, a ‘fragment’, ‘variant’ or ‘homologue’ of a reference protein may optionally be characterised as having at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of the reference protein (*e.g.* a reference isoform). In some embodiments fragments, variants, isoforms and homologues of a reference protein may be characterised by ability to perform a function performed by the reference protein.

In some embodiments, the CD30 is from a mammal (*e.g.* a primate (rhesus, cynomolgous, or human) and/or a rodent (*e.g.* rat or murine) CD30). In preferred embodiments the CD30 is a human CD30.

Isoforms, fragments, variants or homologues may optionally be characterised as having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of an immature or mature CD30 isoform from a given species, *e.g.* human. A fragment of CD30 may have a minimum length of one of 10, 20, 30, 40, 50, 100, 200, 300, 400, 500 or 590 amino acids, and may have a maximum length of one of 10, 20, 30, 40, 50, 100, 200, 300, 400, 500 or 595 amino acids.

In some embodiments, the CD30 comprises, or consists of, an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:87, 88, 89 or 91.

In some embodiments, the CD30 comprises an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:92 or 95. In some embodiments, a fragment of CD30 comprises, or consists of, an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:92 or 95.

Antigen-binding molecules

The present disclosure provides antigen-binding molecules capable of binding to CD30. An antigen-binding molecule that is capable of binding to CD30 may also be described as an antigen-binding molecule that binds to CD30.

An 'antigen-binding molecule' refers to a molecule that binds to a given target antigen. Antigen-binding molecules include antibodies (*i.e.* immunoglobulins (Igs)) and antigen-binding fragments thereof. As used herein, 'antibodies' include monoclonal antibodies, polyclonal antibodies, monospecific and multispecific (*e.g.*, bispecific, trispecific, *etc.*) antibodies, and antibody-derived antigen-binding molecules such as scFv, scFab, diabodies, triabodies, scFv-Fc, minibodies, single domain antibodies (*e.g.* VhH), *etc.*). Antigen-binding fragments of antibodies include *e.g.* Fv, Fab, $F(ab')_2$ and F(ab') fragments. In some embodiments, an antigen-binding molecule may be an antibody or an antigen-binding fragment thereof.

Antigen-binding molecules according to the present disclosure also include antibody-derived molecules, *e.g.* molecules comprising an antigen-binding region/domain derived from an antibody. Antibody-derived antigen-binding molecules may comprise an antigen-binding region/domain that comprises, or consists of, the antigen-binding region of an antibody (*e.g.* an antigen-binding fragment of an antibody). In some embodiments, the antigen-binding region/domain of an antibody-derived antigen-binding molecule may be or comprise the Fv (*e.g.* provided as an scFv) or the Fab region of an antibody, or the whole antibody. For example, antigen-binding molecules according to the present disclosure include antibody-drug conjugates (ADCs) comprising a (cytotoxic) drug moiety (*e.g.* as described hereinbelow). Antigen-binding molecules according to the present disclosure also include

multispecific antigen-binding molecules such as immune cell engager molecules comprising a domain for recruiting (effector) immune cells (reviewed *e.g.* in Goebeler and Bargou, Nat. Rev. Clin. Oncol. (2020) 17: 418–434 and Ellerman, Methods (2019) 154:102-117, both of which are hereby incorporated by reference in their entirety), including BiTEs, BiKEs and TriKEs. Antigen-binding molecules according to the present disclosure also include chimeric antigen receptors (CARs), which are recombinant receptors providing both antigen-binding and T cell activating functions (CAR structure, function and engineering is reviewed *e.g.* in Dotti *et al.*, Immunol Rev (2014) 257(1) and Jayaraman *et al.*, EBioMedicine (2020) 58:102931, both of which are hereby incorporated by reference in their entirety).

The antigen-binding molecule of the present disclosure comprises a moiety or moieties capable of binding to a target antigen(s). In some embodiments, the moiety capable of binding to a target antigen comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL) of an antibody capable of specific binding to the target antigen. In some embodiments, the moiety capable of binding to a target antigen comprises or consists of an aptamer capable of binding to the target antigen, *e.g.* a nucleic acid aptamer (reviewed, for example, in Zhou and Rossi Nat Rev Drug Discov. 2017 16(3):181-202). In some embodiments, the moiety capable of binding to a target antigen comprises or consists of an antigen-binding peptide/polypeptide, *e.g.* a peptide aptamer, thioredoxin, monobody, anticalin, Kunitz domain, avimer, knottin, fynomer, atrimer, DARPin, affibody, nanobody (*i.e.* a single-domain antibody (sdAb)), affilin, armadillo repeat protein (ArmRP), OBody or fibronectin – reviewed *e.g.* in Reverdatto *et al.*, Curr Top Med Chem. 2015; 15(12): 1082–1101, which is hereby incorporated by reference in its entirety (see also *e.g.* Boersma *et al.*, J Biol Chem (2011) 286:41273-85 and Emanuel *et al.*, Mabs (2011) 3:38-48).

As used herein, a ‘peptide’ refers to a chain of two or more amino acid monomers linked by peptide bonds. A peptide typically has a length in the region of about 2 to 50 amino acids. A ‘polypeptide’ is a polymer chain of two or more peptides. Polypeptides typically have a length greater than about 50 amino acids.

The antigen-binding molecules of the present disclosure generally comprise an antigen-binding domain comprising a VH and a VL of an antibody capable of specific binding to the target antigen. The antigen-binding domain formed by a VH and a VL may also be referred to herein as an Fv region.

An antigen-binding molecule may be, or may comprise, an antigen-binding polypeptide, or an antigen-binding polypeptide complex. An antigen-binding molecule may comprise more than one polypeptide which together form an antigen-binding domain. The polypeptides may associate covalently or non-covalently. In some embodiments, the polypeptides form part of a larger polypeptide comprising the polypeptides (*e.g.* in the case of scFv comprising VH and VL, or in the case of scFab comprising VH-CH1 and VL-CL).

An antigen-binding molecule may refer to a non-covalent or covalent complex of more than one polypeptide (*e.g.* 2, 3, 4, 6, or 8 polypeptides), *e.g.* an IgG-like antigen-binding molecule comprising two heavy chain polypeptides and two light chain polypeptides.

5 The antigen-binding molecules of the present disclosure may be designed and prepared using the sequences of monoclonal antibodies (mAbs) capable of binding to CD30. Antigen-binding regions of antibodies, such as single chain variable fragment (scFv), Fab and F(ab')$_2$ fragments may also be used/provided. An 'antigen-binding region' is any fragment of an antibody that binds to the target for which the given antibody is specific.

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Antibodies generally comprise six complementarity-determining regions CDRs; three in the heavy chain variable (VH) region: HC-CDR1, HC-CDR2 and HC-CDR3, and three in the light chain variable (VL) region: LC-CDR1, LC-CDR2, and LC-CDR3. The six CDRs together define the paratope of the antibody, which is the part of the antibody that binds to the target antigen.

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The VH region and VL region comprise framework regions (FRs) either side of each CDR, which provide a scaffold for the CDRs. From N-terminus to C-terminus, VH regions comprise the following structure: N term-[HC-FR1]-[HC-CDR1]-[HC-FR2]-[HC-CDR2]-[HC-FR3]-[HC-CDR3]-[HC-FR4]-C term; and VL regions comprise the following structure: N term-[LC-FR1]-[LC-CDR1]-[LC-FR2]-[LC-
20 CDR2]-[LC-FR3]-[LC-CDR3]-[LC-FR4]-C term.

There are several different conventions for defining antibody CDRs and FRs, such as those described in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991), Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987),
25 and VBASE2, as described in Retter *et al.*, Nucl. Acids Res. (2005) 33 (suppl 1): D671-D674. The CDRs and FRs of the VH regions and VL regions of the antibody clones described herein were defined according to the international IMGT (ImMunoGeneTics) information system (LeFranc *et al.*, Nucleic Acids Res. (2015) 43 (Database issue):D413-22), which uses the IMGT V-DOMAIN numbering rules as described in Lefranc *et al.*, Dev. Comp. Immunol. (2003) 27:55-77. In preferred
30 embodiments, the CDRs and FRs of antigen-binding molecules referred to herein are defined according to the IMGT information system.

In some embodiments, the antigen-binding molecule comprises the CDRs of an antigen-binding molecule that binds to CD30. In some embodiments, the antigen-binding molecule comprises the FRs
35 of an antigen-binding molecule that binds to CD30. In some embodiments, the antigen-binding molecule comprises the CDRs and the FRs of an antigen-binding molecule that binds to CD30. That is, in some embodiments, the antigen-binding molecule comprises the VH region and the VL region of an antigen-binding molecule that binds to CD30.

40 In some embodiments, the antigen-binding molecule comprises the CDRs, FRs and/or the VH and/or VL regions of a CD30-binding antibody described herein, or CDRs, FRs and/or VH and/or VL regions

which are derived from those of a CD30-binding antibody described herein. In some embodiments, a CD30-binding antibody is selected from: VH1VK1, VH1VK2, VH1VK3, VH1VK4, VH1VK5, VH2VK1, VH2VK2, VH2VK3, VH2VK4, VH2VK5, VH3VK1, VH3VK2, VH3VK3, VH3VK4, VH3VK5, VH4VK1, VH4VK2, VH4VK3, VH4VK4, VH4VK5, VH5VK1, VH5VK2, VH5VK3, VH5VK4, VH5VK5, VH1VK1Cys, VH1VK2Cys, VH1VK3Cys, VH1VK4Cys, VH1VK5Cys, VH2VK1Cys, VH2VK2Cys, VH2VK3Cys, VH2VK4Cys, VH2VK5Cys, VH3VK1Cys, VH3VK2Cys, VH3VK3Cys, VH3VK4Cys, VH3VK5Cys, VH4VK1Cys, VH4VK2Cys, VH4VK3Cys, VH4VK4Cys, VH4VK5Cys, VH5VK1Cys, VH5VK2Cys, VH5VK3Cys, VH5VK4Cys, VH5VK5Cys, VH1-5ConVK1-3Con or VH3-5ConVK2-3Con.

In some embodiments, the antigen-binding molecule comprises a VH region according to (1):

(1) a VH region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:2

HC-CDR2 having the amino acid sequence of SEQ ID NO:3

HC-CDR3 having the amino acid sequence of SEQ ID NO:4,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-CDR1, and/or in which 1 or 2 or 3 amino acids in HC-CDR2, and/or in which 1 or 2 or 3 amino acids in HC-CDR3 are substituted with another amino acid.

In some embodiments, the antigen-binding molecule comprises a VH region according to one of (2) to (9) below:

(2) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:46

HC-FR2 having the amino acid sequence of SEQ ID NO:47

HC-FR3 having the amino acid sequence of SEQ ID NO:48

HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

(3) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:22

HC-FR2 having the amino acid sequence of SEQ ID NO:47

HC-FR3 having the amino acid sequence of SEQ ID NO:48

HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

(4) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:5

HC-FR2 having the amino acid sequence of SEQ ID NO:6

HC-FR3 having the amino acid sequence of SEQ ID NO:7

HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

(5) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:18

HC-FR2 having the amino acid sequence of SEQ ID NO:19

HC-FR3 having the amino acid sequence of SEQ ID NO:20

HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

(6) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:22

HC-FR2 having the amino acid sequence of SEQ ID NO:19

HC-FR3 having the amino acid sequence of SEQ ID NO:23

HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

(7) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:22

HC-FR2 having the amino acid sequence of SEQ ID NO:19

HC-FR3 having the amino acid sequence of SEQ ID NO:25

HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

(8) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:22

HC-FR2 having the amino acid sequence of SEQ ID NO:19

HC-FR3 having the amino acid sequence of SEQ ID NO:27

HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

(9) a VH region incorporating the following FRs:

HC-FR1 having the amino acid sequence of SEQ ID NO:22
HC-FR2 having the amino acid sequence of SEQ ID NO:29
HC-FR3 having the amino acid sequence of SEQ ID NO:27
HC-FR4 having the amino acid sequence of SEQ ID NO:8,

or a variant thereof in which 1 or 2 or 3 amino acids in HC-FR1, and/or in which 1 or 2 or 3 amino acids in HC-FR2, and/or in which 1 or 2 or 3 amino acids in HC-FR3, and/or in which 1 or 2 or 3 amino acids in HC-FR4 are substituted with another amino acid.

In some embodiments, the antigen-binding molecule comprises a VH region comprising the CDRs according to (1) above, and the FRs according to any one of (2) to (9) above.

In some embodiments, the antigen-binding molecule comprises a VH region according to one of (10) to (17) below:

(10) a VH region comprising the CDRs according to (1) and the FRs according to (2).

(11) a VH region comprising the CDRs according to (1) and the FRs according to (3).

(12) a VH region comprising the CDRs according to (1) and the FRs according to (4).

(13) a VH region comprising the CDRs according to (1) and the FRs according to (5).

(14) a VH region comprising the CDRs according to (1) and the FRs according to (6).

(15) a VH region comprising the CDRs according to (1) and the FRs according to (7).

(16) a VH region comprising the CDRs according to (1) and the FRs according to (8).

(17) a VH region comprising the CDRs according to (1) and the FRs according to (9).

In some embodiments, the antigen-binding molecule comprises a VH region according to one of (18) to (25) below:

(18) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:45.

(19) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:52.

(20) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:1.

5 (21) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:17.

(22) a VH region comprising an amino acid sequence having at least 70% sequence identity more
10 preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:21.

(23) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,
15 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:24.

(24) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:26.
20

(25) a VH region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:28.

25 In some embodiments, the antigen-binding molecule comprises a VL region according to (26):
(26) a VL region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:10
LC-CDR2 having the amino acid sequence of SEQ ID NO:11
LC-CDR3 having the amino acid sequence of SEQ ID NO:12,
30 or a variant thereof in which 1 or 2 or 3 amino acids in LC-CDR1, and/or in which 1 or 2 or 3 amino acids in LC-CDR2, and/or in which 1 or 2 or 3 amino acids in LC-CDR3 are substituted with another amino acid.

In some embodiments, the antigen-binding molecule comprises a VL region according to one of (27)
35 to (40) below:
(27) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:50
LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:51
40 LC-FR4 having the amino acid sequence of SEQ ID NO:34,

or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

5 (28) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:55

LC-FR2 having the amino acid sequence of SEQ ID NO:32

LC-FR3 having the amino acid sequence of SEQ ID NO:56

LC-FR4 having the amino acid sequence of SEQ ID NO:34,

10 or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

(29) a VL region incorporating the following FRs:

15 LC-FR1 having the amino acid sequence of SEQ ID NO:13

LC-FR2 having the amino acid sequence of SEQ ID NO:14

LC-FR3 having the amino acid sequence of SEQ ID NO:15

LC-FR4 having the amino acid sequence of SEQ ID NO:16,

or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3

20 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

(30) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:31

25 LC-FR2 having the amino acid sequence of SEQ ID NO:32

LC-FR3 having the amino acid sequence of SEQ ID NO:33

LC-FR4 having the amino acid sequence of SEQ ID NO:34,

or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3

30 amino acids in LC-FR4 are substituted with another amino acid.

(31) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:36

LC-FR2 having the amino acid sequence of SEQ ID NO:32

35 LC-FR3 having the amino acid sequence of SEQ ID NO:37

LC-FR4 having the amino acid sequence of SEQ ID NO:34,

or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

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(32) a VL region incorporating the following FRs:

LC-FR1 having the amino acid sequence of SEQ ID NO:39
LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:40
LC-FR4 having the amino acid sequence of SEQ ID NO:34,
5 or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

(33) a VL region incorporating the following FRs:
10 LC-FR1 having the amino acid sequence of SEQ ID NO:39
LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:42
LC-FR4 having the amino acid sequence of SEQ ID NO:34,
or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3
15 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

(34) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:36
20 LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:44
LC-FR4 having the amino acid sequence of SEQ ID NO:34,
or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3
25 amino acids in LC-FR4 are substituted with another amino acid.

(35) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:187
LC-FR2 having the amino acid sequence of SEQ ID NO:14
30 LC-FR3 having the amino acid sequence of SEQ ID NO:15
LC-FR4 having the amino acid sequence of SEQ ID NO:16,
or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.
35
(36) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:188
LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:33
40 LC-FR4 having the amino acid sequence of SEQ ID NO:34,

or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

5 (37) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:189
LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:37
LC-FR4 having the amino acid sequence of SEQ ID NO:34,
10 or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

(38) a VL region incorporating the following FRs:
15 LC-FR1 having the amino acid sequence of SEQ ID NO:190
LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:40
LC-FR4 having the amino acid sequence of SEQ ID NO:34,
or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3
20 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

(39) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:190
25 LC-FR2 having the amino acid sequence of SEQ ID NO:32
LC-FR3 having the amino acid sequence of SEQ ID NO:42
LC-FR4 having the amino acid sequence of SEQ ID NO:34,
or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3
30 amino acids in LC-FR4 are substituted with another amino acid.

(40) a VL region incorporating the following FRs:
LC-FR1 having the amino acid sequence of SEQ ID NO:189
LC-FR2 having the amino acid sequence of SEQ ID NO:32
35 LC-FR3 having the amino acid sequence of SEQ ID NO:44
LC-FR4 having the amino acid sequence of SEQ ID NO:34,
or a variant thereof in which 1 or 2 or 3 amino acids in LC-FR1, and/or in which 1 or 2 or 3 amino acids in LC-FR2, and/or in which 1 or 2 or 3 amino acids in LC-FR3, and/or in which 1 or 2 or 3 amino acids in LC-FR4 are substituted with another amino acid.

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In some embodiments, the antigen-binding molecule comprises a VL region comprising the CDRs according to (26) above, and the FRs according to any one of (27) to (40) above.

In some embodiments, the antigen-binding molecule comprises a VL region according to one of (41)
5 to (54) below:
(41) a VL region comprising the CDRs according to (26) and the FRs according to (27).

(42) a VL region comprising the CDRs according to (26) and the FRs according to (28).

10 (43) a VL region comprising the CDRs according to (26) and the FRs according to (29).

(44) a VL region comprising the CDRs according to (26) and the FRs according to (30).

(45) a VL region comprising the CDRs according to (26) and the FRs according to (31).
15
(46) a VL region comprising the CDRs according to (26) and the FRs according to (32).

(47) a VL region comprising the CDRs according to (26) and the FRs according to (33).

20 (48) a VL region comprising the CDRs according to (26) and the FRs according to (34).

(49) a VL region comprising the CDRs according to (26) and the FRs according to (35).

(50) a VL region comprising the CDRs according to (26) and the FRs according to (36).
25
(51) a VL region comprising the CDRs according to (26) and the FRs according to (37).

(52) a VL region comprising the CDRs according to (26) and the FRs according to (38).

30 (53) a VL region comprising the CDRs according to (26) and the FRs according to (39).

(54) a VL region comprising the CDRs according to (26) and the FRs according to (40).

In some embodiments, the antigen-binding molecule comprises a VL region according to one of (55)
35 to (68) below:
(55) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:49.

(56) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:54.

5 (57) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:9.

(58) a VL region comprising an amino acid sequence having at least 70% sequence identity more
10 preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:30.

(59) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,
15 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:35.

(60) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:38.
20

(61) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:41.

25 (62) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:43.

(63) a VL region comprising an amino acid sequence having at least 70% sequence identity more
30 preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:181.

(64) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,
35 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:182.

(65) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:183.

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(66) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:184.

5 (67) a VL region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:185.

(68) a VL region comprising an amino acid sequence having at least 70% sequence identity more
10 preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:186.

In some embodiments, the antigen-binding molecule comprises a VH region according to any one of (1) to (25) above, and a VL region according to any one of (26) to (68) above.
15

In embodiments in accordance with the present disclosure, one or more amino acids are substituted with another amino acid. A substitution comprises substitution of an amino acid residue with a non-identical 'replacement' amino acid residue. A replacement amino acid residue of a substitution according to the present disclosure may be a naturally-occurring amino acid residue (*i.e.* encoded by
20 the genetic code) which is non-identical to the amino acid residue at the relevant position of the equivalent, unsubstituted amino acid sequence, selected from: alanine (Ala), arginine (Arg), asparagine (Asn), aspartic acid (Asp), cysteine (Cys), glutamine (Gln), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile): leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val). In
25 some embodiments, a replacement amino acid may be a non-naturally occurring amino acid residue – *i.e.* an amino acid residue other than those recited in the preceding sentence. Examples of non-naturally occurring amino acid residues include norleucine, ornithine, norvaline, homoserine, aib, and other amino acid residue analogues such as those described in Ellman, *et al.*, Meth. Enzym. 202 (1991) 301-336.
30

In some embodiments, a substitution may be biochemically conservative. In some embodiments, where an amino acid to be substituted is provided in one of rows 1 to 5 of the table below, the replacement amino acid of the substitution is another, non-identical amino acid provided in the same row:
35

Row	Shared property	Amino acids
1	Hydrophobic	Met, Ala, Val, Leu, Ile, Trp, Tyr, Phe, Norleucine
2	Neutral hydrophilic	Cys, Ser, Thr, Asn, Gln
3	Acidic or negatively-charged	Asp, Glu
4	Basic or positively-charged	His, Lys, Arg

5	Orientation influencing	Gly, Pro

By way of illustration, in some embodiments wherein substitution is of a Met residue, the replacement amino acid may be selected from Ala, Val, Leu, Ile, Trp, Tyr, Phe and Norleucine.

5 In some embodiments, a replacement amino acid in a substitution may have the same side chain polarity as the amino acid residue it replaces. In some embodiments, a replacement amino acid in a substitution may have the same side chain charge (at pH 7.4) as the amino acid residue it replaces:

Amino Acid	**Side-chain polarity**	**Side-chain charge (pH 7.4)**
Alanine	nonpolar	neutral
Arginine	basic polar	positive
Asparagine	polar	neutral
Aspartic acid	acidic polar	negative
Cysteine	nonpolar	neutral
Glutamic acid	acidic polar	negative
Glutamine	polar	neutral
Glycine	nonpolar	neutral
Histidine	basic polar	positive (10%) neutral (90%)
Isoleucine	nonpolar	neutral
Leucine	nonpolar	neutral
Lysine	basic polar	positive
Methionine	nonpolar	neutral
Phenylalanine	nonpolar	neutral
Proline	nonpolar	neutral
Serine	polar	neutral
Threonine	polar	neutral
Tryptophan	nonpolar	neutral
Tyrosine	polar	neutral
Valine	nonpolar	neutral

10 That is, in some embodiments, a nonpolar amino acid is substituted with another, non-identical nonpolar amino acid. In some embodiments, a polar amino acid is substituted with another, non-identical polar amino acid. In some embodiments, an acidic polar amino acid is substituted with another, non-identical acidic polar amino acid. In some embodiments, a basic polar amino acid is substituted with another, non-identical basic polar amino acid. In some embodiments, a neutral amino
15 acid is substituted with another, non-identical neutral amino acid. In some embodiments, a positive amino acid is substituted with another, non-identical positive amino acid. In some embodiments, a negative amino acid is substituted with another, non-identical negative amino acid.

In some embodiments, substitution(s) may be functionally conservative. That is, in some embodiments, the substitution may not affect (or may not substantially affect) one or more functional properties (*e.g.* target binding) of the antigen-binding molecule comprising the substitution as compared to the equivalent unsubstituted molecule.

In some embodiments, the antigen-binding molecule comprises a LC-FR1 having a cysteine residue at the position corresponding to position 23 of SEQ ID NO:9 (*i.e.* Cys23). In some embodiments, the amino acid residue at the position corresponding to position 23 of SEQ ID NO:9 is substituted with a cysteine residue. In some embodiments, the antigen-binding molecule comprises a LC-FR1 region comprising substitution of a tyrosine residue with a cysteine residue at the position corresponding to position 23 of SEQ ID NO:9 (*i.e.* Y23C). In some embodiments, the antigen-binding molecule comprises a VL region having a cysteine residue at the position corresponding to position 23 of SEQ ID NO:9 (*i.e.* Cys23). In some embodiments, the amino acid residue at the position corresponding to position 23 of SEQ ID NO:9 is substituted with a cysteine residue. In some embodiments, the antigen-binding molecule comprises a VL region comprising substitution of a tyrosine residue with a cysteine residue at the position corresponding to position 23 of SEQ ID NO:9 (*i.e.* Y23C).

Introduction of Cys23 into the light chain of HRS3 and humanised variants of HRS3 is predicted to result in the formation of an intra-chain disulfide bond, and is demonstrated herein to confer improved stability.

The VH and VL region of an antigen-binding region of an antibody together constitute the Fv region. In some embodiments, the antigen-binding molecule according to the present disclosure comprises, or consists of, an Fv region that binds to CD30. In some embodiments, the VH and VL regions of the Fv are provided as single polypeptide joined by a linker sequence, *i.e.* a single chain Fv (scFv).

The VL and light chain constant (CL) region, and the VH region and heavy chain constant 1 (CH1) region of an antigen-binding region of an antibody together constitute the Fab region. In some embodiments, the antigen-binding molecule comprises a Fab region comprising a VH, a CH1, a VL and a CL (*e.g.* Cκ or Cλ). In some embodiments, the Fab region comprises a polypeptide comprising a VH and a CH1 (*e.g.* a VH-CH1 fusion polypeptide), and a polypeptide comprising a VL and a CL (*e.g.* a VL-CL fusion polypeptide). In some embodiments, the Fab region comprises a polypeptide comprising a VH and a CL (*e.g.* a VH-CL fusion polypeptide) and a polypeptide comprising a VL and a CH (*e.g.* a VL-CH1 fusion polypeptide); that is, in some embodiments, the Fab region is a CrossFab region. In some embodiments, the VH, CH1, VL and CL regions of the Fab or CrossFab are provided as single polypeptide joined by linker regions, *i.e.* as a single chain Fab (scFab) or a single chain CrossFab (scCrossFab).

In some embodiments, the antigen-binding molecule described herein comprises, or consists of, a whole antibody that binds to CD30. As used herein, 'whole antibody' refers to an antibody having a

structure which is substantially similar to the structure of an immunoglobulin (Ig). Different kinds of immunoglobulins and their structures are described *e.g.* in Schroeder and Cavacini J Allergy Clin Immunol. (2010) 125(202): S41-S52, which is hereby incorporated by reference in its entirety.

5 Immunoglobulins of type G (*i.e.* IgG) are ~150 kDa glycoproteins comprising two heavy chains and two light chains. From N- to C-terminus, the heavy chains comprise a VH followed by a heavy chain constant region comprising three constant domains (CH1, CH2, and CH3), and similarly the light chains comprise a VL followed by a CL. Depending on the heavy chain, immunoglobulins may be classed as IgG (*e.g.* IgG1, IgG2, IgG3, IgG4), IgA (*e.g.* IgA1, IgA2), IgD, IgE, or IgM. The light chain 10 may be kappa (κ) or lambda (λ).

In some embodiments, the antigen-binding molecule described herein comprises, or consists of, an IgG (*e.g.* IgG1, IgG2, IgG3, IgG4), IgA (*e.g.* IgA1, IgA2), IgD, IgE, or IgM that binds to CD30.

15 In some embodiments, the antigen-binding molecule of the present disclosure comprises one or more regions (*e.g.* CH1, CH2, CH3, *etc.*) of an immunoglobulin heavy chain constant sequence. In some embodiments, the immunoglobulin heavy chain constant sequence is, or is derived from, the heavy chain constant sequence of an IgG (*e.g.* IgG1, IgG2, IgG3, IgG4), IgA (*e.g.* IgA1, IgA2), IgD, IgE or IgM, *e.g.* a human IgG (*e.g.* hIgG1, hIgG2, hIgG3, hIgG4), hIgA (*e.g.* hIgA1, hIgA2), hIgD, hIgE or 20 hIgM. In some embodiments, the immunoglobulin heavy chain constant sequence is, or is derived from, the heavy chain constant sequence of a human IgG1 allotype (*e.g.* G1m1, G1m2, G1m3 or G1m17).

In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at 25 least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:96, 101 or 104.

In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at 30 least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:105, 106 or 107.

In some embodiments, the antigen-binding molecule comprises a CH1 region comprising an amino 35 acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:97 or 102. In some embodiments, the antigen-binding molecule comprises a hinge region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 40 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:98. In some embodiments, the antigen-binding molecule comprises a CH2 region

comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:99. In some embodiments, the antigen-binding molecule comprises a CH3 region comprising an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:100 or 103.

It will be appreciated that CH2 and/or CH3 regions may be provided with further substitutions in accordance with modification to an Fc region of the antigen-binding molecule as described herein.

In some embodiments, the antigen-binding molecule of the present disclosure comprises one or more regions of an immunoglobulin light chain constant sequence. In some embodiments, the immunoglobulin light chain constant sequence is human immunoglobulin kappa constant (IGKC; Cκ). In some embodiments, the immunoglobulin light chain constant sequence is a human immunoglobulin lambda constant (IGLC; Cλ), *e.g.* IGLC1, IGLC2, IGLC3, IGLC6 or IGLC7.

In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:108, 109, 110, 111, 112 or 113. In preferred embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 70% sequence identity more preferably one of at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, sequence identity to the amino acid sequence of SEQ ID NO:108.

In some embodiments, the antigen-binding molecule is or comprises a monoclonal antibody, or an antigen-binding fragment thereof.

In some embodiments, the antigen-binding molecule is or comprises a fully human antibody/antibody fragment. A fully human antibody/antibody fragment may be encoded by human nucleic acid sequence(s). A fully human antibody/antibody fragment may be devoid of non-human amino acid sequences. Commonly employed techniques for the production of fully human antibodies include (i) phage display, in which human antibody genes are expressed in phage display libraries, and (ii) production of antibodies in transgenic mice engineered to have human antibody genes (described in Park and Smolen, Advances in Protein Chemistry (2001) 56: 369-421). Briefly, in the human antibody gene-phage display technique, genes encoding the VH and VL chains are generated by PCR amplification and cloning from 'naive' human lymphocytes, and assembled into a library from which they can be expressed either as disulfide-linked Fab fragments or as single-chain Fv (scFv) fragments. The Fab- or scFv-encoding genes are fused to a surface coat protein of filamentous bacteriophage and Fab or scFv capable of binding to the target of interest can then be identified by screening the library with antigen. Molecular evolution or affinity maturation procedures can be

employed to enhance the affinity of the Fab/scFv fragment. In the transgenic mouse technique, mice in which the endogenous murine Ig gene loci have been replaced by homologous recombination with their human homologues are immunised with antigen, and monoclonal antibody is prepared by conventional hybridoma technology, to yield a fully human monoclonal antibody.

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In some embodiments, the antigen-binding molecule of the present disclosure is a mouse antibody/antibody fragment. In some embodiments, the antibody/antibody fragment is obtained from phage display using a human naïve antibody gene library.

10 In some embodiments, the antigen-binding molecule is a mouse/human chimeric antibody/antibody fragment (*i.e.* an antigen-binding molecule comprising mouse antibody variable domains and human antibody constant regions). In some embodiments, the antigen-binding molecule is a humanised antibody/antibody fragment. In some embodiments, the antigen-binding molecule comprises mouse antibody CDRs and human antibody framework and constant regions.

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Mouse/human chimeric antigen-binding molecules can be prepared from mouse antibodies by the process of chimerisation, *e.g.* as described in Human Monoclonal Antibodies: Methods and Protocols, Michael Steinitz (Editor), Methods in Molecular Biology 1060, Springer Protocols, Humana Press (2014), in Chapter 8 thereof, in particular section 3 of Chapter 8.

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Humanised antigen-binding molecules can be prepared from mouse antibodies by the process of humanisation, *e.g.* as described in Human Monoclonal Antibodies: Methods and Protocols, Michael Steinitz (Editor), Methods in Molecular Biology 1060, Springer Protocols, Humana Press (2014), in Chapter 7 thereof, in particular section 3.1 of Chapter 7 entitled ‘Antibody Humanization’. Techniques

25 for antibody humanisation are also described *e.g.* in Safdari *et al.*, Biotechnol Genet Eng Rev (2013) 29:175-86.

Aspects of the present disclosure relate to multispecific antigen-binding molecules. By ‘multispecific’ it is meant that the antigen-binding molecule displays specific binding to more than one target. In some

30 embodiments, the antigen-binding molecule is a bispecific antigen-binding molecule. In some embodiments, the antigen-binding molecule comprises at least two different antigen-binding domains (*i.e.* at least two antigen-binding domains, *e.g.* comprising non-identical VHs and VLs).

In some embodiments, the antigen-binding molecule binds to CD30 and another target (*e.g.* an

35 antigen other than CD30), and so is at least bispecific. The term ‘bispecific’ means that the antigen-binding molecule is able to bind specifically to at least two distinct antigenic determinants.

It will be appreciated that an antigen-binding molecule according to the present disclosure (*e.g.* a multispecific antigen-binding molecule) may comprise antigen-binding molecules capable of binding to

40 the targets for which the antigen-binding molecule is specific. For example, an antigen-binding molecule that binds to CD30 and an antigen other than CD30 may comprise: (i) an antigen-binding

molecule that binds to CD30, and (ii) an antigen-binding molecule that binds to an antigen other than CD30.

It will also be appreciated that an antigen-binding molecule according to the present disclosure (*e.g.* a multispecific antigen-binding molecule) may comprise antigen-binding polypeptides or antigen-binding polypeptide complexes capable of binding to the targets for which the antigen-binding molecule is specific.

In some embodiments, a component antigen-binding molecule of a larger antigen-binding molecule (*e.g.* a multispecific antigen-binding molecule) may be referred to *e.g.* as an ‘antigen-binding domain’ or ‘antigen-binding region’ of the larger antigen-binding molecule.

In some embodiments, the antigen other than CD30 in a multispecific antigen-binding molecule is an immune cell surface molecule. In some embodiments, the antigen is a cancer cell antigen. In some embodiments, the antigen is a receptor molecule, *e.g.* a cell surface receptor. In some embodiments, the antigen is a cell signalling molecule, *e.g.* a cytokine, chemokine, interferon, interleukin or lymphokine. In some embodiments, the antigen is a growth factor or a hormone.

A cancer cell antigen is an antigen which is expressed or over-expressed by a cancer cell. A cancer cell antigen may be any peptide/polypeptide, glycoprotein, lipoprotein, glycan, glycolipid, lipid, or fragment thereof. A cancer cell antigen’s expression may be associated with a cancer. A cancer cell antigen may be abnormally expressed by a cancer cell (*e.g.* the cancer cell antigen may be expressed with abnormal localisation), or may be expressed with an abnormal structure by a cancer cell. A cancer cell antigen may be capable of eliciting an immune response. In some embodiments, the antigen is expressed at the cell surface of the cancer cell (*i.e.* the cancer cell antigen is a cancer cell surface antigen). In some embodiments, the part of the antigen which is bound by the antigen-binding molecule described herein is displayed on the external surface of the cancer cell (*i.e.* is extracellular). The cancer cell antigen may be a cancer-associated antigen. In some embodiments, the cancer cell antigen is an antigen whose expression is associated with the development, progression or severity of symptoms of a cancer. The cancer-associated antigen may be associated with the cause or pathology of the cancer, or may be expressed abnormally as a consequence of the cancer. In some embodiments, the cancer cell antigen is an antigen whose expression is upregulated (*e.g.* at the RNA and/or protein level) by cells of a cancer, *e.g.* as compared to the level of expression by comparable non-cancerous cells (*e.g.* non-cancerous cells derived from the same tissue/cell type). In some embodiments, the cancer-associated antigen may be preferentially expressed by cancerous cells, and not expressed by comparable non-cancerous cells (*e.g.* non-cancerous cells derived from the same tissue/cell type). In some embodiments, the cancer-associated antigen may be the product of a mutated oncogene or mutated tumor suppressor gene. In some embodiments, the cancer-associated antigen may be the product of an overexpressed cellular protein, a cancer antigen produced by an oncogenic virus, an oncofetal antigen, or a cell surface glycolipid or glycoprotein.

Cancer-associated antigens are reviewed by Zarour HM, DeLeo A, Finn OJ, *et al.* Categories of Tumor Antigens. In: Kufe DW, Pollock RE, Weichselbaum RR, *et al.*, editors. Holland-Frei Cancer Medicine. 6th edition. Hamilton (ON): BC Decker; 2003. Cancer-associated antigens include oncofetal antigens: CEA, Immature laminin receptor, TAG-72; oncoviral antigens such as HPV E6 and E7;
5 overexpressed proteins: BING-4, calcium-activated chloride channel 2, cyclin-B1, 9D7, Ep-CAM, EphA3, HER2/neu, telomerase, mesothelin, SAP-1, survivin; cancer-testis antigens: BAGE, CAGE, GAGE, MAGE, SAGE, XAGE, CT9, CT10, NY-ESO-1, PRAME, SSX-2; lineage restricted antigens: MART1, Gp100, tyrosinase, TRP-1/2, MC1R, prostate specific antigen; mutated antigens: β-catenin, BRCA1/2, CDK4, CML66, Fibronectin, MART-2, p53, Ras, TGF-βRII; post-translationally altered
10 antigens: MUC1, idiotypic antigens: Ig, TCR. Other cancer-associated antigens include heat-shock protein 70 (HSP70), heat-shock protein 90 (HSP90), glucose-regulated protein 78 (GRP78), vimentin, nucleolin, feto-acinar pancreatic protein (FAPP), alkaline phosphatase placental-like 2 (ALPPL-2), siglec-5, stress-induced phosphoprotein 1 (STIP1), protein tyrosine kinase 7 (PTK7), and cyclophilin B. In some embodiments the cancer-associated antigen is a cancer-associated antigen described in
15 Zhao and Cao, Front Immunol. 2019; 10: 2250, which is hereby incorporated by reference in its entirety. In some embodiments, a cancer-associated antigen is selected from CD30, CD19, CD20, CD22, B7H3, c-Met, ROR1R, CD4, CD7, CD38, BCMA, Mesothelin, EGFR, GPC3, MUC1, HER2, GD2, CEA, EpCAM, LeY and PSCA. In some embodiments, a cancer-associated antigen is an antigen expressed by cells of a hematological malignancy. In some embodiments, a cancer-
20 associated antigen is selected from CD30, CD19, CD20, CD22, B7H3, c-Met, ROR1R, CD4, CD7, CD38 and BCMA. In some embodiments, a cancer-associated antigen is an antigen expressed by cells of a solid tumor. In some embodiments, a cancer-associated antigen is selected from Mesothelin, EGFR, GPC3, MUC1, HER2, GD2, CEA, EpCAM, LeY and PSCA.

25 An immune cell surface molecule may be any peptide/polypeptide, glycoprotein, lipoprotein, glycan, glycolipid, lipid, or fragment thereof expressed at or on the cell surface of an immune cell. In some embodiments, the part of the immune cell surface molecule which is bound by the antigen-binding molecule of the present disclosure is on the external surface of the immune cell (*i.e.* is extracellular). The immune cell surface molecule may be expressed at the cell surface of any immune cell. In some
30 embodiments, the immune cell may be a cell of hematopoietic origin, *e.g.* a neutrophil, eosinophil, basophil, dendritic cell, lymphocyte, or monocyte. The lymphocyte may be *e.g.* a T cell, B cell, natural killer (NK) cell, NKT cell or innate lymphoid cell (ILC), or a precursor thereof (*e.g.* a thymocyte or pre-B cell).

35 In some embodiments, the antigen-binding molecule is an immune cell engager. Immune cell engagers are reviewed *e.g.* in Goebeler and Bargou, Nat. Rev. Clin. Oncol. (2020) 17: 418–434 and Ellerman, Methods (2019) 154:102-117, both of which are hereby incorporated by reference in their entirety. Immune cell engager molecules comprise an antigen-binding region for a target antigen of interest, and an antigen-binding region for recruiting/engaging an immune cell of interest. Immune cell
40 engagers recruit/engage immune cells through an antigen-binding region specific for an immune cell surface molecule.

The best studied immune cell engagers are bispecific T cell engagers (BiTEs), which comprise a target antigen binding domain, and a CD3 polypeptide (typically CD3ε)-binding domain, through which the BiTE recruits T cells. Binding of the BiTE to its target antigen and to the CD3 polypeptide expressed by the T cell results in activation of the T cell, and ultimately directs T cell effector activity against cells expressing the target antigen. Other kinds of immune cell engagers are well known in the art, and include natural killer cell engagers such as bispecific killer engagers (BiKEs), which recruit and activate NK cells.

In some embodiments, the immune cell engaged by the immune cell engager is a T cell or an NK cell. In some embodiments, the immune cell engager is a T cell-engager.

Multispecific antigen-binding molecules according to the present disclosure may be provided in any suitable format, such as those formats described in described in Brinkmann and Kontermann, MAbs (2017) 9(2): 182-212, which is hereby incorporated by reference in its entirety. Suitable formats include those shown in Figure 2 of Brinkmann and Kontermann, MAbs (2017) 9(2): 182-212: antibody conjugates, *e.g.* IgG_2, $F(ab')_2$ or CovX-Body; IgG or IgG-like molecules, *e.g.* IgG, chimeric IgG, κλ-body common HC; CH1/CL fusion proteins, *e.g.* scFv2-CH1/CL, VHH2-CH1/CL; 'variable domain only' bispecific antigen-binding molecules, *e.g.* tandem scFv (taFV), triplebodies, diabodies (Db), dsDb, Db(kih), DART, scDB, dsFv-dsFv, tandAbs, triple heads, tandem dAb/VHH, tertravalent dAb.VHH; Non-Ig fusion proteins, *e.g.* $scFv_2$-albumin, scDb-albumin, taFv-albumin, taFv-toxin, miniantibody, DNL-Fab_2, DNL-Fab_2-scFv, DNL-Fab_2-IgG-$cytokine_2$, ImmTAC (TCR-scFv); modified Fc and CH3 fusion proteins, *e.g.* scFv-Fc(kih), scFv-Fc(CH3 charge pairs), scFv-Fc (EW-RVT), scFv-fc (HA-TF), scFv-Fc (SEEDbody), taFv-Fc(kih), scFv-Fc(kih)-Fv, Fab-Fc(kih)-scFv, Fab-scFv-Fc(kih), Fab-scFv-Fc(BEAT), Fab-scFv-Fc (SEEDbody), DART-Fc, scFv-CH3(kih), TriFabs; Fc fusions, *e.g.* Di-diabody, scDb-Fc, taFv-Fc, scFv-Fc-scFv, HCAb-VHH, Fab-scFv-Fc, $scFv_4$-Ig, $scFv_2$-Fcab; CH3 fusions, *e.g.* Dia-diabody, scDb-CH3; IgE/IgM CH2 fusions, *e.g.* scFv-EHD2-scFv, scFvMHD2-scFv; Fab fusion proteins, *e.g.* Fab-scFv (bibody), Fab-$scFv_2$ (tribody), Fab-Fv, Fab-dsFv, Fab-VHH, orthogonal Fab-Fab; non-Ig fusion proteins, *e.g.* DNL-Fab_3, DNL-Fab_2-scFv, DNL-Fab_2-IgG-$cytokine_2$; asymmetric IgG or IgG-like molecules, *e.g.* IgG(kih), IgG(kih) common LC, ZW1 IgG common LC, Biclonics common LC, CrossMab, CrossMab(kih), scFab-IgG(kih), Fab-scFab-IgG(kih), orthogonal Fab IgG(kih), DuetMab, CH3 charge pairs + CH1/CL charge pairs, hinge/CH3 charge pairs, SEED-body, Duobody, four-in-one-CrossMab(kih), LUZ-Y common LC; LUZ-Y scFab-IgG, FcFc*; appended and Fc-modified IgGs, *e.g.* IgG(kih)-Fv, IgG HA-TF-Fv, IgG(kih)scFab, scFab-Fc(kih)-scFv2, scFab-Fc(kih)-scFv, half DVD-Ig, DVI-Ig (four-in-one), CrossMab-Fab; modified Fc and CH3 fusion proteins, *e.g.* Fab-Fc(kih)-scFv, Fab-scFv-Fc(kih), Fab-scFv-Fc(BEAT), Fab-scFv-Fc-SEEDbody, TriFab; appended IgGs - HC fusions, *e.g.* IgG-HC, scFv, IgG-dAb, IgG-taFV, IgG-CrossFab, IgG-orthogonal Fab, IgG-(CαCβ) Fab, scFv-HC-IgG, tandem Fab-IgG (orthogonal Fab), Fab-IgG(CαCβ Fab), Fab-IgG(CR3), Fab-hinge-IgG(CR3); appended IgGs - LC fusions, *e.g.* IgG-scFv(LC), scFv(LC)-IgG, dAb-IgG; appended IgGs - HC and LC fusions, *e.g.* DVD-Ig, TVD-Ig, CODV-Ig, $scFv_4$-IgG, Zybody; Fc fusions, *e.g.* Fab-scFv-Fc,

$scFv_4$-Ig; F(ab')2 fusions, *e.g.* $F(ab')_2$-$scFv_2$; CH1/CL fusion proteins *e.g.* $scFv_2$-CH1-hinge/CL; modified IgGs, *e.g.* DAF (two-in one-IgG), DutaMab, Mab^2; and non-Ig fusions, *e.g.* DNL-Fab_4-IgG.

The skilled person is able to design and prepare bispecific antigen-binding molecules. Methods for producing multispecific antigen-binding molecules include chemically crosslinking antigen-binding molecules or antibody fragments, *e.g.* with reducible disulfide or non-reducible thioether bonds, for example as described in Segal and Bast, 2001. Production of Bispecific Antigen-binding molecules. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16, which is hereby incorporated by reference in its entirety. For example, *N*-succinimidyl-3-(-2-pyridyldithio)-propionate (SPDP) can be used to chemically crosslink *e.g.* Fab fragments via hinge region SH- groups, to create disulfide-linked bispecific $F(ab)_2$ heterodimers.

Other methods for producing multispecific antigen-binding molecules include fusing antibody-producing hybridomas *e.g.* with polyethylene glycol, to produce a quadroma cell capable of secreting bispecific antibody, for example as described in D. M. and Bast, B. J. 2001. Production of Bispecific Antigen-binding molecules. Current Protocols in Immunology. 14:IV:2.13:2.13.1–2.13.16.

Multispecific antigen-binding molecules according to the present disclosure can also be produced recombinantly, by expression from *e.g.* a nucleic acid construct encoding polypeptides for the antigen-binding molecules, for example as described in Antibody Engineering: Methods and Protocols, Second Edition (Humana Press, 2012), at Chapter 40: Production of Bispecific Antigen-binding molecules: Diabodies and Tandem scFv (Hornig and Färber-Schwarz), or French, How to make bispecific antigen-binding molecules, Methods Mol. Med. 2000; 40:333-339, the entire contents of both of which are hereby incorporated by reference.

For example, a DNA construct encoding the light and heavy chain variable domains for the two antigen-binding fragments (*i.e.* the light and heavy chain variable domains for the antigen-binding fragment capable of binding CD30, and the light and heavy chain variable domains for the antigen-binding fragment capable of binding to another target protein), and including sequences encoding a suitable linker or dimerisation domain between the antigen-binding fragments can be prepared by molecular cloning techniques. Recombinant bispecific antibody can thereafter be produced by expression (*e.g. in vitro*) of the construct in a suitable host cell (*e.g.* a mammalian host cell), and expressed recombinant bispecific antibody can then optionally be purified.

Fc regions

In some embodiments, the antigen-binding molecules of the present disclosure comprise an Fc region.

An Fc region is composed of CH2 and CH3 regions from one polypeptide, and CH2 and CH3 regions from another polypeptide. The CH2 and CH3 regions from the two polypeptides together form the Fc region.

Fc-mediated functions include Fc receptor binding, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), formation of the membrane attack complex (MAC), cell degranulation, cytokine and/or chemokine production, and antigen processing and presentation. Modifications to antibody Fc regions that influence Fc-mediated functions are known in the art, such as those described *e.g.* in Wang *et al.*, Protein Cell (2018) 9(1):63-73, which is hereby incorporated by reference in its entirety. Exemplary Fc region modifications known to influence antibody effector function are summarised in Table 1 of Wang *et al.*, Protein Cell (2018) 9(1):63-73. In some embodiments, the antigen-binding molecule of the present disclosure comprises an Fc region comprising modification to increase or reduce an Fc-mediated function as compared to an antigen-binding molecule comprising the corresponding unmodified Fc region.

Where an Fc region/CH2/CH3 is described as comprising modification(s) ‘corresponding to’ reference substitution(s), equivalent substitution(s) in the homologous Fc/CH2/CH3 are contemplated. By way of illustration, L234A/L235A substitutions in human IgG1 (numbered according to the EU numbering system as described in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991) correspond to L to A substitutions at positions 117 and 118 of the mouse Ig gamma-2A chain C region (UniProtKB: P01863-1, v1).

Where an Fc region is described as comprising a modification, the modification may be present in one or both of the polypeptide chains which together form the Fc region.

In some embodiments, the antigen-binding molecule of the present disclosure comprises an Fc region comprising modification. In some embodiments, the antigen-binding molecule of the present disclosure comprises an Fc region comprising modification in one or more of the CH2 and/or CH3 regions.

In some embodiments, the Fc region comprises modification to increase an Fc-mediated function. In some embodiments, the Fc region comprises modification to increase ADCC. In some embodiments, the Fc region comprises modification to increase ADCP. In some embodiments, the Fc region comprises modification to increase CDC. An antigen-binding molecule comprising an Fc region comprising modification to increase an Fc-mediated function (*e.g.* ADCC, ADCP, CDC) induces an increased level of the relevant effector function as compared to an antigen-binding molecule comprising the corresponding unmodified Fc region.

In some embodiments, the Fc region comprises modification to increase binding to an Fc receptor. In some embodiments, the Fc region comprises modification to increase binding to an Fcγ receptor. In some embodiments, the Fc region comprises modification to increase binding to one or more of FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa and FcγRIIIb. In some embodiments, the Fc region comprises modification to increase binding to FcγRIIIa. In some embodiments, the Fc region comprises modification to increase binding to FcγRIIa. In some embodiments, the Fc region comprises

modification to increase binding to FcγRIIb. In some embodiments, the Fc region comprises modification to increase binding to FcRn. In some embodiments, the Fc region comprises modification to increase binding to a complement protein. In some embodiments, the Fc region comprises modification to increase binding to C1q. In some embodiments, the Fc region comprises modification to promote hexamerisation of the antigen-binding molecule. In some embodiments, the Fc region comprises modification to increase antigen-binding molecule half-life. In some embodiments, the Fc region comprises modification to increase co-engagement.

In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions F243L/R292P/Y300L/V305I/P396L as described in Stavenhagen *et al.* Cancer Res. (2007) 67:8882–8890. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions S239D/I332E or S239D/I332E/A330L as described in Lazar *et al.*, Proc Natl Acad Sci USA. (2006)103:4005–4010. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions S298A/E333A/K334A as described in Shields *et al.*, J Biol Chem. (2001) 276:6591–6604. In some embodiments, the Fc region comprises modification to one of heavy chain polypeptides corresponding to the combination of substitutions L234Y/L235Q/G236W/S239M/H268D/D270E/S298A, and modification to the other heavy chain polypeptide corresponding to the combination of substitutions D270E/K326D/A330M/K334E, as described in Mimoto *et al.*, MAbs. (2013): 5:229–236. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions G236A/S239D/I332E as described in Richards *et al.*, Mol Cancer Ther. (2008) 7:2517–2527.

In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions K326W/E333S as described in Idusogie *et al.* J Immunol. (2001) 166(4):2571-5. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions S267E/H268F/S324T as described in Moore *et al.* MAbs. (2010) 2(2):181-9. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions described in Natsume *et al.*, Cancer Res. (2008) 68(10):3863-72. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions E345R/E430G/S440Y as described in Diebolder *et al.* Science (2014) 343(6176):1260-3.

In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions M252Y/S254T/T256E as described in Dall'Acqua *et al.* J Immunol. (2002) 169:5171–5180. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions M428L/N434S as described in Zalevsky *et al.* Nat Biotechnol. (2010) 28:157–159.

In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions S267E/L328F as described in Chu *et al.*, Mol Immunol. (2008) 45:3926–3933. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions N325S/L328F as described in Shang *et al.* Biol Chem. (2014) 289:15309–15318.

In some embodiments, the Fc region comprises modification to reduce/prevent an Fc-mediated function. In some embodiments, the Fc region comprises modification to reduce/prevent ADCC. In some embodiments, the Fc region comprises modification to reduce/prevent ADCP. In some embodiments, the Fc region comprises modification to reduce/prevent CDC. An antigen-binding molecule comprising an Fc region comprising modification to reduce/prevent an Fc-mediated function (*e.g.* ADCC, ADCP, CDC) induces an reduced level of the relevant effector function as compared to an antigen-binding molecule comprising the corresponding unmodified Fc region.

In some embodiments, the Fc region comprises modification to reduce/prevent binding to an Fc receptor. In some embodiments, the Fc region comprises modification to reduce/prevent binding to an Fcγ receptor. In some embodiments, the Fc region comprises modification to reduce/prevent binding to one or more of FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa and FcγRIIIb. In some embodiments, the Fc region comprises modification to reduce/prevent binding to FcγRIIIa. In some embodiments, the Fc region comprises modification to reduce/prevent binding to FcγRIIa. In some embodiments, the Fc region comprises modification to reduce/prevent binding to FcγRIIb. In some embodiments, the Fc region comprises modification to reduce/prevent binding to a complement protein. In some embodiments, the Fc region comprises modification to reduce/prevent binding to C1q. In some embodiments, the Fc region comprises modification to reduce/prevent glycosylation of the amino acid residue corresponding to N297.

In some embodiments, the Fc region is not able to induce one or more Fc-mediated functions (*i.e.* lacks the ability to elicit the relevant Fc-mediated function(s)). Accordingly, antigen-binding molecules comprising such Fc regions also lack the ability to induce the relevant function(s). Such antigen-binding molecules may be described as being devoid of the relevant function(s).

In some embodiments, the Fc region is not able to induce ADCC. In some embodiments, the Fc region is not able to induce ADCP. In some embodiments, the Fc region is not able to induce CDC. In some embodiments, the Fc region is not able to induce ADCC and/or is not able to induce ADCP and/or is not able to induce CDC.

In some embodiments, the Fc region is not able to bind to an Fc receptor. In some embodiments, the Fc region is not able to bind to an Fcγ receptor. In some embodiments, the Fc region is not able to bind to one or more of FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa and FcγRIIIb. In some embodiments, the Fc region is not able to bind to FcγRIIIa. In some embodiments, the Fc region is not able to bind to FcγRIIa. In some embodiments, the Fc region is not able to bind to FcγRIIb. In some embodiments, the Fc region is not able to bind to FcRn. In some embodiments, the Fc region is not able to bind to a complement protein. In some embodiments, the Fc region is not able to bind to C1q. In some embodiments, the Fc region is not glycosylated at the amino acid residue corresponding to N297.

In some embodiments, the Fc region comprises modification corresponding to N297A or N297Q or N297G as described in Leabman *et al.*, MAbs. (2013) 5:896–903. In some embodiments, the Fc region comprises modification corresponding to L235E as described in Alegre *et al.*, J Immunol. (1992) 148:3461–3468. In some embodiments, the Fc region comprises modification corresponding to
5 the combination of substitutions L234A/L235A or F234A/L235A as described in Xu *et al.*, Cell Immunol. (2000) 200:16–26. In some embodiments, the Fc region comprises modification corresponding to P329A or P329G as described in Schlothauer *et al.*, Protein Engineering, Design and Selection (2016), 29(10):457–466. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions L234A/L235A/P329G as described in Lo *et al.* J.
10 Biol. Chem (2017) 292(9):3900-3908. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions described in Rother *et al.*, Nat Biotechnol. (2007) 25:1256–1264. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions S228P/L235E as described in Newman *et al.*, Clin. Immunol. (2001) 98:164–174. In some embodiments, the Fc region comprises modification corresponding to the
15 combination of substitutions H268Q/V309L/A330S/P331S as described in An *et al.*, MAbs. (2009) 1:572–579. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions V234A/G237A/P238S/H268A/V309L/A330S/P331S as described in Vafa *et al.*, Methods. (2014) 65:114–126. In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions L234A/L235E/G237A/A330S/P331S as described in
20 US 2015/0044231 A1.

The combination of substitutions ‘L234A/L235A’ and corresponding substitutions (such as *e.g.* F234A/L235A in human IgG4) are known to disrupt binding of Fc to Fcγ receptors and inhibit ADCC, ADCP, and also to reduce C1q binding and thus CDC (Schlothauer *et al.*, Protein Engineering, Design
25 and Selection (2016), 29(10):457–466, hereby incorporated by reference in entirety). The substitutions ‘P329G’ and ‘P329A’ reduce C1q binding (and thereby CDC). Substitution of ‘N297’ with ‘A’, ‘G’ or ‘Q’ is known to eliminate glycosylation, and thereby reduce Fc binding to C1q and Fcγ receptors, and thus CDC and ADCC. Lo *et al.* J. Biol. Chem (2017) 292(9):3900-3908 (hereby incorporated by reference in its entirety) reports that the combination of substitutions L234A/L235A/P329G eliminated
30 complement binding and fixation as well as Fc γ receptor dependent, antibody-dependent, cell-mediated cytotoxicity in both murine IgG2a and human IgG1.

The combination of substitutions L234A/L235E/G237A/A330S/P331S in IgG1 Fc is disclosed in US 2015/0044231 A1 to abolish induction of phagocytosis, ADCC and CDC.

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In some embodiments, the Fc region comprises modification corresponding to the substitution S228P as described in Silva *et al.*, J Biol Chem. (2015) 290(9):5462-5469. The substitution S228P in IgG4 Fc reduces Fab-arm exchange (Fab arm exchange can be undesirable).

40 In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions L234A/L235A. In some embodiments, the Fc region comprises modification

corresponding to the substitution P329G. In some embodiments, the Fc region comprises modification corresponding to the substitution N297Q.

In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions L234A/L235A/P329G.

In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions L234A/L235A/P329G/N297Q.

In some embodiments, the Fc region comprises modification corresponding to the combination of substitutions L234A/L235E/G237A/A330S/P331S.

In some embodiments, the Fc region comprises modification corresponding to the substitution S228P, *e.g.* in IgG4.

In some embodiments – particularly embodiments in which the antigen-binding molecule is a multispecific (*e.g.* bispecific) antigen-binding molecule – the antigen-binding molecule comprises an Fc region comprising modification in one or more of the CH2 and CH3 regions promoting association of the Fc region. Recombinant co-expression of constituent polypeptides of an antigen-binding molecule and subsequent association leads to several possible combinations. To improve the yield of the desired combinations of polypeptides in antigen-binding molecules in recombinant production, it is advantageous to introduce in the Fc regions modification(s) promoting association of the desired combination of heavy chain polypeptides. Modifications may promote *e.g.* hydrophobic and/or electrostatic interaction between CH2 and/or CH3 regions of different polypeptide chains. Suitable modifications are described *e.g.* in Ha *et al.*, Front. Immnol (2016) 7:394, which is hereby incorporated by reference in its entirety.

In some embodiments, the antigen-binding molecule of the present disclosure comprises an Fc region comprising paired substitutions in the CH3 regions of the Fc region according to one of the following formats, as shown in Table 1 of Ha *et al.*, Front. Immnol (2016) 7:394: KiH, KiH_{s-s}, HA-TF, ZW1, 7.8.60, DD-KK, EW-RVT, $EW\text{-}RVT_{s-s}$, SEED or A107.

Functional properties of the antigen-binding molecules

The antigen-binding molecules described herein may be characterised by reference to certain functional properties. In some embodiments, the antigen-binding molecule described herein may possess one or more of the following properties:

binds to CD30 (*e.g.* human CD30);
binds to CD30-expressing cells;
does not bind to cells that do not express CD30;
increases killing of cells expressing CD30;
does not increase killing of cells that do not express CD30;

increases ADCC of cells expressing CD30;
does not increase ADCC of cells that do not express CD30;
inhibits tumor growth, *e.g.* of CD30-expressing cancer;
increases survival of subjects having a cancer, *e.g.* a CD30-expressing cancer;
5 is not predicted to be highly immunogenic in humans.

It will be appreciated that a given antigen-binding molecule may display more than one of the properties recited in the preceding paragraph. A given antigen-binding molecule may be evaluated for the properties recited in the preceding paragraph using suitable assays. For example, the assays may 10 be *e.g. in vitro* assays, optionally cell-based assays or cell-free assays. In some embodiments, the assays may be *e.g. in vivo* assays, *i.e.* performed in non-human animals. In some embodiments, the assays may be *e.g. ex vivo* assays, *i.e.* performed using cells/tissue/an organ obtained from a subject.

Where assays are cell-based assays, they may comprise treating cells with a given antigen-binding 15 molecule in order to determine whether the antigen-binding molecule displays one or more of the recited properties. Assays may employ species labelled with detectable entities in order to facilitate their detection. Assays may comprise evaluating the recited properties following treatment of cells separately with a range of quantities/concentrations of a given antigen-binding molecule (*e.g.* a dilution series). It will be appreciated that the cells preferably express the target antigen for the 20 antigen-binding molecule (*i.e.* CD30).

Analysis of the results of such assays may comprise determining the concentration at which 50% of the maximal level of the relevant activity is attained. The concentration of a given agent at which 50% of the maximal level of the relevant activity is attained may be referred to as the ‘half-maximal effective 25 concentration’ of the agent in relation to the relevant activity, which may also be referred to as the ‘EC_{50}’. By way of illustration, the EC_{50} of a given antigen-binding molecule for binding to human CD30 may be the concentration of the antigen-binding molecule at which 50% of the maximal level of binding to human CD30 is achieved.

30 Depending on the property, the EC_{50} may also be referred to as the ‘half-maximal inhibitory concentration’ or ‘IC_{50}’, this being the concentration of the agent at which 50% of the maximal level of inhibition of a given property is observed.

The antigen-binding molecules described herein bind to CD30. The antigen-binding molecules and 35 antigen-binding domains described herein preferably display specific binding to CD30. As used herein, ‘specific binding’ refers to binding which is selective for the antigen, and which can be discriminated from non-specific binding to non-target antigen. An antigen-binding molecule/domain that specifically binds to a target molecule preferably binds the target with greater affinity, and/or with greater duration than it binds to other, non-target molecules.

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The ability of a given polypeptide to bind specifically to a given molecule can be determined by analysis according to methods known in the art, such as by ELISA, Surface Plasmon Resonance (SPR; see *e.g.* Hearty *et al.*, Methods Mol Biol (2012) 907:411-442), Bio-Layer Interferometry (see *e.g.* Lad *et al.*, (2015) J Biomol Screen 20(4): 498-507), flow cytometry, or by a radiolabeled antigen-binding assay (RIA) enzyme-linked immunosorbent assay. Through such analysis binding to a given molecule can be measured and quantified. In some embodiments, the binding may be the response detected in a given assay.

In some embodiments, the extent of binding of the antigen-binding molecule to a non-target molecule is less than about 10% of the binding of the antibody to the target molecule as measured, *e.g.* by ELISA, SPR, Bio-Layer Interferometry or by RIA. Alternatively, binding specificity may be reflected in terms of binding affinity where the antigen-binding molecule binds with a dissociation constant (K_D) that is at least 0.1 order of magnitude (*i.e.* 0.1×10^n, where n is an integer representing the order of magnitude) greater than the K_D of the antigen-binding molecule towards a non-target molecule. This may optionally be one of at least 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, or 2.0.

The affinity of binding to a given target antigen for an antigen-binding molecule described herein may be determined by SPR, *e.g.* as described in the Examples of the present disclosure.

In some embodiments, the antigen-binding molecule described herein binds to CD30 with an affinity in the micromolar range, *i.e.* $K_D = 9.9 \times 10^{-4}$ to 1×10^{-6} M. In some embodiments, the antigen-binding molecule described herein binds to CD30 with sub-micromolar affinity, *i.e.* $K_D < 1 \times 10^{-6}$ M. In some embodiments, the antigen-binding molecule described herein binds to CD30 with an affinity in the nanomolar range, *i.e.* $K_D = 9.9 \times 10^{-7}$ to 1×10^{-9} M. In some embodiments, the antigen-binding molecule described herein binds to CD30 with sub-nanomolar affinity, *i.e.* $K_D < 1 \times 10^{-9}$ M. In some embodiments, the antigen-binding molecule described herein binds to CD30 with an affinity in the picomolar range, *i.e.* $K_D = 9.9 \times 10^{-10}$ to 1×10^{-12} M. In some embodiments, the antigen-binding molecule described herein binds to CD30 with sub-picomolar affinity, *i.e.* $K_D < 1 \times 10^{-12}$ M.

In some embodiments, the antigen-binding molecule described herein binds to human CD30 with a K_D of 10 μM or less, preferably one of ≤5 μM, ≤2 μM, ≤1 μM, ≤500 nM, ≤100 nM, ≤75 nM, ≤50 nM, ≤40 nM, ≤30 nM, ≤20 nM, ≤15 nM, ≤12.5 nM, ≤10 nM, ≤9 nM, ≤8 nM, ≤7 nM, ≤6 nM, ≤5 nM, ≤4 nM ≤3 nM, ≤2 nM, ≤1 nM, ≤500 pM, ≤400 pM, ≤300 pM, ≤200 pM, ≤100 pM, ≤50 pM, ≤40 pM, ≤30 pM, ≤20 pM, ≤10 pM or ≤1 pM (*e.g.* as determined by analysis as described in Example 1.3 herein). In some embodiments, the antigen-binding molecule described herein binds to human CD30 with a K_D of 100 nM or less, preferably one of ≤50 nM, ≤40 nM, ≤30 nM, ≤20 nM, ≤15 nM, ≤12.5 nM, ≤10 nM, ≤9 nM, ≤8 nM, ≤7 nM, ≤6 nM, ≤5 nM, ≤4 nM ≤3 nM, ≤2 nM, ≤1 nM, ≤500 pM, ≤400 pM, ≤300 pM, ≤200 pM, ≤100 pM, ≤50 pM, ≤40 pM, ≤30 pM, ≤20 pM, ≤10 pM or ≤1 pM (*e.g.* as determined by analysis as described in Example 1.3 herein).

In some embodiments, the antigen-binding molecule described herein binds to human CD30 with an EC_{50} of 10 μM or less, preferably one of ≤5 μM, ≤2 μM, ≤1 μM, ≤500 nM, ≤100 nM, ≤75 nM, ≤50 nM, ≤40 nM, ≤30 nM, ≤20 nM, ≤15 nM, ≤12.5 nM, ≤10 nM, ≤9 nM, ≤8 nM, ≤7 nM, ≤6 nM, ≤5 nM, ≤4 nM ≤3 nM, ≤2 nM, ≤1 nM, ≤500 pM, ≤400 pM, ≤300 pM, ≤200 pM, ≤100 pM, ≤50 pM, ≤40 pM, ≤30 pM, ≤20 pM, ≤10 pM or ≤1 pM (*e.g.* as determined by analysis as described in Example 1.3 herein).

The antigen-binding molecules of the present disclosure may bind to a particular region of interest of CD30. Antigen-binding molecules according to the present disclosure may bind to linear epitope of CD30, consisting of a contiguous sequence of amino acids (*i.e.* an amino acid primary sequence). In some embodiments, an antigen-binding molecules may bind to a conformational epitope of CD30, consisting of a discontinuous sequence of amino acids of the amino acid sequence.

The region of a given target molecule to which an antigen-binding molecule binds can be determined by the skilled person using various methods well known in the art, including X-ray co-crystallography analysis of antibody-antigen complexes, peptide scanning, mutagenesis mapping, hydrogen-deuterium exchange analysis by mass spectrometry, phage display, competition ELISA and proteolysis-based 'protection' methods. Such methods are described, for example, in Gershoni *et al.*, BioDrugs, 2007, 21(3):145-156, which is hereby incorporated by reference in its entirety.

In some embodiments, the antigen-binding molecule of the present disclosure binds to the extracellular domain of CD30. In some embodiments, the antigen-binding molecule binds to the region of CD30 shown in SEQ ID NO:92. In some embodiments, the antigen-binding molecule binds to a polypeptide comprising or consisting of the amino acid sequence shown in SEQ ID NO:92.

In some embodiments, the antigen-binding molecule is capable of binding to the epitope of CD30 which is bound by antibody HRS3, *e.g.* within the region of amino acid positions 185-335 of human CD30 numbered according to SEQ ID NO:87, shown in SEQ ID NO:95 (Schlapschy *et al.*, Protein Engineering, Design and Selection (2004) 17(12): 847–860, hereby incorporated by reference in its entirety).

In some embodiments, the antigen-binding molecule binds to the region of CD30 shown in SEQ ID NO:95. In some embodiments, the antigen-binding molecule contacts the region of CD30 shown in SEQ ID NO:95. In some embodiments, the antigen-binding molecule binds to CD30 via contact with one or more amino acids of the region shown in SEQ ID NO:95. In some embodiments, the epitope of the antigen-binding molecule comprises or consists of the amino acid sequence shown in SEQ ID NO:95. In some embodiments, the antigen-binding molecule binds to a polypeptide comprising or consisting of the amino acid sequence shown in SEQ ID NO:95.

The ability of an antigen-binding molecule to bind to a given peptide/polypeptide can be analysed by methods well known to the skilled person, including analysis by ELISA, immunoblot (*e.g.* western blot), immunoprecipitation, surface plasmon resonance and biolayer interferometry.

In some embodiments, the antigen-binding molecule is capable of binding the same region of CD30, or an overlapping region of CD30, to the region of CD30 which is bound by an antibody comprising the VH and VL regions (see *e.g.* Table C) of one of VH1VK1, VH1VK2, VH1VK3, VH1VK4, VH1VK5, VH2VK1, VH2VK2, VH2VK3, VH2VK4, VH2VK5, VH3VK1, VH3VK2, VH3VK3, VH3VK4, VH3VK5, VH4VK1, VH4VK2, VH4VK3, VH4VK4, VH4VK5, VH5VK1, VH5VK2, VH5VK3, VH5VK4, VH5VK5, VH1VK1Cys, VH1VK2Cys, VH1VK3Cys, VH1VK4Cys, VH1VK5Cys, VH2VK1Cys, VH2VK2Cys, VH2VK3Cys, VH2VK4Cys, VH2VK5Cys, VH3VK1Cys, VH3VK2Cys, VH3VK3Cys, VH3VK4Cys, VH3VK5Cys, VH4VK1Cys, VH4VK2Cys, VH4VK3Cys, VH4VK4Cys, VH4VK5Cys, VH5VK1Cys, VH5VK2Cys, VH5VK3Cys, VH5VK4Cys, VH5VK5Cys, VH1-5ConVK1-3Con or VH3-5ConVK2-3Con.

Whether a test antigen-binding molecule binds to the same or an overlapping region of a given target as a reference antigen-binding molecule can be evaluated, for example, by analysis of (i) interaction between the test antigen-binding molecule and the target in the absence of the reference binding molecule, and (ii) interaction between the test antigen-binding molecule in the presence of the reference antigen-binding molecule, or following incubation of the target with the reference antigen-binding molecule. Determination of a reduced level of interaction between the test antigen-binding molecule and the target following analysis according to (ii) as compared to (i) might support an inference that the test and reference antigen-binding molecule bind to the same or an overlapping region of the target. Suitable assays for such analysis include *e.g.* competition ELISA assays and epitope binning assays.

In some embodiments, the antigen-binding molecule of the present disclosure binds to CD30 in a region which is accessible to an antigen-binding molecule (*i.e.*, an extracellular antigen-binding molecule) when CD30 is expressed at the cell surface (*i.e.* in or at the cell membrane). In some embodiments, the antigen-binding molecule binds to CD30 expressed at the cell surface of a cell expressing CD30. In some embodiments, the antigen-binding molecule binds to CD30-expressing cells (*e.g.* cells of a hematopoietic malignancy, *e.g.* anaplastic large cells lymphoma or Hodgkin's lymphoma (*e.g.* KM-H2 cells). In some embodiments, the antigen-binding molecule does not bind (*i.e.* does not substantially bind) to cells lacking surface expression of CD30 (*e.g.* Raji cells).

The ability of an antigen-binding molecule to bind to a given cell type (*e.g.* cells expressing CD30, or cells not expressing CD30) can be analysed by contacting cells with the antigen-binding molecule, and detecting antigen-binding molecule bound to the cells, *e.g.* after a washing step to remove unbound antigen-binding molecule. The ability of an antigen-binding molecule to bind to a given cell type can be analysed by methods such as flow cytometry (*e.g.* as described in Example 1.3 herein) and immunofluorescence microscopy.

In some embodiments, an antigen-binding molecule according to the present disclosure may potentiate (*i.e.* upregulate, enhance) cell killing of cells comprising/expressing CD30. In some embodiments, the antigen-binding molecule potentiates cell killing of CD30-expressing cells of a

hematopoietic malignancy (*e.g.* anaplastic large cells lymphoma or Hodgkin's lymphoma (*e.g.* KM-H2 cells)). In some embodiments, the antigen-binding molecule does not potentiate (*i.e.* does not substantially potentiate) cell killing of cells lacking surface expression of CD30 (*e.g.* Raji cells).

5 In some embodiments, an antigen-binding molecule according to the present disclosure may inhibit growth or reduce metastasis of a cancer comprising cells comprising/expressing CD30. In some embodiments, an antigen-binding molecule according to the present disclosure may potentiate (i.e. upregulate, enhance) cell killing of cells comprising/expressing CD30. In some embodiments, an antigen-binding molecule according to the present disclosure may inhibit growth or reduce metastasis 10 of a cancer comprising cells comprising/expressing CD30.

Cell killing can be investigated, for example, using any of the methods reviewed in Zaritskaya *et al.*, Expert Rev Vaccines (2011), 9(6):601-616, hereby incorporated by reference in its entirety. Examples of *in vitro* assays of cytotoxicity/cell killing assays include release assays such as the ^{51}Cr release 15 assay, the lactate dehydrogenase (LDH) release assay, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) release assay, and the calcein-acetoxymethyl (calcein-AM) release assay. These assays measure cell killing based on the detection of factors released from lysed cells. Cell killing of a given test cell type by a given effector immune cell type can be analysed *e.g.* by co-culturing the test cells with the effector immune cells, and measuring the number/proportion of 20 viable/dead (*e.g.* lysed) test cells after a suitable period of time. Other suitable assays include the xCELLigence real-time cytolytic *in vitro* potency assay described in Cerignoli *et al.*, PLoS One. (2018) 13(3): e0193498 (hereby incorporated by reference in its entirety).

In some embodiments an antigen-binding molecule according to the present disclosure is capable of 25 reducing the number/proportion of cells expressing CD30. In some embodiments an antigen-binding molecule according to the present disclosure is capable of reducing the number/proportion of cells expressing CD30. In some embodiments, an antigen-binding molecule according to the present disclosure is capable of depleting/enhancing depletion of such cells.

30 Antigen-binding molecules according to the present disclosure may comprise one or more moieties for potentiating a reduction in the number/proportion of cells expressing CD30. For example, an antigen-binding molecule according to the present disclosure may *e.g.* comprise an Fc region and/or a drug moiety.

35 Fc regions provide for interaction with Fc receptors and other molecules of the immune system to bring about functional effects. IgG Fc-mediated effector functions are reviewed *e.g.* in Jefferis *et al.*, Immunol Rev 1998 163:59-76 (hereby incorporated by reference in its entirety), and are brought about through Fc-mediated recruitment and activation of immune cells (*e.g.* macrophages, dendritic cells, neutrophils, basophils, eosinophils, platelets, mast cells, NK cells and T cells) through interaction 40 between the Fc region and Fc receptors expressed by the immune cells, recruitment of complement pathway components through binding of the Fc region to complement protein C1q, and consequent

activation of the complement cascade. Fc-mediated functions include Fc receptor binding, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cell-mediated phagocytosis (ADCP), complement-dependent cytotoxicity (CDC), formation of the membrane attack complex (MAC), cell degranulation, cytokine and/or chemokine production, and antigen processing and presentation.

5

In some embodiments, an antigen-binding molecule according to the present disclosure comprises an Fc region capable of potentiating/directing one or more of ADCC, ADCP, CDC against, and/or potentiating formation of a MAC on or cell degranulation of, a cell expressing CD30 (*e.g.* a cell expressing CD30 at the cell surface).

10

In some embodiments, an antigen-binding molecule according to the present disclosure is capable of potentiating/directing ADCC against a cell expressing CD30.

The ability of, and extent to which, a given antigen-binding molecule is able to induce ADCC of a given
15 target cell type can be analysed *e.g.* according to the method described in Yamashita *et al.*, Scientific Reports (2016) 6:19772 (hereby incorporated by reference in its entirety), or by ^{51}Cr release assay as described *e.g.* in Jedema *et al.*, Blood (2004) 103: 2677–82 (hereby incorporated by reference in its entirety). The ability of, and extent to which, a given antigen-binding molecule is able to induce ADCP can be analysed *e.g.* according to the method described in Kamen *et al.*, J Immunol (2017) 198 (1
20 Supplement) 157.17 (hereby incorporated by reference in its entirety). The ability of, and extent to which, a given antigen-binding molecule is able to induce CDC can be analysed *e.g.* using a C1q binding assay, *e.g.* as described in Schlothauer *et al.*, Protein Engineering, Design and Selection (2016), 29(10):457–466 (hereby incorporated by reference in its entirety).

25 In some embodiments, an antigen-binding molecule according to the present disclosure comprises a drug moiety. The antigen-binding molecule may be conjugated to the drug moiety. Antibody-drug conjugates are reviewed *e.g.* in Parslow *et al.*, Biomedicines. 2016 Sep; 4(3):14 (hereby incorporated by reference in its entirety). In some embodiments, the drug moiety is or comprises a cytotoxic agent, such that the antigen-binding molecule displays cytotoxicity to a cell expressing CD30 (*e.g.* a cell
30 expressing CD30 at the cell surface). In some embodiments, the drug moiety is or comprises a chemotherapeutic agent.

In some embodiments, an antigen-binding molecule according to the present disclosure comprises an immune cell-engaging moiety. In some embodiments, the antigen-binding molecule comprises a CD3
35 polypeptide-binding moiety (*e.g.* an antigen-binding domain capable of binding to a CD3 polypeptide).

In some embodiments, an antigen-binding molecule according to the present disclosure is capable of potentiating/directing T cell-mediated cytolytic activity against a cell expressing CD30.

40 In some embodiments, the antigen-binding molecule of the present disclosure displays anticancer activity. In some embodiments, the antigen-binding molecule of the present disclosure increases killing

of cancer cells. In some embodiments, the antigen-binding molecule of the present disclosure causes a reduction in the number of cancer cells *in vivo*, *e.g.* as compared to an appropriate control condition. The cancer may be a cancer expressing CD30.

5 In some embodiments, an antigen-binding molecule according to the present disclosure reduces/inhibits growth of a cancer and/or of a tumor of a cancer. In some embodiments, an antigen-binding molecule reduces tissue invasion by cells of a cancer. In some embodiments, an antigen-binding molecule reduces metastasis of a cancer. In some embodiments, the antigen-binding molecule displays anticancer activity. In some embodiments, the antigen-binding molecule reduces
10 the growth/proliferation of cancer cells. In some embodiments, the antigen-binding molecule reduces the survival of cancer cells. In some embodiments, the antigen-binding molecule increases the killing of cancer cells. In some embodiments, the antigen-binding molecule of the present disclosure causes a reduction in the number of cancer cells *e.g. in vivo*. The cancer may be a cancer comprising cells expressing CD30.
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The antigen-binding molecule of the present disclosure may be analysed for the properties described in the preceding paragraph in appropriate assays. Such assays include *e.g. in vivo* models.

In some embodiments, administration of an antigen-binding molecule according to the present
20 disclosure may cause one or more of: inhibition of the development/progression of the cancer, a delay to/prevention of onset of the cancer, a reduction in/delay to/prevention of tumor growth, a reduction in/delay to/prevention of tissue invasion, a reduction in/delay to/prevention of metastasis, a reduction in the severity of the symptoms of the cancer, a reduction in the number of cancer cells, a reduction in tumour size/volume, and/or an increase in survival (*e.g.* progression free survival or overall survival),
25 *e.g.* as determined in an appropriate model.

In some embodiments, the antigen-binding molecule of the present disclosure is capable of reducing/inhibiting tumor growth (*e.g.* in an *in vivo* model, *e.g.* of a CD30-expressing cancer) to less than 1 times, *e.g.* ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7
30 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the tumor growth observed in the absence of treatment with the antigen-binding molecule (or following treatment with an appropriate control antigen-binding molecule known not to influence tumor growth), in a given assay.

35 In some embodiments, the antigen-binding molecule of the present disclosure is capable of reducing/inhibiting metastasis (*e.g.* in an *in vivo* model, *e.g.* of a CD30-expressing cancer) to less than 1 times, *e.g.* ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the level of metastasis
40 observed in the absence of treatment with the antigen-binding molecule (or following treatment with an appropriate control antigen-binding molecule known not to influence metastasis), in a given assay.

In some embodiments, the antigen-binding molecule of the present disclosure is capable of increasing survival of subjects having a cancer (*e.g.* in an *in vivo* model, *e.g.* of a CD30-expressing cancer) to more than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level of survival observed in the absence of treatment with the antigen-binding molecule (or following treatment with an appropriate control antigen-binding molecule known not to influence survival), in a given assay.

In some embodiments, the antigen-binding molecule of the present disclosure is not predicted to be highly immunogenic in humans. Predicted immunogenicity in humans can be evaluated *e.g.* by analysis using iTope software (Abzena), which is described *e.g.* in Bryson, BioDrugs (2010) 24(1):1-8. iTope models binding between amino acid side chains of peptides and binding pockets within the binding grooves of 34 human MHC class II alleles. iTope data has a high correlation with peptide-MHC class II binding *in vitro* and can be used to successfully discriminate between peptides that either bind or do not bind MHC class II molecules.

In some embodiments, the VH of an antigen-binding molecule of the present disclosure comprises 7 or fewer, *e.g.* ≤6, ≤5, ≤4, ≤3 or ≤2 peptides predicted by iTope to be ‘Promiscuous High’. In some embodiments, the VH of an antigen-binding molecule of the present disclosure comprises 8 or fewer, *e.g.* ≤7, ≤6, ≤5 or ≤4 peptides predicted by iTope to be ‘Promiscuous Moderate’.

In some embodiments, the VL of an antigen-binding molecule of the present disclosure comprises 5 or fewer, *e.g.* ≤4, ≤3, ≤2 or ≤1 peptides predicted by iTope to be ‘Promiscuous High’. In some embodiments, the VL of an antigen-binding molecule of the present disclosure comprises 6 or fewer, *e.g.* ≤5, ≤4, ≤3 or ≤2 peptides predicted by iTope to be ‘Promiscuous Moderate’.

The antigen-binding molecules of the present disclosure preferably possess novel and/or improved properties compared to known antigen-binding molecules that bind to CD30. Known antigen-binding molecules that bind to CD30 include HRS3 and HRS4 (described *e.g.* in Hombach *et al.*, Scand J Immunol. (1998) 48(5):497-501), HRS3 derivatives described in Schlapschy *et al.*, Protein Engineering, Design and Selection (2004) 17(12): 847–860, BerH2 (MBL International Cat# K0145-3, RRID:AB_590975), SGN-30 (also known as cAC10, described *e.g.* in Forero-Torres *et al.*, Br J Haematol (2009) 146:171–9), MDX-060 (described *e.g.* in Ansell *et al.*, J Clin Oncol (2007) 25:2764–9; also known as 5F11, iratumumab), and MDX-1401 (described *e.g.* in Cardarelli *et al.*, Clin Cancer Res. (2009) 15(10):3376-83), and anti-CD30 antibodies described in WO 2020/068764 A1, WO 2003/059282 A2, WO 2006/089232 A2, WO 2007/084672 A2, WO 2007/044616 A2, WO 2005/001038 A2, US 2007/166309 A1, US 2007/258987 A1, WO 2004/010957 A2 and US 2005/009769 A1.

In particular, the antigen-binding molecules of the present disclosure preferably possess novel and/or improved properties compared to HRS3. The amino acid sequence of the VH of HRS3 is shown in SEQ ID NO:1, and the VL of HRS3 is shown in SEQ ID NO:9. Herein, reference to 'HRS3' may refer to a CD30-binding antibody comprising a VH having the amino acid sequence of SEQ ID NO:1, and a VL having the amino acid sequence of SEQ ID NO:9 (*i.e.* an antibody comprising the VH and VL of HRS3). In some embodiments, the 'HRS3' may be an antibody formed of a polypeptide having the amino acid sequence of SEQ ID NO:59 (*i.e.* HRS3 scFv). In some embodiments, the 'HRS3' may be an antibody formed of an antibody heavy chain polypeptide having the amino acid sequence of SEQ ID NO:179, and an antibody light chain polypeptide having the amino acid sequence of SEQ ID NO:180 (*i.e.* HRS3 hIgG1).

Herein, where an antigen-binding molecule of the present disclosure is compared to an HRS3 antibody, the antibodies of the comparison are preferably provided in the same format. For example, where the antigen-binding molecule of the disclosure is provided in scFv format, the HRS3 of the comparison is preferably HRS3 scFv. Similarly, where the antigen-binding molecule of the disclosure is provided in hIgG1 format, the HRS3 of the comparison is preferably HRS3 hIgG1.

In some embodiments, an antigen-binding molecule according to the present disclosure:

binds to CD30 (*e.g.* human CD30) with an affinity which is similar to, or greater than, that of a known antibody to CD30 (*e.g.* HRS3);

has a thermostability which is similar to, or greater than, that of a known antibody to CD30 (*e.g.* HRS3);

increases killing of cells expressing CD30 with a potency/to an extent which is similar to, or greater than, that of a known antibody to CD30 (*e.g.* HRS3);

increases ADCC of cells expressing CD30 with a potency/to an extent which is similar to, or greater than, that of a known antibody to CD30 (*e.g.* HRS3);

inhibits tumor growth, *e.g.* of CD30-positive cancer with a potency/to an extent which is similar to, or greater than, that of a known antibody to CD30 (*e.g.* HRS3);

increases survival of subjects having a cancer, *e.g.* a CD30-positive cancer to an extent which is similar to, or greater than, that of a known antibody to CD30 (*e.g.* HRS3);

is predicted to be less immunogenic in humans than a known antibody to CD30 (*e.g.* HRS3).

In some embodiments, an antigen-binding molecule according to the present disclosure binds to CD30 with an EC_{50} which is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the EC_{50} of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay. In some embodiments, an antigen-binding molecule according to the present disclosure binds to CD30 with an EC_{50} which is less than 1 times, *e.g.* one of ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times,

≤0.05 times, or ≤0.01 the EC_{50} of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay.

In some embodiments, an antigen-binding molecule according to the present disclosure binds to CD30 with a K_D which is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the K_D of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay. In some embodiments, an antigen-binding molecule according to the present disclosure binds to CD30 with a K_D which is less than 1 times, *e.g.* one of ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 the K_D of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay.

Thermostability of antigen-binding molecules can be analysed by methods well known to the skilled person, including Differential Scanning Fluorimetry and Differential Scanning Calorimetry (DSC), which are described *e.g.* in He *et al.*, J Pharm Sci. (2010) which is hereby incorporated by reference in its entirety. Thermostability may be reflected in terms of a melting temperature (T_m), unfolding temperature or disassembly temperature (expressed *e.g.* in °C or F°). In some embodiments, thermostability is evaluated using the UNcle biostability platform as described in Example 1.4 herein.

In some embodiments, an antigen-binding molecule according to the present disclosure has a T_m which is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the T_m of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay. In some embodiments, an antigen-binding molecule according to the present disclosure has a T_m which is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the T_m of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay.

In some embodiments, an antigen-binding molecule according to the present disclosure provided in scFv-mFc format has a T_m which is similar to, or greater than, the T_m of HRS3 provided in scFv-mFc format.

In some embodiments, an antigen-binding molecule according to the present increases killing or ADCC of cells expressing CD30, to ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level of killing/ADCC achieved by treatment with a comparable

concentration of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay. In some embodiments, an antigen-binding molecule according to the present increases killing or ADCC of cells expressing CD30, to more than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level of killing/ADCC achieved by treatment with a comparable concentration of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay.

In some embodiments, an antigen-binding molecule according to the present disclosure inhibits tumor growth (*e.g.* in an *in vivo* model, *e.g.* of a CD30-expressing cancer) to ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level to which tumor growth is inhibited by treatment with a comparable concentration of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay. In some embodiments, an antigen-binding molecule according to the present disclosure inhibits tumor growth (*e.g.* in an *in vivo* model, *e.g.* of a CD30-expressing cancer) to less than 1 times, *e.g.* one of ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 the level to which tumor growth is inhibited by treatment with a comparable concentration of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay.

In some embodiments, an antigen-binding molecule according to the present disclosure reduces metastasis (*e.g.* in an *in vivo* model, *e.g.* of a CD30-expressing cancer) to ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level to which metastasis is reduced by treatment with a comparable concentration of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay. In some embodiments, an antigen-binding molecule according to the present disclosure reduces metastasis (*e.g.* in an *in vivo* model, *e.g.* of a CD30-expressing cancer) to less than 1 times, *e.g.* one of ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 the level to which metastasis is reduced by treatment with a comparable concentration of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay.

In some embodiments, an antigen-binding molecule according to the present increases survival of subjects having a cancer to ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level of survival achieved by treatment with a comparable concentration

of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay. In some embodiments, an antigen-binding molecule according to the present increases survival of subjects having a cancer to more than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level of survival achieved by treatment with a comparable concentration of a known antibody to CD30 (*e.g.* HRS3), as determined in a given assay.

In some embodiments, the antigen-binding molecule of the present disclosure predicted to be less immunogenic in humans than a known antibody to CD30 (*e.g.* HRS3). Predicted immunogenicity in humans can be evaluated *e.g.* by analysis using iTope software (Abzena).

In some embodiments, the VH of an antigen-binding molecule of the present disclosure comprises fewer peptides predicted by iTope to be ‘Promiscuous High’ than the VH of a known antibody to CD30 (*e.g.* HRS3, *i.e.* the VH of SEQ ID NO:1). In some embodiments, the VH of an antigen-binding molecule of the present disclosure comprises fewer peptides predicted by iTope to be ‘Promiscuous Medium’ than the VH of a known antibody to CD30 (*e.g.* HRS3, *i.e.* the VH of SEQ ID NO:1).

In some embodiments, the VL of an antigen-binding molecule of the present disclosure comprises fewer peptides predicted by iTope to be ‘Promiscuous High’ than the VL of a known antibody to CD30 (*e.g.* HRS3, *i.e.* the VL of SEQ ID NO:9). In some embodiments, the VL of an antigen-binding molecule of the present disclosure comprises fewer peptides predicted by iTope to be ‘Promiscuous Medium’ than the VL of a known antibody to CD30 (*e.g.* HRS3, *i.e.* the VL of SEQ ID NO:9).

Chimeric antigen receptors (CARs)

In some aspects and embodiments in accordance with the present disclosure, the antigen-binding molecule is a chimeric antigen receptor (CAR). In some aspects and embodiments, the present disclosure provides a chimeric antigen receptor comprising an antigen-binding molecule or polypeptide according to the present disclosure.

CARs are recombinant receptors that provide both antigen-binding and T cell activating functions. CAR structure and engineering is reviewed, for example, in Dotti *et al.*, Immunol Rev (2014) 257(1), hereby incorporated by reference in its entirety. CARs comprise an antigen-binding domain linked via a transmembrane domain to a signalling domain. An optional hinge or spacer domain may provide separation between the antigen-binding domain and transmembrane domain and may act as a flexible linker. When expressed by a cell, the antigen-binding domain is provided in the extracellular space, and the signalling domain is intracellular.

The antigen-binding domain mediates binding to the target antigen for which the CAR is specific. The antigen-binding domain of a CAR may be based on the antigen-binding region of an antibody which is specific for the antigen to which the CAR is targeted. For example, the antigen-binding domain of a

CAR may comprise amino acid sequences for the complementarity-determining regions (CDRs) of an antibody which binds specifically to the target antigen. The antigen-binding domain of a CAR may comprise or consist of the light chain and heavy chain variable region amino acid sequences of an antibody which binds specifically to the target antigen. The antigen-binding domain may be provided as a single chain variable fragment (scFv) comprising the sequences of the light chain and heavy chain variable region amino acid sequences of an antibody. Antigen-binding domains of CARs may target antigens based on other protein:protein interactions, such as ligand:receptor binding; for example an IL-13Rα2-targeted CAR has been developed using an antigen-binding domain based on IL-13 (see *e.g.* Kahlon *et al.* 2004 Cancer Res 64(24): 9160-9166).

The CAR of the present disclosure comprises an antigen-binding domain which comprises or consists of the antigen-binding molecule of the present disclosure, or which comprises or consists of a polypeptide according to the present disclosure.

An optional spacer domain may provide separation between the antigen-binding domain and the transmembrane domain, and may act as a flexible linker. Such domains may be or comprise flexible regions allowing the binding moiety to orient in different directions. Spacer domains may be derived from IgG.

The transmembrane domain is provided between the antigen-binding domain and the signalling domain of the CAR. The transmembrane domain provides for anchoring the CAR to the cell membrane of a cell expressing a CAR, with the antigen-binding domain in the extracellular space and signalling domain inside the cell. Transmembrane domains of CARs may be derived from transmembrane region sequences for cell membrane-bound proteins (*e.g.* CD28, CD8, CD4, CD3-ζ *etc.*).

The signalling domain comprises amino acid sequences required for activation of immune cell function. The CAR signalling domains may comprise the amino acid sequence of the intracellular domain of CD3-ζ, which provides immunoreceptor tyrosine-based activation motifs (ITAMs) for phosphorylation and activation of the CAR-expressing cell. Signalling domains comprising sequences of other ITAM-containing proteins have also been employed in CARs, such as domains comprising the ITAM containing region of FcγRI (Haynes *et al.*, 2001 J Immunol 166(1):182-187). CARs comprising a signalling domain derived from the intracellular domain of CD3-ζ are often referred to as first generation CARs.
The signalling domains of CARs typically also comprise the signalling domain of a costimulatory protein (*e.g.* CD28, 4-1BB *etc.*), for providing the costimulation signal necessary for enhancing immune cell activation and effector function. CARs having a signalling domain including additional costimulatory sequences are often referred to as second generation CARs. In some cases, CARs are engineered to provide for costimulation of different intracellular signalling pathways. For example, CD28 costimulation preferentially activates the phosphatidylinositol 3-kinase (P13K) pathway, whereas 4-1BB costimulation triggers signalling is through TNF receptor associated factor (TRAF)

adaptor proteins. Signalling domains of CARs therefore sometimes contain costimulatory sequences derived from signalling domains of more than one costimulatory molecule. CARs comprising a signalling domain with multiple costimulatory sequences are often referred to as third generation CARs.

Throughout this specification, polypeptides, domains and amino acid sequences which are 'derived from' a reference polypeptide/domain/amino acid sequence have at least 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of the reference polypeptide/domain/amino acid sequence. Polypeptides, domains and amino acid sequences which are 'derived from' a reference polypeptide/domain/amino acid sequence preferably retain the functional and/or structural properties of the reference polypeptide/domain/amino acid sequence.

By way of illustration, an amino acid sequence derived from the intracellular domain of CD28 may comprise an amino acid sequence having 60%, preferably one of 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the intracellular domain of CD28, *e.g.* as shown in SEQ ID NO:138. Furthermore, an amino acid sequence derived from the intracellular domain of CD28 preferably retains the functional properties of the amino acid sequence of SEQ ID NO:138, *i.e.* the ability to activate CD28-mediated signalling.

The amino acid sequence of a given polypeptide or domain thereof can be retrieved from, or determined from a nucleic acid sequence retrieved from, databases known to the person skilled in the art. Such databases include GenBank, EMBL and UniProt.

Through engineering to express a CAR specific for a particular target antigen, immune cells (typically T cells, but also other immune cells such as NK cells) can be directed to kill cells expressing the target antigen. Binding of a CAR-expressing T cell (CAR-T cell) to the target antigen for which it is specific triggers intracellular signalling, and consequently activation of the T cell. The activated CAR-T cell is stimulated to divide and produce factors resulting in killing of the cell expressing the target antigen.

Antigen-binding domain

The antigen-binding domain of a CAR according to the present disclosure comprises or consists of an antigen-binding molecule that binds to CD30 as described herein. Accordingly, a CAR according to the present disclosure comprises an antigen-binding molecule as described herein.

It will be appreciated that an antigen-binding molecule according to the present disclosure forms, or is comprised in, the antigen-binding domain of the CAR. Accordingly, in some embodiments, the antigen-binding molecule of the present disclosure is comprised in a CAR.

It will also be appreciated that an antigen-binding molecule according to the present disclosure may be a CAR. A CAR having an antigen-binding domain comprising or consisting of an antigen-binding

molecule of the present disclosure (*e.g.* a CD30-binding Fv) is an antigen-binding molecule. The antigen-binding domain of the CAR of the present disclosure may be provided with any suitable format, *e.g.* scFv, scFab, *etc.*

5 In some embodiments, the antigen-binding domain comprises, or consists of, a CD30-binding Fv (*i.e.* a VH and VL pair) as described herein. In some embodiments, the antigen-binding domain comprises, or consists of, a CD30-binding Fv, in which the VH and VL are covalently linked. In some embodiments, the VH and VL of a CD30-binding Fv sequences are linked by a flexible linker sequence, *e.g.* a flexible linker sequence as described herein. The flexible linker sequence may be 10 joined to ends of the VH sequence and VL sequence, thereby linking the VH and VL sequences.

In some embodiments, the antigen-binding domain comprises, or consists of, a CD30-binding scFv as described herein.

15 Spacer domain

In some embodiments, the CAR comprises a spacer domain. The spacer domain may be provided between the antigen-binding domain and the transmembrane domain. The spacer domain may also be referred to as a hinge domain. A spacer domain is an amino acid sequence which provides for flexible linkage of the antigen-binding and transmembrane domains of the CAR.

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The presence, absence and length of spacer domains has been shown to influence CAR function (reviewed *e.g.* in Dotti *et al.*, Immunol Rev (2014) 257(1) and Jayaraman *et al.*, EBioMedicine (2020) 58:102931, *supra*). Spacer length can be varied to control synaptic cleft distances, which might in turn regulate signalling. Flexible spacers can enable access to sterically hindered epitopes on the target 25 antigen. Multimerisation of spacer domains (*e.g.* through homotypic associations) results in increased signal strength and activation stimulus.

In some embodiments, a spacer domain according to the present disclosure comprises, or consists of, an amino acid sequence which is, or which is derived from: the CH2-CH3 region of human IgG1 (e.g. 30 as shown in SEQ ID NO:128), the CH2-CH3 region of human IgG1 (e.g. as shown in SEQ ID NO:130), the CH1-CH2 hinge region of human IgG1, a spacer domain derived from CD8α, *e.g.* as described in WO 2012/031744 A1, or a spacer domain derived from CD28, *e.g.* as described in WO 2011/041093 A1. Hombach *et al.*, Gene Therapy (2010) 17:1206-1213 describes a variant CH2-CH3 region for reduced activation of FcγR-expressing cells such as monocytes and NK cells. The amino 35 acid sequence of the variant CH2-CH3 region is shown in SEQ ID NO:129.

In some embodiments, the spacer domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:128. In some embodiments, the 40 spacer domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity

to the amino acid sequence of SEQ ID NO:129. In some embodiments, the spacer domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:130. In some embodiments, the spacer domain comprises, or consists of, an amino
5 acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:131. In some embodiments, the spacer domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:132. In some embodiments, the
10 spacer domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:133. In some embodiments, the spacer domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence
15 of SEQ ID NO:134.

Transmembrane domain

The CAR of the present disclosure comprises a transmembrane domain. A transmembrane domain refers to any three-dimensional structure formed by a sequence of amino acids which is
20 thermodynamically stable in a biological membrane, *e.g.* a cell membrane. In connection with the present disclosure, the transmembrane domain may be an amino acid sequence which spans the cell membrane of a cell expressing the CAR.

The transmembrane domain may comprise or consist of a sequence of amino acids which forms a
25 hydrophobic alpha helix or beta-barrel. The amino acid sequence of the transmembrane domain of the CAR of the present disclosure may be, or may be derived from, the amino acid sequence of a transmembrane domain of a protein comprising a transmembrane domain. Transmembrane domains are recorded in databases such as GenBank, UniProt, Swiss-Prot, TrEMBL, Protein Information Resource, Protein Data Bank, Ensembl, and InterPro, and/or can be identified/predicted *e.g.* using
30 amino acid sequence analysis tools such as TMHMM (Krogh *et al.*, 2001 J Mol Biol 305: 567-580).

In some embodiments, the amino acid sequence of the transmembrane domain of the CAR of the present disclosure may be, or may be derived from, the amino acid sequence of the transmembrane domain of a protein expressed at the cell surface. In some embodiments the protein expressed at the
35 cell surface is a receptor or ligand, *e.g.* an immune receptor or ligand. In some embodiments the amino acid sequence of the transmembrane domain may be, or may be derived from, the amino acid sequence of the transmembrane domain of one of ICOS, ICOSL, CD86, CTLA-4, CD28, CD80, MHC class I α, MHC class II α, MHC class II β, CD3ε, CD3δ, CD3γ, CD3-ζ, TCRα TCRβ, CD4, CD8α, CD8β, CD40, CD40L, PD-1, PD-L1, PD-L2, 4-1BB, 4-1BBL, OX40, OX40L, GITR, GITRL, TIM-3,
40 Galectin 9, LAG3, CD27, CD70, LIGHT, HVEM, TIM-4, TIM-1, ICAM1, LFA-1, LFA-3, CD2, BTLA, CD160, LILRB4, LILRB2, VTCN1, CD2, CD48, 2B4, SLAM, CD30, CD30L, DR3, TL1A, CD226,

CD155, CD112 and CD276. In some embodiments, the transmembrane is, or is derived from, the amino acid sequence of the transmembrane domain of CD28, CD3-ζ, CD8α, CD8β or CD4. In some embodiments, the transmembrane is, or is derived from, the amino acid sequence of the transmembrane domain of CD28.

5

In some embodiments, the transmembrane domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:135.

10 In some embodiments, the transmembrane domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:136.

In some embodiments, the transmembrane domain comprises, or consists of, an amino acid sequence
15 having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:137.

Signalling domain

The chimeric antigen receptor of the present disclosure comprises a signalling domain. The signalling
20 domain provides sequences for initiating intracellular signalling in cells expressing the CAR.

ITAM-containing sequence:

The signalling domain comprises ITAM-containing sequence. An ITAM-containing sequence comprises one or more immunoreceptor tyrosine-based activation motifs (ITAMs). ITAMs comprise the
25 amino acid sequence YXXL/I (SEQ ID NO:141), wherein 'X' denotes any amino acid. In ITAM-containing proteins, sequences according to SEQ ID NO:141 are often separated by 6 to 8 amino acids; YXXL/I$(X)_{6-8}$YXXL/I (SEQ ID NO:142). When phosphate groups are added to the tyrosine residue of an ITAM by tyrosine kinases, a signalling cascade is initiated within the cell.

30 In some embodiments, the signalling domain comprises one or more copies of an amino acid sequence according to SEQ ID NO:141 or SEQ ID NO:142. In some embodiments, the signalling domain comprises at least 1, 2, 3, 4, 5 or 6 copies of an amino acid sequence according to SEQ ID NO:141. In some embodiments, the signalling domain comprises at least 1, 2, or 3 copies of an amino acid sequence according to SEQ ID NO:142.

35

In some embodiments, the signalling domain comprises an ITAM-containing sequence which is, or which is derived from, the amino acid sequence of an ITAM-containing sequence of a protein having an ITAM-containing amino acid sequence. In some embodiments the signalling domain comprises an ITAM-containing sequence which is, or which is derived from, the amino acid sequence of the
40 intracellular domain of one of CD3-ζ, FcγRI, CD3ε, CD3δ, CD3γ, CD79α, CD79β, FcγRIIA, FcγRIIC, FcγRIIIA, FcγRIV or DAP12. In some embodiments the signalling domain comprises an ITAM-

containing sequence which is, or which is derived from, the amino acid sequence of the intracellular domain of CD3-ζ.

In some embodiments, the signalling domain comprises an ITAM-containing sequence which comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:143.

Costimulatory sequence:

The signalling domain may additionally comprise one or more costimulatory sequences. A costimulatory sequence is an amino acid sequence which provides for costimulation of the cell expressing the CAR of the present disclosure. Costimulation promotes proliferation and survival of a CAR-expressing cell upon binding to the target antigen, and may also promote cytokine production, differentiation, cytotoxic function and memory formation by the CAR-expressing cell. Molecular mechanisms of T cell costimulation are reviewed in Chen and Flies, (2013) Nat Rev Immunol 13(4):227-242.

A costimulatory sequence may be, or may be derived from, the amino acid sequence of a costimulatory protein. In some embodiments the costimulatory sequence is an amino acid sequence which is, or which is derived from, the amino acid sequence of the intracellular domain of a costimulatory protein.

Upon binding of the CAR to the target antigen, the costimulatory sequence provides costimulation to the cell expressing the CAR of the kind which would be provided by the costimulatory protein from which the costimulatory sequence is derived upon ligation by its cognate ligand. By way of example in the case of a CAR comprising a signalling domain comprising a costimulatory sequence derived from CD28, binding to the target antigen triggers signalling in the cell expressing the CAR of the kind that would be triggered by binding of CD80 and/or CD86 to CD28. Thus, a costimulatory sequence is capable of delivering the costimulation signal of the costimulatory protein from which the costimulatory sequence is derived.

In some embodiments, the costimulatory protein may be a member of the B7-CD28 superfamily (*e.g.* CD28, ICOS), or a member of the TNF receptor superfamily (*e.g.* 4-1BB, OX40, CD27, DR3, GITR, CD30, HVEM). In some embodiments, the costimulatory sequence is, or is derived from, the intracellular domain of one of CD28, 4-1BB, ICOS, CD27, OX40, HVEM, CD2, SLAM, TIM-1, CD30, GITR, DR3, CD226 and LIGHT. In some embodiments, the costimulatory sequence is, or is derived from, the intracellular domain of CD28.

In some embodiments the signalling domain comprises more than one costimulatory sequence. In some embodiments the signalling domain comprises 1, 2, 3, 4, 5 or 6 costimulatory sequences. Plural costimulatory sequences may be provided in tandem.

Whether a given amino acid sequence is capable of initiating signalling mediated by a given costimulatory protein can be investigated *e.g.* by analysing a correlate of signalling mediated by the costimulatory protein (*e.g.* expression/activity of a factor whose expression/activity is upregulated or downregulated as a consequence of signalling mediated by the costimulatory protein).

Costimulatory proteins upregulate expression of genes promoting cell growth, effector function and survival through several transduction pathways. For example, CD28 and ICOS signal through phosphatidylinositol 3 kinase (PI3K) and AKT to upregulate expression of genes promoting cell growth, effector function and survival through NF-κB, mTOR, NFAT and AP1/2. CD28 also activates AP1/2 via CDC42/RAC1 and ERK1/2 via RAS, and ICOS activates C-MAF. 4-1BB, OX40, and CD27 recruit TNF receptor associated factor (TRAF) and signal through MAPK pathways, as well as through PI3K.

In some embodiments the signalling domain comprises a costimulatory sequence which is, or which is derived from CD28.

Kofler *et al.* Mol. Ther. (2011) 19: 760-767 describes a variant CD28 intracellular domain in which the lck kinase binding site is mutated in order to reduce induction of IL-2 production on CAR ligation, in order to minimise regulatory T cell-mediated suppression of CAR-T cell activity. The amino acid sequence of the variant CD28 intracellular domain is shown in SEQ ID NO:139.

In some embodiments, the signalling domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:138. In some embodiments, the signalling domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:139. In some embodiments, the signalling domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:140.

In some embodiments, the signalling domain comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:147.

In some embodiments, the CAR comprises an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:148.

Linkers and additional sequences

The antigen-binding molecules, polypeptides and CARs of the present disclosure may additionally comprise further amino acids or sequences of amino acids.

The antigen-binding molecules, polypeptides and CARs of the present disclosure may comprise one or more linker sequences between sequences of amino acids. For example, a linker sequence may be provided between a VH sequence and a VL sequence, providing linkage between the VH and VL (*e.g.* as in an scFv molecule). By way of further example, a linker sequence may be provided between domains of a CAR (*e.g.* between the antigen-binding domain and spacer domain, and/or between the spacer domain and the transmembrane domain, and/or between the transmembrane domain and the signalling domain). By way of further example, a linker sequence may be provided between subsequences of the domains of a CAR (*e.g.* between VH and VL of an antigen-binding domain, and/or between the costimulatory and sequence and ITAM-containing sequence of a signalling domain).

Linker sequences are known to the skilled person, and are described, for example in Chen *et al.*, Adv Drug Deliv Rev (2013) 65(10): 1357-1369, which is hereby incorporated by reference in its entirety. In some embodiments, a linker sequence may be a flexible linker sequence. Flexible linker sequences allow for relative movement of the amino acid sequences which are linked by the linker sequence. Flexible linkers are known to the skilled person, and several are identified in Chen *et al.*, Adv Drug Deliv Rev (2013) 65(10): 1357-1369. Flexible linker sequences often comprise high proportions of glycine and/or serine residues.

In some embodiments, the linker sequence comprises at least one glycine residue and/or at least one serine residue. In some embodiments, the linker sequence comprises or consists of glycine and serine residues. In some embodiments, the linker sequence has the structure: (GxS)n or (GxS)nGm; wherein G = glycine, S = serine, x = 3 or 4, n = 2, 3, 4, 5 or 6, and m = 0, 1, 2 or 3. In some embodiments, the linker sequence comprises one or more (*e.g.* 1, 2, 3, 4, 5 or 6) copies (*e.g.* in tandem) of the sequence motif G_4S. In some embodiments, the linker sequence comprises or consists of $(G_4S)_4$ or $(G_4S)_6$. In some embodiments, the linker sequence has a length of 1-2, 1-3, 1-4, 1-5, 1-10, 1-15, 1-20, 1-25, or 1-30 amino acids.

In some embodiments, the linker sequence comprises one or more copies of an amino acid sequence according to SEQ ID NO:57. In some embodiments, the linker sequence comprises at least 1, 2, 3 or 4 copies of an amino acid sequence according to SEQ ID NO:57.

In some embodiments, the linker sequence comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:58.

The antigen-binding molecules, polypeptides and CARs of the present disclosure may comprise amino acid sequence(s) to facilitate expression, folding, trafficking, processing, purification or detection of the antigen-binding molecule/polypeptide. For example, antigen-binding molecules and polypeptides of the present disclosure may additionally comprise a sequence of amino acids forming a detectable
5 moiety, *e.g.* as described hereinbelow.

The antigen-binding molecules, polypeptides and CARs of the present disclosure may additionally comprise a signal peptide (also known as a leader sequence or signal sequence). Signal peptides normally consist of a sequence of 5-30 hydrophobic amino acids, which form a single alpha helix.
10 Secreted proteins and proteins expressed at the cell surface often comprise signal peptides. Signal peptides are known for many proteins, and are recorded in databases such as GenBank, UniProt and Ensembl, and/or can be identified/predicted *e.g.* using amino acid sequence analysis tools such as SignalP (Petersen *et al.*, 2011 Nature Methods 8: 785-786) or Signal-BLAST (Frank and Sippl, 2008 Bioinformatics 24: 2172-2176).
15

The signal peptide may be present at the N-terminus of the antigen-binding molecule/polypeptide/CAR, and may be present in the newly synthesised antigen-binding molecule/polypeptide/CAR. The signal peptide provides for efficient trafficking of the antigen-binding molecule/polypeptide/CAR. Signal peptides are often removed by cleavage, and thus are not
20 comprised in the mature antigen-binding molecule/polypeptide/CAR.

Signal peptides are known for many proteins, and are recorded in databases such as GenBank, UniProt, Swiss-Prot, TrEMBL, Protein Information Resource, Protein Data Bank, Ensembl, and InterPro, and/or can be identified/predicted *e.g.* using amino acid sequence analysis tools such as
25 SignalP (Petersen *et al.*, 2011 Nature Methods 8: 785-786) or Signal-BLAST (Frank and Sippl, 2008 Bioinformatics 24: 2172-2176).

In some embodiments, the signal peptide comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or
30 100% sequence identity to the amino acid sequence of SEQ ID NO:144. In some embodiments, the signal peptide comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:145. In some embodiments, the signal peptide comprises, or consists of, an amino acid sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%,
35 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:146.

Labels and conjugates

In some embodiments, the antigen-binding molecule, polypeptide or CAR of the present disclosure
40 additionally comprise a detectable moiety.

In some embodiments, a detectable moiety is a fluorescent label, phosphorescent label, luminescent label, immuno-detectable label (*e.g.* an epitope tag), radiolabel, chemical, nucleic acid or enzymatic label. The antigen-binding molecule, polypeptide or CAR may be covalently or non-covalently labelled with the detectable moiety.

Fluorescent labels include *e.g.* fluorescein, rhodamine, allophycocyanin, eosine and NDB, green fluorescent protein (GFP), chelates of rare earths such as europium (Eu), terbium (Tb) and samarium (Sm), tetramethyl rhodamine, Texas Red, 4-methyl umbelliferone, 7-amino-4-methyl coumarin, Cy3, and Cy5. Radiolabels include radioisotopes such as Hydrogen^{3}, Sulfur^{35}, Carbon^{14}, Phosphorus^{32}, Iodine^{123}, Iodine^{125}, Iodine^{126}, Iodine^{131}, Iodine^{133}, Bromine^{77}, Technetium^{99m}, Indium^{111}, Indium^{113m}, Gallium^{67}, Gallium^{68}, Ruthenium^{95}, Ruthenium^{97}, Ruthenium^{103}, Ruthenium^{105}, Mercury^{207}, Mercury^{203}, Rhenium^{99m}, Rhenium^{101}, Rhenium^{105}, Scandium^{47}, Tellurium^{121m}, Tellurium^{122m}, Tellurium^{125m}, Thulium^{165}, Thuliuml^{167}, Thulium^{168}, Copper^{67}, Fluorine^{18}, Yttrium^{90}, Palladium^{100}, Bismuth^{217} and Antimony^{211}. Luminescent labels include as radioluminescent, chemiluminescent (*e.g.* acridinium ester, luminol, isoluminol) and bioluminescent labels. Immuno-detectable labels include haptens, peptides/polypeptides, antibodies, receptors and ligands such as biotin, avidin, streptavidin or digoxigenin. Nucleic acid labels include aptamers.

In some embodiments, the antigen-binding molecule/polypeptide/CAR comprises an epitope tag, *e.g.* a His, (*e.g.* 6XHis), FLAG, c-Myc, StrepTag, haemagglutinin, E, calmodulin-binding protein (CBP), glutathione-s-transferase (GST), maltose-binding protein (MBP), thioredoxin, S-peptide, T7 peptide, SH2 domain, avidin, streptavidin, and haptens (*e.g.* biotin, digoxigenin, dinitrophenol), optionally at the N- or C- terminus of the antigen-binding molecule/polypeptide/CAR.

In some embodiments, the antigen-binding molecule/polypeptide/CAR comprises a moiety having a detectable activity, *e.g.* an enzymatic moiety. Enzymatic moieties include *e.g.* luciferases, glucose oxidases, galactosidases (*e.g.* beta-galactosidase), glucorinidases, phosphatases (*e.g.* alkaline phosphatase), peroxidases (*e.g.* horseradish peroxidase) and cholinesterases.

In some embodiments, the antigen-binding molecule/polypeptide/CAR of the present disclosure is conjugated to a chemical moiety. The chemical moiety may be a moiety for providing a therapeutic effect, *i.e.* a drug moiety. A drug moiety may be a small molecule (*e.g.* a low molecular weight (< 1000 daltons, typically between ~300-700 daltons) organic compound). Drug moieties are described *e.g.* in Parslow *et al.*, Biomedicines. 2016 Sep; 4(3):14 (hereby incorporated by reference in its entirety). In some embodiments, a drug moiety may be or comprise a cytotoxic agent. In some embodiments, a drug moiety may be or comprise a chemotherapeutic agent. Drug moieties include *e.g.* calicheamicin, DM1, DM4, monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), SN-38, doxorubicin, duocarmycin, D6.5 and PBD.

Particular exemplary polypeptides, antigen-binding molecules and CARs

The present disclosure also provides polypeptide constituents of antigen-binding molecules. The polypeptides may be provided in isolated or substantially purified form.

5 The antigen-binding molecule of the present disclosure may be, or may comprise, a complex of polypeptides.

In the present specification where a polypeptide comprises more than one domain or region, it will be appreciated that the plural domains/regions are preferably present in the same polypeptide chain. That 10 is, the polypeptide comprising more than one domain or region is a fusion polypeptide comprising the domains/regions.

In some embodiments a polypeptide according to the present disclosure comprises, or consists of, a VH as described herein. In some embodiments a polypeptide according to the present disclosure 15 comprises, or consists of, a VL as described herein.

In some embodiments, the polypeptide additionally comprises one or more antibody heavy chain constant regions (CH). In some embodiments, the polypeptide additionally comprises one or more antibody light chain constant regions (CL). In some embodiments, the polypeptide comprises a CH1, 20 CH2 region and/or a CH3 region of an immunoglobulin (Ig).

In some embodiments a polypeptide according to the present disclosure comprises a linker sequence (linker) as described herein.

25 In some embodiments, the polypeptide according to the present disclosure comprises a structure from N- to C-terminus according to one of the following:

(i) VH
(ii) VL
(iii) VH-CH1
30 (iv) VL-CL
(v) VL-CH1
(vi) VH-CL
(vii) VH-CH1-CH2-CH3
(viii) VL-CL-CH2-CH3
35 (ix) VL-CH1-CH2-CH3
(x) VH-CL-CH2-CH3
(xi) VH-linker-VL
(xii) VL-linker-VH

Also provided by the present disclosure are antigen-binding molecules composed of the polypeptides of the present disclosure. In some embodiments, the antigen-binding molecule of the present disclosure comprises one of the following combinations of polypeptides:

(A) VH + VL
5 (B) VH-CH1 + VL-CL
(C) VL-CH1 + VH-CL
(D) VH-CH1-CH2-CH3 + VL-CL
(E) VH-CL-CH2-CH3 + VL-CH1
(F) VL-CH1-CH2-CH3 + VH-CL
10 (G) VL-CL-CH2-CH3 + VH-CH1
(H) VH-CH1-CH2-CH3 + VL-CL-CH2-CH3
(I) VH-CL-CH2-CH3 + VL-CH1-CH2-CH3

In some embodiments, the antigen-binding molecule comprises more than one of a polypeptide of the
15 combinations shown in (A) to (I) above. By way of example, with reference to (D) above, in some embodiments, the antigen-binding molecule comprises two polypeptides comprising the structure VH-CH1-CH2-CH3, and two polypeptides comprising the structure VL-CL.

In each of (i) to (xii) and (A) to (I) above: 'VH' refers to refers to the VH of an antigen-binding molecule
20 capable of binding to CD30 as described herein, *e.g.* as defined in one of (1) to (25); and 'VL' refers to refers to the VL of an antigen-binding molecule capable of binding to CD30 as described herein, *e.g.* as defined in one of (26) to (68).

In some embodiments, the antigen-binding molecule of the present disclosure comprises a
25 polypeptide which comprises or consists of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:1, 17, 21, 24, 26, 28, 45 or 52.

In some embodiments, the antigen-binding molecule of the present disclosure comprises a
30 polypeptide which comprises or consists of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:9, 30, 35, 38, 41, 43, 49, 54, 181, 182, 183, 184, 185 or 186.

In some embodiments, the antigen-binding molecule of the present disclosure comprises a
35 polypeptide which comprises or consists of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO:179, 114, 115, 116, 117, 118, 119 or 120.

In some embodiments, the antigen-binding molecule of the present disclosure comprises a
40 polypeptide which comprises or consists of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid

sequence identity to SEQ ID NO:180, 121, 122, 123, 124, 125, 126, 127, 252, 217, 218, 219, 220 or 221.

In some embodiments, the antigen-binding molecule of the present disclosure comprises a polypeptide or polypeptides comprising: (i) a VH region comprising HC-CDR1, HC-CDR2 and HC-CDR3 as indicated in column A of Table A, and (ii) a VL region comprising LC-CDR1, LC-CDR2 and LC-CDR3 as indicated in column B of Table A, wherein the sequences of columns A and B are selected from the same row of Table A. That is, in some embodiments, the antigen-binding molecule comprises a polypeptide or polypeptides comprising: (i) a VH region comprising HC-CDR1 = SEQ ID NO:2, HC-CDR2 = SEQ ID NO:3 and HC-CDR3 = SEQ ID NO:4, and (ii) a VL region comprising LC-CDR1 = SEQ ID NO:10, LC-CDR2 = SEQ ID NO:11 and LC-CDR3 SEQ ID NO:12.

In some embodiments, the antigen-binding molecule of the present disclosure comprises a polypeptide or polypeptides comprising a VH region comprising the heavy chain FRs, and a VL region comprising the light chain FRs, of an antibody selected from VH1VK1, VH1VK2, VH1VK3, VH1VK4, VH1VK5, VH2VK1, VH2VK2, VH2VK3, VH2VK4, VH2VK5, VH3VK1, VH3VK2, VH3VK3, VH3VK4, VH3VK5, VH4VK1, VH4VK2, VH4VK3, VH4VK4, VH4VK5, VH5VK1, VH5VK2, VH5VK3, VH5VK4, VH5VK5, VH1VK1Cys, VH1VK2Cys, VH1VK3Cys, VH1VK4Cys, VH1VK5Cys, VH2VK1Cys, VH2VK2Cys, VH2VK3Cys, VH2VK4Cys, VH2VK5Cys, VH3VK1Cys, VH3VK2Cys, VH3VK3Cys, VH3VK4Cys, VH3VK5Cys, VH4VK1Cys, VH4VK2Cys, VH4VK3Cys, VH4VK4Cys, VH4VK5Cys, VH5VK1Cys, VH5VK2Cys, VH5VK3Cys, VH5VK4Cys, VH5VK5Cys, VH1-5ConVK1-3Con or VH3-5ConVK2-3Con, as shown in Table B herein. That is, in some embodiments, the antigen-binding molecule comprises a polypeptide or polypeptides comprising: (i) a VH region comprising HC-FR1, HC-FR2, HC-FR3 and HC-FR4 as indicated in column A of Table B, and (ii) a VL region comprising LC-FR1, LC-FR2, LC-FR3, and LC-FR4 as indicated in column B of Table B, wherein the sequences of columns A and B are selected from the same row of Table B.

In some embodiments, the antigen-binding molecule of the present disclosure comprises a polypeptide or polypeptides comprising: (i) an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to an amino acid sequence indicated in column A of Table C, and (ii) an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to an amino acid sequence indicated in column B of Table C, wherein the sequences of columns A and B are selected from the same row of Table C.

In some embodiments, the antigen-binding molecule of the present disclosure comprises a polypeptide or polypeptides comprising a VH region and a VL region of an antibody selected from VH1VK1, VH1VK2, VH1VK3, VH1VK4, VH1VK5, VH2VK1, VH2VK2, VH2VK3, VH2VK4, VH2VK5, VH3VK1, VH3VK2, VH3VK3, VH3VK4, VH3VK5, VH4VK1, VH4VK2, VH4VK3, VH4VK4, VH4VK5, VH5VK1, VH5VK2, VH5VK3, VH5VK4, VH5VK5, VH1VK1Cys, VH1VK2Cys, VH1VK3Cys,

VH1VK4Cys, VH1VK5Cys, VH2VK1Cys, VH2VK2Cys, VH2VK3Cys, VH2VK4Cys, VH2VK5Cys, VH3VK1Cys, VH3VK2Cys, VH3VK3Cys, VH3VK4Cys, VH3VK5Cys, VH4VK1Cys, VH4VK2Cys, VH4VK3Cys, VH4VK4Cys, VH4VK5Cys, VH5VK1Cys, VH5VK2Cys, VH5VK3Cys, VH5VK4Cys, VH5VK5Cys, VH1-5ConVK1-3Con or VH3-5ConVK2-3Con, as shown in Table C herein. That is, in some embodiments, the antigen-binding molecule comprises a polypeptide or polypeptides comprising: (i) an amino acid sequence indicated in column A of Table C, and (ii) an amino acid sequence indicated in column B of Table C, wherein the sequences of columns A and B are selected from the same row of Table C.

In some embodiments, the antigen-binding molecule of the present disclosure comprises: (i) a polypeptide comprising or consisting of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to an amino acid sequence indicated in column A of Table D, and (ii) a polypeptide comprising or consisting of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to an amino acid sequence indicated in column B of Table D, wherein the sequences of columns A and B are selected from the same row of Table D.

In some embodiments, the antigen-binding molecule of the present disclosure comprises the polypeptides of an antigen-binding molecule as detailed in Table D herein. That is, in some embodiments, the antigen-binding molecule comprises: (i) a polypeptide comprising or consisting of an amino acid sequence indicated in column A of Table D, and (ii) a polypeptide comprising or consisting of an amino acid sequence indicated in column B of Table D, wherein the sequences of columns A and B are selected from the same row of Table D.

In some embodiments, the antigen-binding molecule of the present disclosure comprises or consists of a polypeptide comprising or consisting of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 191, 192, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215 or 216.

In some embodiments, the antigen-binding molecule or CAR of the present disclosure comprises or consists of a polypeptide comprising or consisting of an amino acid sequence having at least 70%, preferably one of 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to the amino acid sequence of SEQ ID NO:149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250 or 251.

In some embodiments, the antigen-binding molecule of the present disclosure does not comprise, or does not consist of, a polypeptide having the amino acid sequence of SEQ ID NO:59.

In some embodiments, the antigen-binding molecule of the present disclosure does not comprise a polypeptide having the amino acid sequence of SEQ ID NO:179. In some embodiments, the antibody of the present disclosure does not comprise a polypeptide having the amino acid sequence of SEQ ID NO:180.

In some embodiments, a VH region according to the present disclosure does not comprise, or does not consist of, the amino acid sequence of SEQ ID NO:1. In some embodiments, a VH region according to the present disclosure does not comprise, or does not consist of, the amino acid sequence of SEQ ID NO:9.

In some embodiments, a VH region according to the present disclosure does not comprise HC-FR1 having the amino acid sequence of SEQ ID NO:5. In some embodiments, a VH region does not comprise HC-FR2 having the amino acid sequence of SEQ ID NO:6. In some embodiments, a VH region does not comprise HC-FR3 having the amino acid sequence of SEQ ID NO:7.

In some embodiments, a VL region according to the present disclosure does not comprise LC-FR1 having the amino acid sequence of SEQ ID NO:13. In some embodiments, a VL region does not comprise LC-FR2 having the amino acid sequence of SEQ ID NO:14. In some embodiments, a VL region does not comprise LC-FR3 having the amino acid sequence of SEQ ID NO:15.

Nucleic acids and vectors

The present disclosure provides a nucleic acid, or a plurality of nucleic acids, encoding an antigen-binding molecule, polypeptide or CAR according to the present disclosure. In some embodiments, the nucleic acid(s) comprise or consist of DNA and/or RNA.

In some embodiments, the nucleic acid(s) may be, or may be comprised in, a vector, or a plurality of vectors. That is, the nucleotide sequence(s) of the nucleic acid(s) may be contained in vector(s). The antigen-binding molecule, polypeptide or CAR according to the present disclosure may be produced within a cell by transcription from a vector encoding the antigen-binding molecule, polypeptide or CAR, and subsequent translation of the transcribed RNA.

Accordingly, the present disclosure also provides a vector, or plurality of vectors, comprising the nucleic acid or plurality of nucleic acids according to the present disclosure. The vector may facilitate delivery of the nucleic acid(s) encoding an antigen-binding molecule, polypeptide or CAR according to the present disclosure. The vector may be an expression vector comprising elements required for expressing nucleic acid(s) comprising/encoding an antigen-binding molecule, polypeptide or CAR according to the present disclosure.

Nucleic acids and vectors according to the present disclosure may be provided in purified or isolated form, *i.e.* from other nucleic acid, or naturally-occurring biological material.

The nucleotide sequence may be contained in a vector, *e.g.* an expression vector. A 'vector' as used herein is a nucleic acid molecule used as a vehicle to transfer exogenous nucleic acid into a cell. The vector may be a vector for expression of the nucleic acid in the cell. Such vectors may include a promoter sequence operably linked to the nucleotide sequence encoding the sequence to be expressed. A vector may also include a termination codon and expression enhancers. Any suitable vectors, promoters, enhancers and termination codons known in the art may be used to express a peptide or polypeptide from a vector according to the present disclosure.

The term 'operably linked' may include the situation where a selected nucleic acid sequence and regulatory nucleic acid sequence (*e.g.* promoter and/or enhancer) are covalently linked in such a way as to place the expression of nucleic acid sequence under the influence or control of the regulatory sequence (thereby forming an expression cassette). Thus a regulatory sequence is operably linked to the selected nucleic acid sequence if the regulatory sequence is capable of effecting transcription of the nucleic acid sequence. The resulting transcript(s) may then be translated into a desired peptide(s)/polypeptide(s).

Suitable vectors include plasmids, binary vectors, DNA vectors, mRNA vectors, viral vectors (*e.g.* retroviral vectors, *e.g.* gammaretroviral vectors (*e.g.* murine Leukemia virus (MLV)-derived vectors, *e.g.* SFG vector), lentiviral vectors, adenovirus vectors, adeno-associated virus vectors, vaccinia virus vectors and herpesvirus vectors), transposon-based vectors, and artificial chromosomes (*e.g.* yeast artificial chromosomes), *e.g.* as described in Maus *et al.*, Annu Rev Immunol (2014) 32:189-225 or Morgan and Boyerinas, Biomedicines (2016) 4:9, which are both hereby incorporated by reference in their entirety.

In some embodiments, the vector may be a eukaryotic vector, *e.g.* a vector comprising the elements necessary for expression of protein from the vector in a eukaryotic cell. In some embodiments, the vector may be a mammalian vector, *e.g.* comprising a cytomegalovirus (CMV) or SV40 promoter to drive protein expression.

Constituent polypeptides of an antigen-binding molecule/CAR according to the present disclosure may be encoded by different nucleic acids of the plurality of nucleic acids, or by different vectors of the plurality of vectors.

<u>Producing the antigen-binding molecules and polypeptides</u>

Antigen-binding molecules, polypeptides and CARs according to the present disclosure may be prepared according to methods for the production of polypeptides known to the skilled person.

Antigen-binding molecules, polypeptides and CARs may be prepared by chemical synthesis, *e.g.* liquid or solid phase synthesis. For example, peptides/polypeptides can be synthesised using the methods described in, for example, Chandrudu *et al.*, Molecules (2013), 18: 4373-4388, which is hereby incorporated by reference in its entirety.

Alternatively, antigen-binding molecules, polypeptides and CARs may be produced by recombinant expression. Molecular biology techniques suitable for recombinant production of polypeptides are well known in the art, such as those set out in Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th Edition), Cold Spring Harbor Press, 2012, and in Nat Methods. (2008); 5(2): 135-146 both of which are hereby incorporated by reference in their entirety. Methods for the recombinant production of antigen-binding molecules are also described in Frenzel *et al.*, Front Immunol. (2013); 4: 217 and Kunert and Reinhart, Appl Microbiol Biotechnol. (2016) 100: 3451–3461, both of which are hereby incorporated by reference in their entirety.

In some cases, the antigen-binding molecules and CARs of the present disclosure are comprised of more than one polypeptide chain. In such cases, production of the antigen-binding molecule/CAR may comprise transcription and translation of more than one polypeptide, and subsequent association of the polypeptide chains to form the antigen-binding molecule/CAR.

For recombinant production according to the present disclosure, any cell suitable for the expression of polypeptides may be used. The cell may be a prokaryote or eukaryote. In some embodiments, the cell is a prokaryotic cell, such as a cell of archaea or bacteria. In some embodiments, the bacteria may be Gram-negative bacteria such as bacteria of the family Enterobacteriaceae, for example *Escherichia coli*. In some embodiments, the cell is a eukaryotic cell such as a yeast cell, a plant cell, insect cell or a mammalian cell, *e.g.* a cell described hereinabove.

In some cases, the cell is not a prokaryotic cell because some prokaryotic cells do not allow for the same folding or post-translational modifications as eukaryotic cells. In addition, very high expression levels are possible in eukaryotes and proteins can be easier to purify from eukaryotes using appropriate tags. Specific plasmids may also be utilised which enhance secretion of the protein into the media.

In some embodiments polypeptides may be prepared by cell-free-protein synthesis (CFPS), *e.g.* according to a system described in Zemella *et al.* Chembiochem (2015) 16(17): 2420-2431, which is hereby incorporated by reference in its entirety.

Production may involve culture or fermentation of a eukaryotic cell modified to express the polypeptide(s) of interest. The culture or fermentation may be performed in a bioreactor provided with an appropriate supply of nutrients, air/oxygen and/or growth factors. Secreted proteins can be collected by partitioning culture media/fermentation broth from the cells, extracting the protein content, and separating individual proteins to isolate secreted polypeptide(s). Culture, fermentation and

separation techniques are well known to those of skill in the art, and are described, for example, in Green and Sambrook, Molecular Cloning: A Laboratory Manual (4th Edition; incorporated by reference herein above).

5 Bioreactors include one or more vessels in which cells may be cultured. Culture in the bioreactor may occur continuously, with a continuous flow of reactants into, and a continuous flow of cultured cells from, the reactor. Alternatively, the culture may occur in batches. The bioreactor monitors and controls environmental conditions such as pH, oxygen, flow rates into and out of, and agitation within the vessel such that optimum conditions are provided for the cells being cultured.

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Following culturing the cells that express the polypeptide(s), the polypeptide(s) of interest may be isolated. Any suitable method for separating proteins from cells known in the art may be used. In order to isolate the polypeptide, it may be necessary to separate the cells from nutrient medium. If the polypeptide(s) are secreted from the cells, the cells may be separated by centrifugation from the 15 culture media that contains the secreted polypeptide(s) of interest. If the polypeptide(s) of interest collect within the cell, protein isolation may comprise centrifugation to separate cells from cell culture medium, treatment of the cell pellet with a lysis buffer, and cell disruption *e.g.* by sonification, rapid freeze-thaw or osmotic lysis.

20 It may then be desirable to isolate the polypeptide(s) of interest from the supernatant or culture medium, which may contain other protein and non-protein components. A common approach to separating protein components from a supernatant or culture medium is by precipitation. Proteins of different solubilities are precipitated at different concentrations of precipitating agent such as ammonium sulfate. For example, at low concentrations of precipitating agent, water soluble proteins 25 are extracted. Thus, by adding different increasing concentrations of precipitating agent, proteins of different solubilities may be distinguished. Dialysis may be subsequently used to remove ammonium sulfate from the separated proteins.

Other methods for distinguishing different proteins are known in the art, for example ion exchange 30 chromatography and size chromatography. These may be used as an alternative to precipitation or may be performed subsequently to precipitation.

Once the polypeptide(s) of interest have been isolated from culture it may be desired or necessary to concentrate the polypeptide(s). A number of methods for concentrating proteins are known in the art, 35 such as ultrafiltration or lyophilisation.

Cells comprising/expressing the antigen-binding molecules and polypeptides

The present disclosure also provides a cell comprising or expressing an antigen-binding molecule, polypeptide or CAR according to the present disclosure. Also provided is a cell comprising or 40 expressing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present disclosure.

It will be appreciated that where cells are referred to herein in the singular (*i.e.* ‘a/the cell’), pluralities/populations of such cells are also contemplated.

5 The cell may be a eukaryotic cell, *e.g.* a mammalian cell. The mammal may be a primate (rhesus, cynomolgous, non-human primate or human) or a non-human mammal (*e.g.* rabbit, guinea pig, rat, mouse or other rodent (including any animal in the order Rodentia), cat, dog, pig, sheep, goat, cattle (including cows, *e.g.* dairy cows, or any animal in the order Bos), horse (including any animal in the order Equidae), donkey, and non-human primate).

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In some embodiments, the cell is, or is derived from, a cell type commonly used for the expression of polypeptides for use in therapy in humans. Exemplary cells are described *e.g.* in Kunert and Reinhart, Appl Microbiol Biotechnol. (2016) 100:3451–3461 (hereby incorporated by reference in its entirety), and include *e.g.* CHO, HEK 293, PER.C6, NS0 and BHK cells. In preferred embodiments, the cell is,
15 or is derived from, a CHO cell.

The present disclosure also provides a method for producing a cell comprising a nucleic acid(s) or vector(s) according to the present disclosure, comprising introducing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present disclosure into a cell. In some
20 embodiments, introducing an isolated nucleic acid(s) or vector(s) according to the present disclosure into a cell comprises transformation, transfection, electroporation or transduction (*e.g.* retroviral transduction).

The present disclosure also provides a method for producing a cell expressing/comprising an antigen-
25 binding molecule, polypeptide or CAR according to the present disclosure, comprising introducing a nucleic acid, a plurality of nucleic acids, a vector or a plurality of vectors according to the present disclosure in a cell. In some embodiments, the methods additionally comprise culturing the cell under conditions suitable for expression of the nucleic acid(s) or vector(s) by the cell. In some embodiments, the methods are performed *in vitro*.

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The present disclosure also provides cells obtained or obtainable by the methods according to the present disclosure.

Cells expressing the CARs of the disclosure

35 In some aspects and embodiments, the present disclosure provides a cell comprising a CAR according to the present disclosure. The CAR according to the present disclosure may be used to generate CAR-expressing cells, *e.g.* CAR-expressing immune cells (*e.g.* CAR-T or CAR-NK cells).

CAR-expressing cells may comprise or express nucleic acid encoding a CAR according to the present
40 disclosure. It will be appreciated that a CAR-expressing cell comprises the CAR it expresses. It will

also be appreciated that a cell expressing nucleic acid encoding a CAR also expresses and comprises the CAR encoded by the nucleic acid.

CAR-expressing cell is preferably an immune cell. An immune cell may be a cell of hematopoietic origin, *e.g.* a neutrophil, eosinophil, basophil, dendritic cell, lymphocyte, or monocyte. A lymphocyte may be *e.g.* a T cell, B cell, NK cell, NKT cell or innate lymphoid cell (ILC), or a precursor thereof. The immune cell may express *e.g.* CD3 polypeptides (*e.g.* CD3γ CD3ε CD3ζ or CD3δ), TCR polypeptides (TCRα or TCRβ), CD27, CD28, CD4 or CD8. In some embodiments, the immune cell is a T cell, *e.g.* a CD3+ T cell. In some embodiments, the T cell is a CD3+, CD4+ T cell. In some embodiments, the T cell is a CD3+, CD8+ T cell. In some embodiments, the T cell is a T helper cell (T_H cell). In some embodiments, the T cell is a cytotoxic T cell (*e.g.* a cytotoxic T lymphocyte (CTL)).

Aspects and embodiments of the present disclosure relate particularly to T cells comprising/expressing CD30-specific CARs according to the present disclosure.

In some aspects and embodiments, the immune cell may be a virus-specific immune cell. A 'virus-specific immune cell' as used herein refers to an immune cell which is specific for a virus. A virus-specific immune cell expresses/comprises a receptor (preferably a T cell receptor) capable of recognising a peptide of an antigen of a virus (*e.g.* when presented by an MHC molecule). The virus-specific immune cell may express/comprise such a receptor as a result of expression of endogenous nucleic acid encoding such antigen receptor, or as a result of having been engineered to express such a receptor. The virus-specific immune cell preferably expresses/comprises a TCR specific for a peptide of an antigen of a virus. A virus-specific T cell may display certain functional properties of a T cell in response to the viral antigen for which the T cell is specific, or in response a cell comprising/expressing the virus/antigen. In some embodiments, the properties are functional properties associated with effector T cells, *e.g.* cytotoxic T cells.

In some embodiments, a virus-specific T cell may display one or more of the following properties: cytotoxicity to a cell comprising/expressing the virus /the viral antigen for which the T cell is specific; proliferation, IFNγ expression, CD107a expression, IL-2 expression, TNFα expression, perforin expression, granzyme expression, granulysin expression, and/or FAS ligand (FASL) expression in response to stimulation with the virus/the viral antigen for which the T cell is specific, or in response to exposure to a cell comprising/expressing the virus /the viral antigen for which the T cell is specific.

Virus-specific T cells express/comprise a TCR capable of recognising a peptide of the viral antigen for which the T cell is specific when presented by the appropriate MHC molecule. Virus-specific T cells may be CD4+ T cells and/or CD8+ T cells.

The virus for which the virus-specific immune cell is specific may be any virus. For example, the virus may be a dsDNA virus (*e.g.* adenovirus, herpesvirus, poxvirus), ssRNA virus (*e.g.* parvovirus), dsRNA virus (*e.g.* reovirus), (+)ssRNA virus (*e.g.* picornavirus, togavirus), (-)ssRNA virus (*e.g.*

orthomyxovirus, rhabdovirus), ssRNA-RT virus (*e.g.* retrovirus) or dsDNA-RT virus (*e.g.* hepadnavirus). In particular, the present disclosure contemplates viruses of the families adenoviridae, herpesviridae, papillomaviridae, polyomaviridae, poxviridae, hepadnaviridae, parvoviridae, astroviridae, caliciviridae, picornaviridae, coronaviridae, flaviviridae, togaviridae, hepeviridae, retroviridae, orthomyxoviridae, arenaviridae, bunyaviridae, filoviridae, paramyxoviridae, rhabdoviridae and reoviridae. In some embodiments the virus is selected from Epstein-Barr virus, adenovirus, Herpes simplex type 1 virus, Herpes simplex type 2 virus, Varicella-zoster virus, Human cytomegalovirus, Human herpesvirus type 8, Human papillomavirus, BK virus, JC virus, Smallpox, Hepatitis B virus, Parvovirus B19, Human Astrovirus, Norwalk virus, coxsackievirus, hepatitis A virus, poliovirus, rhinovirus, severe acute respiratory syndrome virus, Hepatitis C virus, yellow fever virus, dengue virus, West Nile virus, TBE virus, Rubella virus, Hepatitis E virus, Human immunodeficiency virus, influenza virus, lassa virus, Crimean-Congo hemorrhagic fever virus, Hantaan virus, ebola virus, Marburg virus, measles virus, mumps virus, parainfluenza virus, picornavirus, respiratory syncytial virus, rabies virus, hepatitis D virus, rotavirus, orbivirus, coltivirus, and banna virus.

In some embodiments, the virus is selected from Epstein-Barr virus (EBV), adenovirus, cytomegalovius (CMV), human papilloma virus (HPV), influenza virus, measles virus, hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), lymphocytic choriomeningitis virus (LCMV), or herpes simplex virus (HSV).

In some embodiments, the virus-specific immune cell may be specific for a peptide/polypeptide of a virus *e.g.* selected from Epstein-Barr virus (EBV), adenovirus, cytomegalovius (CMV), human papilloma virus (HPV), influenza virus, measles virus, hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), lymphocytic choriomeningitis virus (LCMV), or herpes simplex virus (HSV).

A T cell which is specific for an antigen of a virus may be referred to herein as a virus-specific T cell (VST). A T cell which is specific for an antigen of a particular virus may be described as being specific for the relevant virus; for example, a T cell which is specific for an antigen of EBV may be referred to as an EBV-specific T cell, or 'EBVST'.

Accordingly, in some embodiments the virus-specific immune cell is an Epstein-Barr virus-specific T cell (EBVST), adenovirus-specific T cell (AdVST), cytomegalovius-specific T cell (CMVST), human papilloma virus (HPVST), influenza virus-specific T cell, measles virus-specific T cell, hepatitis B virus-specific T cell (HBVST), hepatitis C virus-specific T cell (HCVST), human immunodeficiency virus-specific T cell (HIVST), lymphocytic choriomeningitis virus-specific T cell (LCMVST), or herpes simplex virus-specific T cell (HSVST).

In some preferred embodiments, the virus-specific immune cell is specific for a peptide/polypeptide of an EBV antigen. In preferred embodiments the virus-specific immune cell is an Epstein-Barr virus-specific T cell (EBVST).

EBV virology is described *e.g.* in Stanfield and Luftiq, F1000Res. (2017) 6:386 and Odumade *et al.*, Clin Microbiol Rev (2011) 24(1):193-209, both of which are hereby incorporated by reference in their entirety.

EBV infects epithelial cells via binding of viral protein BMFR2 to β1 integrins, and binding of viral protein gH/gL with integrins avβ6 and avβ8. EBV infects B cells through interaction of viral glycoprotein gp350 with CD21 and/or CD35, followed by interaction of viral gp42 with MHC class II. These interactions trigger fusion of the viral envelope with the cell membrane, allowing the virus to enter the cell. Once inside, the viral capsid dissolves and the viral genome is transported to the nucleus.

EBV has two modes of replication; latent and lytic. The latent cycle does not result in production of virions, and can take place in place B cells and epithelial cells. The EBV genomic circular DNA resides in the cell nucleus as an episome and is copied by the host cell's DNA polymerase. In latency, only a fraction of EBV's genes are expressed, in one of three different patterns known as latency programs, which produce distinct sets of viral proteins and RNAs. The latent cycle is described *e.g.* in Amon and Farrell, Reviews in Medical Virology (2004) 15(3): 149–56, which is hereby incorporated by reference in its entirety.

EBNA1 protein and non-coding RNA EBER are expressed in each of latency programs I-III. Latency programs II and III further involve expression of EBNALP, LMP1, LMP2A and LMP2B proteins, and latency program III further involves expression of EBNA2, EBNA3A, EBNA3B and EBNA3C.

EBNA1 is multifunctional, and has roles in gene regulation, extrachromosomal replication, and maintenance of the EBV episomal genome through positive and negative regulation of viral promoters (Duellman *et al.*, J Gen Virol. (2009); 90(Pt 9): 2251–2259). EBNA2 is involved in the regulation of latent viral transcription and contributes to the immortalisation of cells infected with EBV (Kempkes and Ling, Curr Top Microbiol Immunol. (2015) 391:35-59). EBNA-LP is required for transformation of native B cells, and recruits transcription factors for viral replication (Szymula *et al.*, PLoS Pathog. (2018);14(2):e1006890). EBNA3A, 3B and 3C interact with RBPJ to influence gene expression, contributing to survival and growth of infected cells (Wang *et al.*, J Virol. (2016) 90(6):2906–2919). LMP1 regulates expression of genes involved in B cell activation (Chang *et al.*, J. Biomed. Sci. (2003) 10(5): 490–504). LMP2A and LMP2B inhibit normal B cell signal transduction by mimicking the activated B cell receptor (Portis and Longnecker, Oncogene (2004) 23(53): 8619–8628). EBERs form ribonucleoprotein complexes with host cell proteins, and are proposed to have roles in cell transformation.

The latent cycle can progress according to any of latency programs I to III in B cells, and usually progresses from III to II to I. Upon infection of a resting naïve B cell, EBV enters latency program III. Expression of latency III genes activates the B cell, which becomes a proliferating blast. EBV then

typically progresses to latency II by restricting expression to a subset of genes, which cause differentiation of the blast to a memory B cell. Further restriction of gene expression causes EBV to enter latency I. EBNA1 expression allows EBV to replicate when the memory B cell divides. In epithelial cells, only latency II occurs.

In primary infection, EBV replicates in oropharyngeal epithelial cells and establishes Latency III, II, and I infections in B-lymphocytes. EBV latent infection of B-lymphocytes is necessary for virus persistence, subsequent replication in epithelial cells, and release of infectious virus into saliva. EBV Latency III and II infections of B-lymphocytes, Latency II infection of oral epithelial cells, and Latency II infection of NK- or T cell can result in malignancies, marked by uniform EBV genome presence and gene expression.

Latent EBV in B cells can be reactivated to switch to lytic replication. The lytic cycle results in the production of infectious virions and can take place in place B cells and epithelial cells, and is reviewed *e.g.* by Kenney in Chapter 25 of Arvin *et al.*, Human Herpesviruses: Biology, Therapy and Immunoprophylaxis; Cambridge University Press (2007), which is hereby incorporated by reference in its entirety.

Lytic replication requires the EBV genome to be linear. The latent EBV genome is episomal, and so it must be linearised for lytic reactivation. In B cells, lytic replication normally only takes place after reactivation from latency.

Immediate-early lytic gene products such as BZFL1 and BRLF1 act as transactivators, enhancing their own expression, and the expression of later lytic cycle genes.

Early lytic gene products have roles in viral replication (*e.g.* EBV DNA polymerase catalytic component BALF5; DNA polymerase processivity factor BMRF1, DNA binding protein BALF2, helicase BBLF4, primase BSLF1, and primase-associated protein BBLF2/3) and deoxynucleotide metabolism (*e.g.* thymidine kinase BXLF1, dUTPase BORF2). Other early lytic gene products act transcription factors (*e.g.* BMRF1, BRRF1), have roles in RNA stability and processing (*e.g.* BMLF1), or are involved in immune evasion (*e.g.* BHRF1, which inhibits apoptosis).

Late lytic gene products are traditionally classed as those expressed after the onset of viral replication. They generally encode structural components of the virion such as nucleocapsid proteins, as well as glycoproteins which mediate EBV binding and fusion (*e.g.* gp350/220, gp85, gp42, gp25). Other late lytic gene products have roles in immune evasion; BCLF1 encodes a viral homologue of IL-10, and BALF1 encodes a protein with homology to the anti-apoptotic protein Bcl2.

An ‘EBV-specific immune cell’ as used herein refers to an immune cell which is specific for Epstein-Barr virus (EBV). An EBV-specific immune cell expresses/comprises a receptor (preferably a T cell receptor) capable of recognising a peptide of an antigen of EBV (*e.g.* when presented by an MHC

molecule). The EBV-specific immune cell preferably expresses/comprises a TCR specific for a peptide of an EBV antigen presented by MHC class I.

In some embodiments, the EBV-specific immune cell is a T cell, *e.g.* a CD3+ T cell. In some
5 embodiments, the T cell is a CD3+, CD4+ T cell. In some embodiments, the T cell is a CD3+, CD8+ T cell. In some embodiments, the T cell is a T helper cell (T_H cell)). In some embodiments, the T cell is a cytotoxic T cell (*e.g.* a cytotoxic T lymphocyte (CTL)).

EBV-specific T cells preferably express/comprise a TCR capable of recognising a peptide of the EBV
10 antigen for which the T cell is specific when presented by the appropriate MHC molecule. EBV-specific T cells may be CD4+ T cells and/or CD8+ T cells.

An immune cell specific for EBV may be specific for any EBV antigen, *e.g.* an EBV antigen described herein. A population of immune cell specific for EBV, or a composition comprising a plurality of
15 immune cells specific for EBV, may comprise immune cells specific for one or more EBV antigens.

In some embodiments, an EBV antigen is an EBV latent antigen, *e.g.* a type III latency antigen (*e.g.* EBNA1, EBNA-LP, LMP1, LMP2A, LMP2B, BARF1, EBNA2, EBNA3A, EBNA3B or EBNA3C), a type II latency antigen (*e.g.* EBNA1, EBNA-LP, LMP1, LMP2A, LMP2B or BARF1), or a type I latency
20 antigen, (*e.g.* EBNA1 or BARF1). In some embodiments, an EBV antigen is an EBV lytic antigen, *e.g.* an immediate-early lytic antigen (*e.g.* BZLF1, BRLF1 or BMRF1), an early lytic antigen (*e.g.* BMLF1, BMRF1, BXLF1, BALF1, BALF2, BARF1, BGLF5, BHRF1, BNLF2A, BNLF2B, BHLF1, BLLF2, BKRF4, BMRF2, FU or EBNA1-FUK), or a late lytic antigen (*e.g.* BALF4, BILF1, BILF2, BNFR1, BVRF2, BALF3, BALF5, BDLF3 or gp350).
25

In some embodiments in accordance with the various aspects of the present disclosure, cells may comprise/express more than one (*e.g.* 2, 3, 4, *etc.*) CAR.

In some embodiments, the cells may comprise/express more than one, non-identical CAR. Cells
30 comprising/expressing more than one non-identical CAR may comprise/express CARs specific for non-identical target antigens. In some embodiments, each non-identical target antigen is independently a cancer cell antigen as described herein.

Functional properties of cells expressing the CARs of the disclosure
35 Cells (*e.g.* immune cells, *e.g.* T cells) expressing a CAR according to the present disclosure may display certain functional properties in response to CD30, or in response a cell comprising/expressing CD30. In some embodiments, the properties are functional properties associated with effector T cells, *e.g.* cytotoxic T cells.

40 Cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure may display one or more of the following properties:

expression of one or more cytotoxic/effector factors (*e.g.* IFNγ, TNFα, GM-CSF), proliferation/population expansion, and/or growth factor (*e.g.* IL-2) expression in response to cells expressing CD30;
cytotoxicity to cells expressing CD30;
5 no cytotoxicity (*i.e.* above baseline) to cells which do not express CD30;
anti-cancer activity (*e.g.* cytotoxicity to cancer cells, tumor growth inhibition, reduction of metastasis, *etc.*) against cancer comprising cells expressing CD30;
cytotoxicity to alloreactive immune cells, *e.g.* alloreactive immune cells expressing CD30.

10 In some embodiments, a CD30-specific CAR-expressing T cell may display one or more of the following properties: cytotoxicity to a cell comprising/expressing CD30; proliferation, IFNγ expression, CD107a expression, IL-2 expression, TNFα expression, perforin expression, granzyme expression, granulysin expression, and/or FAS ligand (FASL) expression in response to stimulation with CD30, or in response to exposure to a cell comprising/expressing CD30; anti-cancer activity (*e.g.* cytotoxicity to 15 cancer cells, tumor growth inhibition, reduction of metastasis, *etc.*) against cancer comprising cells expressing CD30.

Cell proliferation/population expansion can be investigated by analysing cell division or the number of cells over a period of time. Cell division can be analysed, for example, by *in vitro* analysis of 20 incorporation of ^{3}H-thymidine or by CFSE dilution assay, *e.g.* as described in Fulcher and Wong, Immunol Cell Biol (1999) 77(6): 559-564, hereby incorporated by reference in entirety. Proliferating cells can also be identified by analysis of incorporation of 5-ethynyl-2′-deoxyuridine (EdU) by an appropriate assay, as described *e.g.* in Buck *et al.*, Biotechniques. 2008 Jun; 44(7):927-9, and Sali and Mitchison, PNAS USA 2008 Feb 19; 105(7): 2415–2420, both hereby incorporated by reference in 25 their entirety.

As used herein, ‘expression’ may be gene or protein expression. Gene expression encompasses transcription of DNA to RNA, and can be measured by various means known to those skilled in the art, for example by measuring levels of mRNA by quantitative real-time PCR (qRT-PCR), or by reporter- 30 based methods. Similarly, protein expression can be measured by various methods well known in the art, *e.g.* by antibody-based methods, for example by western blot, immunohistochemistry, immunocytochemistry, flow cytometry, ELISA, ELISPOT, or reporter-based methods.

Cytotoxicity and cell killing can be investigated, for example, using any of the methods reviewed in 35 Zaritskaya *et al.*, Expert Rev Vaccines (2011), 9(6):601-616, hereby incorporated by reference in its entirety. Examples of *in vitro* assays of cytotoxicity/cell killing assays include release assays such as the ^{51}Cr release assay, the lactate dehydrogenase (LDH) release assay, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) release assay, and the calcein-acetoxymethyl (calcein-AM) release assay. These assays measure cell killing based on the detection of factors released from 40 lysed cells. Cell killing by a given cell type can be analysed *e.g.* by co-culturing the test cells with the

given cell type, and measuring the number/proportion of viable/dead test cells after a suitable period of time.

In some embodiments, cell killing of cells expressing CD30 by CAR-expressing cells may be evaluated by xCELLigence assay as described in Example 1.9 herein. Cell killing by CAR-expressing cells can also be evaluated *in vivo*, *e.g.* by evaluating the number/proportion of cells expressing the target antigen for the CAR, and inferring their killing/depletion by CAR-expressing cells.

Cells may be evaluated for anti-cancer activity by analysis in an appropriate *in vitro* assays or *in vivo* models of the relevant cancer.

Cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure preferably possess novel and/or improved properties compared to cells comprising/comprising nucleic acid encoding a known CD30-specific CAR. Known CD30-specific CARs include *e.g.* CD30-specific CARs described in Hombach *et al.* Cancer Res. (1998) 58(6):1116-9, Hombach *et al.* Gene Therapy (2000) 7:1067-1075, Hombach *et al.* J Immunother. (1999) 22(6):473-80, Hombach *et al.* Cancer Res. (2001) 61:1976-1982, Hombach *et al.* J Immunol (2001) 167:6123-6131, Savoldo *et al.* Blood (2007) 110(7):2620-30, Koehler *et al.* Cancer Res. (2007) 67(5):2265-2273, Di Stasi *et al.* Blood (2009) 113(25):6392-402, Hombach *et al.* Gene Therapy (2010) 17:1206-1213, Chmielewski *et al.* Gene Therapy (2011) 18:62-72, Kofler *et al.* Mol. Ther. (2011) 19(4):760-767, Gilham, Abken and Pule. Trends in Mol. Med. (2012) 18(7):377-384, Chmielewski *et al.* Gene Therapy (2013) 20:177-186, Hombach *et al.* Mol. Ther. (2016) 24(8):1423-1434,
Ramos *et al.* J. Clin. Invest. (2017) 127(9):3462-3471, WO 2015/028444 A1, WO 2016/008973 A1, WO 2021/222927 A1 and WO 2021/222928 A1, all of which are hereby incorporated by reference in their entirety.

In particular, the cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure preferably possess novel and/or improved properties compared to an equivalent CAR construct identical in all respects with the exception that the antigen-binding domain of the CAR comprises the VH and VL sequences of HRS3 (i.e. SEQ ID NOs:1 and 9, respectively) in place of those of the CD30-binding domain of the CAR of the present disclosure. By way of illustration, the CAR construct of SEQ ID NO:149 is identical to that of SEQ ID NO:154, with the exception that the CAR of SEQ ID NO:149 comprises the VH and VL sequences of HRS3, whereas the CAR of SEQ ID NO:154 comprises the VH and VL sequences of VH3VK3.

For conciseness, in the following paragraphs, such reference HRS3 CARs may be referred to simply as ‘an equivalent CAR comprising the VH and VL of HRS3’.

In some embodiments, cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure:

expand and/or proliferate to an extent which is similar to, or greater than, the extent to which cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3 expand/proliferate;

express cytotoxic/effector factors (*e.g.* IFNγ, TNFα, GM-CSF) in response to stimulation with cells expressing CD30 to an extent which is similar to, or greater than, the extent to which cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3 express such factors;

kill cells expressing CD30 with potency/rate which is similar to, or greater than, the potency/rate of killing of such cells by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3;

display off-target cytotoxicity which is similar to, or less than, the off-target cytotoxicity displayed by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3;

induce systemic inflammation to a recipient subject to an extent which is similar to, or less than, the systemic inflammation induced by administration of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3;

display tumor infiltration which is similar to, or greater than, the tumor infiltration displayed by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3;

inhibit tumor growth, *e.g.* of CD30-positive cancer with a potency/to an extent which is similar to, or greater than, tumor growth inhibition by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3;

increase survival of subjects having a cancer*, e.g.* a CD30-positive cancer to an extent which is similar to, or greater than, the extent to which survival is increased by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3;

display persistence *in vivo* which is similar to, or greater than, the *in vivo* persistence of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3.

Cell proliferation and cell population expansion can be measured as described hereinabove. The rate of cell proliferation/population expansion for a given cell type can be determined by analysing the number of such cells over time, *e.g.* at different time points. Cell proliferation/population expansion may be measured *in vitro*, or alternatively *in vivo*, *e.g.* following administration to a subject (*e.g.* a subject having a CD30+ cancer).

In some embodiments, the rate of cell proliferation or population expansion of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure (*e.g. in vitro* or *in vivo*) is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the rate of cell proliferation/population expansion of cells comprising a CAR/nucleic acid encoding an equivalent CAR

comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, the rate of cell proliferation/population expansion of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure (*e.g. in vitro* or *in vivo*) is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the rate of cell proliferation/population expansion of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

In some embodiments, the level of expression of one or more cytotoxic/effector factors (*e.g.* IFNγ, TNFα, GM-CSF) by cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure in response to stimulation with cells expressing CD30 is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level of expression of such factor(s) by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3 in response to stimulation with cells expressing CD30, as determined in a given assay. In some embodiments, the level of expression of one or more cytotoxic/effector factors (*e.g.* IFNγ, TNFα, GM-CSF) by cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure in response to stimulation with cells expressing CD30 is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level of expression of such factor(s) by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3 in response to stimulation with cells expressing CD30, as determined in a given assay.

In some embodiments, the potency or rate of cell killing of cells expressing CD30 by cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure (*e.g. in vitro* or *in vivo*) is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the potency or rate of cell killing of such cells by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, the potency or rate of cell killing of cells expressing CD30 by cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure (*e.g. in vitro* or *in vivo*) is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the potency or rate of cell killing of such cells by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

In some embodiments, the cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure induce cell killing of cells not expressing CD30 to an extent which is similar to or less than the extent to which cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3 induce cell killing of cells not expressing CD30.

In some embodiments, the cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure induce cell killing of cells not expressing CD30 (*e.g. in vitro* or *in vivo*) to a level which is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level of cell killing of such cells by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, the cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure induce cell killing of cells not expressing CD30 (*e.g. in vitro* or *in vivo*) to a level which is less than 1 times, *e.g.* ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the level of cell killing of such cells by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

In some embodiments, cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure induce systemic inflammation (*e.g.* cytokine release syndrome) in a receipt subject of such cells to an extent which is similar to, or less than, that of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3. Induction of systemic inflammation by CAR-expressing cells in a recipient subject can be evaluated by measuring one or more markers of systemic inflammation in a recipient subject following administration of such cells. Markers of systemic inflammation include *e.g.* levels of proinflammatory cytokines (*e.g.* IL-6, IL-8 and TNFα and GM-CSF) in the peripheral blood. In some embodiments, cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure induce the expression of one or more proinflammatory cytokines (*e.g.* selected from IL-6, IL-8 and TNFα and GM-CSF) to an extent which is similar to, or less than, that of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3.

In some embodiments, the cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure induce the expression of one or more proinflammatory cytokines (*e.g.* selected from IL-6, IL-8 and TNFα and GM-CSF) to a level which is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level to which their expression is induced by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, the cells comprising a CAR/nucleic

acid encoding a CAR according to the present disclosure induce the expression of one or more proinflammatory cytokines (*e.g.* selected from IL-6, IL-8 and TNF- α and GM-CSF) to a level which is less than 1 times, *e.g.* ≤0.99 times, ≤0.95 times, ≤0.9 times, ≤0.85 times, ≤0.8 times, ≤0.75 times, ≤0.7 times, ≤0.65 times, ≤0.6 times, ≤0.55 times, ≤0.5 times, ≤0.45 times, ≤0.4 times, ≤0.35 times, ≤0.3 times, ≤0.25 times, ≤0.2 times, ≤0.15 times, ≤0.1 times, ≤0.05 times, or ≤0.01 times the level to which their expression is induced by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

Tumor infiltration by, inhibition of tumor growth by, survival of subjects following treatment with and *in vivo* persistence of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure can be measured *e.g.* in suitable experiments *in vivo*, *e.g.* performed in animal models of CD30+ cancer. For example, these functional properties of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure may be evaluated as described in the experimental examples of the present disclosure, particularly Examples 1.15 and 1.16.

In some embodiments, the cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure display a level of tumor infiltration which is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level of tumor infiltration displayed by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, the cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure display a level of tumor infiltration which is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level of tumor infiltration displayed by cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

In some embodiments, the level of inhibition of tumor growth (*e.g.* of a CD30+ tumor) achieved by cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level achieved by administration of a comparable quantity of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, the level of inhibition of tumor growth (*e.g.* of a CD30+ tumor) achieved by cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level achieved by

administration of a comparable quantity of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

In some embodiments, administration of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure increases survival of recipient subjects having a CD30+ cancer to ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the level of survival achieved by administration of a comparable quantity of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, administration of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure increases survival of recipient subjects having a CD30+ cancer to greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the level of survival achieved by administration of a comparable quantity of cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

In some embodiments, the *in vivo* persistence of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure following administration to a subject (*e.g.* a subject having a cancer, *e.g.* a CD30+ cancer) is ≥ 0.5 times and ≤ 2 times, *e.g.* one of ≥ 0.55 times and ≤ 1.9 times, ≥ 0.6 times and ≤ 1.8 times, ≥ 0.65 times and ≤ 1.7 times, ≥ 0.7 times and ≤ 1.6 times, ≥ 0.75 times and ≤ 1.5 times, ≥ 0.8 times and ≤ 1.4 times, ≥ 0.85 times and ≤ 1.3 times, ≥ 0.9 times and ≤ 1.2 times, ≥ 0.95 times and ≤ 1.1 times the *in vivo* persistence observed for cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay. In some embodiments, the *in vivo* persistence of cells comprising a CAR/nucleic acid encoding a CAR according to the present disclosure following administration to a subject (*e.g.* a subject having a cancer, *e.g.* a CD30+ cancer) is greater than 1 times, *e.g.* one of ≥1.01 times, ≥1.02 times, ≥1.03 times, ≥1.04 times, ≥1.05 times, ≥1.1 times, ≥1.2 times, ≥1.3 times, ≥1.4 times, ≥1.5 times, ≥1.6 times, ≥1.7 times, ≥1.8 times, ≥1.9 times, ≥2 times, ≥3 times, ≥4 times, ≥5 times, ≥6 times, ≥7 times, ≥8 times, ≥9 times or ≥10 times the *in vivo* persistence observed for cells comprising a CAR/nucleic acid encoding an equivalent CAR comprising the VH and VL of HRS3, as determined in a given assay.

Producing cells expressing the CARs of the disclosure

Methods for producing CAR-expressing cells are well known to the skilled person. They generally involve modifying cells (*e.g.* immune cells, *e.g.* T cells or NK cells) to express/comprise a CAR, e.g. introducing nucleic acid encoding a CAR into the immune cells.

Immune cells may be modified to comprise/express a CAR or nucleic acid encoding a CAR described herein according to methods that are well known to the skilled person. The methods generally

comprise nucleic acid transfer for permanent (stable) or transient expression of the transferred nucleic acid.

Any suitable genetic engineering platform may be used to modify a cell according to the present disclosure. Suitable methods for modifying a cell include the use of genetic engineering platforms such as gammaretroviral vectors, lentiviral vectors, adenovirus vectors, DNA transfection, transposon-based gene delivery and RNA transfection, for example as described in Maus *et al.*, Annu Rev Immunol (2014) 32:189-225, hereby incorporated by reference in its entirety.

Methods also include those described *e.g.* in Wang and Rivière Mol Ther Oncolytics. (2016) 3:16015, which is hereby incorporated by reference in its entirety. Suitable methods for introducing nucleic acid(s)/vector(s) into cells include transduction, transfection and electroporation.

Methods for generating/expanding populations of CAR-expressing immune cells *in vitro*/*ex vivo* are well known to the skilled person. Suitable culture conditions (*i.e.* cell culture media, additives, stimulations, temperature, gaseous atmosphere), cell numbers, culture periods and methods for introducing nucleic acid encoding a CAR into cells, *etc.* can be determined by reference *e.g.* to Hombach *et al.* J Immunol (2001) 167:6123-6131, Ramos *et al.* J. Clin. Invest. (2017) 127(9):3462-3471 and WO 2015/028444 A1, all of which are hereby incorporated by reference in their entirety.

Conveniently, cultures of cells according to the present disclosure may be maintained at 37°C in a humidified atmosphere containing 5% CO_2. The cells of cell cultures can be established and/or maintained at any suitable density, as can readily be determined by the skilled person.

Cultures can be performed in any vessel suitable for the volume of the culture, *e.g.* in wells of a cell culture plate, cell culture flasks, a bioreactor, *etc.* In some embodiments cells are cultured in a bioreactor, *e.g.* a bioreactor described in Somerville and Dudley, Oncoimmunology (2012) 1(8):1435-1437, which is hereby incorporated by reference in its entirety. In some embodiments cells are cultured in a GRex cell culture vessel, *e.g.* a GRex flask or a GRex 100 bioreactor.

Immune cells (*e.g.* T cells) may be activated prior to introduction of nucleic acid encoding the CAR. For example, T cells within a population of PBMCs may be non-specifically activated by stimulation *in vitro* with agonist anti-CD3 and agonist anti-CD28 antibodies, in the presence of IL-2.

Introducing nucleic acid(s)/vector(s) into a cell may comprise transduction, *e.g.* retroviral transduction. Accordingly, in some embodiments the nucleic acid(s) is/are comprised in a viral vector(s), or the vector(s) is/are a viral vector(s). Transduction of immune cells with viral vectors is described *e.g.* in Simmons and Alberola-Ila, Methods Mol Biol. (2016) 1323:99-108, which is hereby incorporated by reference in its entirety.

Agents may be employed to enhance the efficiency of transduction. Hexadimethrine bromide (polybrene) is a cationic polymer which is commonly used to improve transduction, through neutralising charge repulsion between virions and sialic acid residues expressed on the cell surface. Other agents commonly used to enhance transduction include *e.g.* the poloxamer-based agents such as LentiBOOST (Sirion Biotech), Retronectin (Takara), Vectofusin (Miltenyi Biotech) and also SureENTRY (Qiagen) and ViraDuctin (Cell Biolabs).

In some embodiments the methods comprise centrifuging the cells into which it is desired to introduce nucleic acid encoding the CAR in the presence of cell culture medium comprising viral vector comprising the nucleic acid (referred to in the art as 'spinfection').

In some embodiments, the methods comprise introducing a nucleic acid or vector according to the present disclosure into an immune cell by electroporation, *e.g.* as described in Koh *et al.*, Molecular Therapy – Nucleic Acids (2013) 2, e114, which is hereby incorporated by reference in its entirety.

The methods generally comprise introducing a nucleic acid encoding a CAR into a cell, and culturing the cell under conditions suitable for expression of the nucleic acid/CAR by the cell. In some embodiments, the methods comprise culturing immune cells into which nucleic acid encoding a CAR has been introduced in order to expand their number. In some embodiments, the methods comprise culturing immune cells into which nucleic acid encoding a CAR has been introduced in the presence of IL-7 and/or IL-15 (e.g. recombinant IL-7 and/or IL-15).

In some embodiments the methods further comprise purifying/isolating CAR-expressing cells, *e.g.* from other cells (*e.g.* cells which do not express the CAR). Methods for purifying/isolating immune cells from heterogeneous populations of cells are well known in the art, and may employ *e.g.* FACS- or MACS-based methods for sorting populations of cells based on the expression of markers of the immune cells. In some embodiments the methods purifying/isolating cells of a particular type, *e.g.* CAR-expressing CD8+ T cells, CAR-expressing CTLs.

In preferred embodiments, CD30-specific CAR-expressing T cells may be generated from T cells within populations of PBMCs by a process comprising: stimulating PBMCs with antagonist anti-CD3 and andti-CD28 antibodies, transducing the cells with a viral vector (*e.g.* a gamma-retroviral vector) encoding the CD30-specific CAR, and subsequently culturing the cells in the presence of IL-7 and IL-15.

Aspects and embodiments of the present disclosure relate particularly to EBV-specific immune cells. Methods for generating/expanding populations of EBV-specific immune cells are described *e.g.* in WO 2013/088114 A1, Lapteva and Vera, Stem Cells Int. (2011): 434392, Straathof *et al.*, Blood (2005) 105(5): 1898–1904, WO 2017/202478 A1, WO 2018/052947 A1 and WO 2020/214479 A1, all of which are hereby incorporated by reference in their entirety. The methods typically comprise stimulating immune cells specific for a virus/viral antigen by contacting populations of immune cells with

peptide(s) corresponding to EBV antigen(s) or APCs presenting peptide(s) corresponding to viral antigen(s).

The present disclosure also provides CAR-expressing cells obtained or obtainable by the methods according to the present disclosure.

Compositions

The present disclosure also provides compositions comprising the antigen-binding molecules, polypeptides, CARs, nucleic acids, expression vectors and cells described herein.

The antigen-binding molecules, polypeptides, CARs, nucleic acids, expression vectors and cells described herein may be formulated as pharmaceutical compositions or medicaments for clinical use and may comprise a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.
The compositions of the present disclosure may comprise one or more pharmaceutically-acceptable carriers (*e.g.* liposomes, micelles, microspheres, nanoparticles), diluents/excipients (*e.g.* starch, cellulose, a cellulose derivative, a polyol, dextrose, maltodextrin, magnesium stearate), adjuvants, fillers, buffers, preservatives (*e.g.* vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium, cysteine, methionine, citric acid, sodium citrate, methyl paraben, propyl paraben), anti-oxidants (*e.g.* vitamin A, vitamin E, vitamin C, retinyl palmitate, selenium), lubricants (*e.g.* magnesium stearate, talc, silica, stearic acid, vegetable stearin), binders (*e.g.* sucrose, lactose, starch, cellulose, gelatin, polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), xylitol, sorbitol, mannitol), stabilisers, solubilisers, surfactants (*e.g.*, wetting agents), masking agents or colouring agents (*e.g.* titanium oxide).

The term ‘pharmaceutically-acceptable’ as used herein pertains to compounds, ingredients, materials, compositions, dosage forms, *etc.*, which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of the subject in question (*e.g.* a human subject) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, diluent, excipient, adjuvant, filler, buffer, preservative, anti-oxidant, lubricant, binder, stabiliser, solubiliser, surfactant, masking agent, colouring agent, flavouring agent or sweetening agent of a composition according to the present disclosure must also be ‘acceptable’ in the sense of being compatible with the other ingredients of the formulation. Suitable carriers, diluents, excipients, adjuvants, fillers, buffers, preservatives, anti-oxidants, lubricants, binders, stabilisers, solubilisers, surfactants, masking agents, colouring agents, flavouring agents or sweetening agents can be found in standard pharmaceutical texts, for example, Remington’s ‘The Science and Practice of Pharmacy’ (Ed. A. Adejare), 23rd Edition (2020), Academic Press.

Compositions may be formulated for topical, parenteral, systemic, intracavitary, intravenous, intra-arterial, intramuscular, intrathecal, intraocular, intraconjunctival, intratumoral, subcutaneous, intradermal, intrathecal, oral or transdermal routes of administration. In some embodiments, a pharmaceutical composition/medicament may be formulated for administration by injection or infusion, or administration by ingestion.

Suitable formulations may comprise the relevant article in a sterile or isotonic medium. Medicaments and pharmaceutical compositions may be formulated in fluid, including gel, form. Fluid formulations may be formulated for administration by injection or infusion (*e.g.* via catheter) to a selected region of the human or animal body.

In some embodiments, the composition is formulated for injection or infusion, *e.g.* into a blood vessel, tissue/organ of interest, or tumor.

The present disclosure also provides methods for the production of pharmaceutically useful compositions, such methods of production may comprise one or more steps selected from: producing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein; isolating an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein; and/or mixing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

For example, a further aspect the present disclosure relates to a method of formulating or producing a medicament or pharmaceutical composition for use in the treatment of a disease/condition (*e.g.* a cancer), the method comprising formulating a pharmaceutical composition or medicament by mixing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof) or cell described herein with a pharmaceutically acceptable carrier, adjuvant, excipient or diluent.

Therapeutic and prophylactic applications

The antigen-binding molecules, polypeptides, CARs, nucleic acids, expression vectors, cells and compositions described herein find use in therapeutic and prophylactic methods.

The present disclosure provides an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein for use in a method of medical treatment or prophylaxis. Also provided is an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein for use in a method of treating or preventing a disease or condition described herein. Also provided is the use of an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein in the manufacture of a medicament for treating or preventing a disease or condition described herein. Also provided is a method of treating or preventing a disease or condition described herein, comprising administering to a subject a therapeutically or prophylactically effective amount of an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein.

The methods may be effective to reduce the development or progression of a disease/condition, alleviation of the symptoms of a disease/condition or reduction in the pathology of a disease/condition. The methods may be effective to prevent progression of the disease/condition, *e.g.* to prevent worsening of, or to slow the rate of development of, the disease/condition. In some embodiments, the methods may lead to an improvement in the disease/condition, *e.g.* a reduction in the symptoms of the disease/condition or reduction in some other correlate of the severity/activity of the disease/condition. In some embodiments, the methods may prevent development of the disease/condition a later stage (*e.g.* a chronic stage or metastasis).

It will be appreciated that the articles of the present disclosure may be used for the treatment/prevention of any disease/condition that would derive therapeutic or prophylactic benefit from a reduction in the level/activity of CD30, or a reduction in the number or activity of cells comprising/expressing CD30.

For example, the disease/condition may be a disease/condition in which CD30, or cells comprising/expressing CD30 are pathologically-implicated, *e.g.* a disease/condition in which an increased level/activity of CD30, or an increase in the number/proportion of cells comprising/expressing CD30 is positively associated with the onset, development or progression of the disease/condition, and/or severity of one or more symptoms of the disease/condition. In some embodiments, an increased level/activity of CD30, or an increase in the number/proportion of cells comprising/expressing CD30 may be a risk factor for the onset, development or progression of the disease/condition.

In some embodiments, the disease/condition to be treated/prevented in accordance with the present disclosure is a disease/condition characterised by an increase in the level of expression or activity of CD30, *e.g.* as compared to the level of expression/activity in the absence of the disease/condition. In some embodiments, the disease/condition to be treated/prevented is a disease/condition characterised by an increase in the number/proportion/activity of cells expressing CD30, *e.g.* as compared to the level/number/proportion/activity in the absence of the disease/condition (*e.g.* in a healthy subject, or in equivalent non-diseased tissue). Where the disease/condition is a cancer, the level of expression or activity of CD30 may be greater than the level of expression or activity of CD30 in equivalent non-cancerous cells/non-tumor tissue. A cancer/cell thereof may comprise one or more mutations (*e.g.* relative to equivalent non-cancerous cells/non-tumor tissue) causing upregulation of expression or activity of CD30.

Treatment in accordance with the methods of the present disclosure may achieve one or more of the following in a subject (compared to an equivalent untreated subject, or subject treated with an appropriate control): a reduction in the level of CD30; a reduction in the activity of CD30; and/or a reduction in the number/proportion of cells comprising/expressing CD30.

The biology of CD30 and intervention targeting CD30 for the treatment and prevention of disease is reviewed *e.g.* in van der Weyden *et al.*, Blood Cancer Journal (2017) 7:e603 and Muta and Podack, Immunol Res (2013), 57(1-3):151-8, both of which are hereby incorporated by reference in their entirety.

In aspects and embodiments according to the present disclosure, cells (particularly immune cells, more particularly T cells) comprising/expressing a CAR according to the present disclosure are provided for therapeutic and prophylactic use. It will be appreciated that the methods generally comprise administering a population of immune cells expressing a CAR according to the present disclosure to a subject. In some embodiments, immune cells expressing a CAR according to the present disclosure may be administered in the form of a pharmaceutical composition comprising such cells.

In particular, use of immune cells expressing a CAR according to the present disclosure in methods to treat/prevent diseases/conditions by adoptive cell transfer (ACT) is contemplated.

Adoptive cell transfer generally refers to a process by which cells (*e.g.* immune cells) are obtained from a subject, typically by drawing a blood sample from which the cells are isolated. The cells are then typically modified and/or expanded, and then administered either to the same subject (in the case of adoptive transfer of autologous/autogeneic cells) or to a different subject (in the case of adoptive transfer of allogeneic cells). The treatment is typically aimed at providing a population of cells with certain desired characteristics to a subject, or increasing the frequency of such cells with such characteristics in that subject. Adoptive transfer may be performed with the aim of introducing a cell or population of cells into a subject, and/or increasing the frequency of a cell or population of cells in a subject.

Adoptive transfer of immune cells is described, for example, in Kalos and June (2013), Immunity 39(1): 49-60, and Davis *et al.* (2015), Cancer J. 21(6): 486–491, both of which are hereby incorporated by reference in their entirety. The skilled person is able to determine appropriate reagents and procedures for adoptive transfer of cells according to the present disclosure, for example by reference to Dai *et al.*, 2016 J Nat Cancer Inst 108(7): djv439, which is incorporated by reference in its entirety.

The utility of immune cells expressing CD30-specific CARs in the treatment/prevention of cancer is described *e.g.* in Hombach *et al.* Cancer Res. (1998) 58(6):1116-9, Hombach *et al.* Gene Therapy (2000) 7:1067-1075, Hombach *et al.* J Immunother. (1999) 22(6):473-80, Hombach *et al.* Cancer Res. (2001) 61:1976-1982, Hombach *et al.* J Immunol (2001) 167:6123-6131, Savoldo *et al.* Blood (2007) 110(7):2620-30, Koehler *et al.* Cancer Res. (2007) 67(5):2265-2273, Di Stasi *et al.* Blood (2009) 113(25):6392-402, Hombach *et al.* Gene Therapy (2010) 17:1206-1213, Chmielewski *et al.* Gene Therapy (2011) 18:62-72, Kofler *et al.* Mol. Ther. (2011) 19(4):760-767, Gilham, Abken and Pule. Trends in Mol. Med. (2012) 18(7):377-384, Chmielewski *et al.* Gene Therapy (2013) 20:177-186, Hombach *et al.* Mol. Ther. (2016) 24(8):1423-1434, Ramos *et al.* J. Clin. Invest. (2017) 127(9):3462-

3471, WO 2015/028444 A1, WO 2016/008973 A1, WO 2021/222927 A1 and WO 2021/222928 A1, incorporated by reference hereinabove.

The immune cells expressing a CAR according to the present disclosure may be employed in the
5 treatment/prevention of diseases/conditions by allotransplantation or autotransplantation.

As used herein, 'allotransplantation' refers to the transplantation to a recipient subject of cells, tissues or organs which are genetically non-identical to the recipient subject. The cells, tissues or organs may be from, or may be derived from, cells, tissues or organs of a donor subject that is genetically non-
10 identical to the recipient subject. Allotransplantation is distinct from autotransplantation, which refers to the transplantation of cells, tissues or organs which are from/derived from a donor subject genetically identical to the recipient subject (*i.e.* autologous material). It will be appreciated that adoptive transfer of allogeneic immune cells is a form of allotransplantation, and that adoptive transfer of autologous immune cells is a form of autotransplantation.
15

The present disclosure provides methods comprising administering immune cells comprising/expressing a CAR according to the present disclosure, or immune cells comprising/expressing nucleic acid encoding a CAR according to the present disclosure, to a subject.

20 In some embodiments, the methods comprise modifying an immune cell to comprise/express a CAR according to the present disclosure. In some embodiments, the methods comprise modifying an immune cell specific for a virus to comprise/express nucleic acid encoding a CAR according to the present disclosure.

25 In some embodiments, the methods comprise:

(a) modifying an immune cell to express or comprise a CAR according to the present disclosure, or to express or comprise nucleic acid encoding a CAR according to the present disclosure, and

(b) administering the immune cell specific for a virus modified to express or comprise a CAR
30 according to the present disclosure, or modified to express or comprise a nucleic acid encoding a CAR according to the present disclosure, to a subject.

In some embodiments, the methods comprise:

(a) isolating or obtaining immune cells;

35 (b) modifying an immune cell to express or comprise a CAR according to the present disclosure, or to express or comprise nucleic acid encoding a CAR according to the present disclosure, and

(c) administering the immune cell modified to express or comprise a CAR according to the present disclosure, or modified to express or comprise a nucleic acid encoding a CAR
40 according to the present disclosure, to a subject.

In some embodiments, the methods comprise:

(a) isolating immune cells (*e.g.* PBMCs) from a subject;

(b) generating/expanding a population of immune cells specific for a virus;

5 (c) modifying an immune cell specific for a virus to express or comprise a CAR according to the present disclosure, or to express or comprise nucleic acid encoding a CAR according to the present disclosure, and

(d) administering the immune cell specific for a virus modified to express or comprise a CAR according to the present disclosure, or modified to express or comprise a nucleic acid

10 encoding a CAR according to the present disclosure, to a subject.

In some embodiments, the methods comprise administering to a subject an EBV-specific immune cell modified to express or comprise a CD30-specific CAR according to the present disclosure, or modified to express or comprise a nucleic acid encoding a CD30-specific CAR according to the present

15 disclosure.

In some embodiments, the subject from which the immune cells (*e.g.* PBMCs) are isolated is the same subject to which cells are administered (*i.e.*, adoptive transfer may be of autologous/autogeneic cells). In some embodiments, the subject from which the immune cells (*e.g.* PBMCs) are isolated is a

20 different subject to the subject to which cells are administered (*i.e.*, adoptive transfer may be of allogeneic cells).

In some embodiments the methods may comprise one or more of:

obtaining a blood sample from a subject;

25 isolating immune cells (*e.g.* PBMCs) from a blood sample which has been obtained from a subject;

generating/expanding a population of immune cells;

culturing the immune cells in *in vitro* or *ex vivo* cell culture;

modifying an immune cell to express or comprise a CAR according to the present disclosure,

30 or to express or comprise a nucleic acid encoding a CAR according to the present disclosure (*e.g.* by transduction with a viral vector encoding such CAR, or a viral vector comprising such nucleic acid);

culturing immune cells expressing/comprising a CAR according to the present disclosure, or expressing/comprising a nucleic acid encoding a CAR according to the present disclosure in *in vitro* or *ex vivo* cell culture;

35 collecting/isolating immune cells expressing/comprising a CAR according to the present disclosure, or expressing/comprising a nucleic acid encoding a CAR according to the present disclosure;

formulating immune cells expressing/comprising a CAR according to the present disclosure, or a nucleic acid encoding a CAR according to the present disclosure to a pharmaceutical

40 composition, *e.g.* by mixing the cells with a pharmaceutically acceptable adjuvant, diluent, or carrier;

administering immune cells expressing/comprising a CAR according to the present disclosure, or expressing/comprising a nucleic acid encoding a CAR according to the present disclosure, or a pharmaceutical composition comprising such cells, to a subject.

5 In some embodiments, the methods may additionally comprise treating the cells or subject to induce/enhance expression of CAR and/or to induce/enhance proliferation or survival of virus-specific immune cells comprising/expressing the CAR.

Cancer

10 In some embodiments, the disease to be treated/prevented in accordance with the present disclosure is a cancer.

Cancer may refer to any unwanted cell proliferation (or any disease manifesting itself by unwanted cell proliferation), neoplasm or tumor. The cancer may be benign or malignant and may be primary or
15 secondary (metastatic). A neoplasm or tumor may be any abnormal growth or proliferation of cells and may be located in any tissue. The cancer may be of tissues/cells derived from *e.g.* the adrenal gland, adrenal medulla, anus, appendix, bladder, blood, bone, bone marrow, brain, breast, cecum, central nervous system (including or excluding the brain) cerebellum, cervix, colon, duodenum, endometrium, epithelial cells (*e.g.* renal epithelia), gallbladder, oesophagus, glial cells, heart, ileum, jejunum, kidney,
20 lacrimal glad, larynx, liver, lung, lymph, lymph node, lymphoblast, maxilla, mediastinum, mesentery, myometrium, nasopharynx, omentum, oral cavity, ovary, pancreas, parotid gland, peripheral nervous system, peritoneum, pleura, prostate, salivary gland, sigmoid colon, skin, small intestine, soft tissues, spleen, stomach, testis, thymus, thyroid gland, tongue, tonsil, trachea, uterus, vulva, and/or white blood cells.

25

Tumors may be nervous or non-nervous system tumors. Nervous system tumors may originate either in the central or peripheral nervous system, *e.g.* glioma, medulloblastoma, meningioma, neurofibroma, ependymoma, Schwannoma, neurofibrosarcoma, astrocytoma and oligodendroglioma. Non-nervous system cancers/tumors may originate in any other non-nervous tissue, examples include melanoma,
30 mesothelioma, lymphoma, myeloma, leukemia, Non-Hodgkin's lymphoma (NHL), Hodgkin's lymphoma, chronic myelogenous leukemia (CML), acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), cutaneous T cell lymphoma (CTCL), chronic lymphocytic leukemia (CLL), hepatoma, epidermoid carcinoma, prostate carcinoma, breast cancer, lung cancer , colon cancer, ovarian cancer, pancreatic cancer, thymic carcinoma, NSCLC, hematologic cancer and sarcoma.

35

In some embodiments the cancer is selected from the group consisting of: a solid cancer, a hematological cancer, gastric cancer (*e.g.* gastric carcinoma, gastric adenocarcinoma, gastrointestinal adenocarcinoma), liver cancer (hepatocellular carcinoma, cholangiocarcinoma), head and neck cancer (*e.g.* head and neck squamous cell carcinoma), oral cavity cancer (*e.g.* oropharyngeal cancer (*e.g.*
40 oropharyngeal carcinoma), oral cancer, laryngeal cancer, nasopharyngeal carcinoma, oesophageal cancer), colorectal cancer (*e.g.* colorectal carcinoma), colon cancer, colon carcinoma, cervical

carcinoma, prostate cancer, lung cancer (*e.g.* NSCLC, small cell lung cancer, lung adenocarcinoma, squamous lung cell carcinoma), bladder cancer, urothelial carcinoma, skin cancer (*e.g.* melanoma, advanced melanoma), renal cell cancer (*e.g.* renal cell carcinoma), ovarian cancer (*e.g.* ovarian carcinoma), mesothelioma, breast cancer, brain cancer (*e.g.* glioblastoma), prostate cancer,
5 pancreatic cancer, a myeloid hematologic malignancy, a lymphoblastic hematologic malignancy, myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), lymphoma, non-Hodgkin's lymphoma (NHL), thymoma or multiple myeloma (MM).

10 In some embodiments the cancer is a cancer in which CD30 is pathologically implicated. That is, in some embodiments the cancer is a cancer which is caused or exacerbated by the expression of CD30, a cancer for which expression of CD30 is a risk factor and/or a cancer for which expression of CD30 is positively associated with onset, development, progression, severity or metastasis of the cancer. The cancer may be characterised by expression of CD30, *e.g.* the cancer may comprise cells
15 expressing CD30. Such cancers may be referred to as being positive for CD30. A cancer which is 'positive' for the CD30 may be a cancer comprising cells expressing CD30 (*e.g.* at the cell surface). A cancer which is 'positive' for CD30 may overexpress CD30.

CD30-positive cancers are described *e.g.* in van der Weyden *et al.*, Blood Cancer Journal (2017)
20 7:e603 and Muta and Podack, Immunol Res (2013), 57(1-3):151-8. CD30 is expressed on small subsets of activated T and B lymphocytes, and by various lymphoid neoplasms including classical Hodgkin's lymphoma and anaplastic large cell lymphoma. Variable expression of CD30 has also been shown for peripheral T cell lymphoma, not otherwise specified (PTCL-NOS), adult T cell leukemia/lymphoma, cutaneous T cell lymphoma (CTCL), extra-nodal NK-T cell lymphoma, various B
25 cell non-Hodgkin's lymphomas (including diffuse large B cell lymphoma, particularly EBV-positive diffuse large B cell lymphoma), and advanced systemic mastocytosis. CD30 expression has also been observed in some non-hematopoietic malignancies, including germ cell tumors and testicular embryonal carcinomas.

30 The transmembrane glycoprotein CD30, is a member of the tumor necrosis factor receptor superfamily (Falini et al., Blood (1995) 85(1):1-14). Members of the TNF/TNF-receptor (TNF-R) superfamily coordinate the immune response at multiple levels and CD30 plays a role in regulating the function or proliferation of normal lymphoid cells. CD30 was originally described as an antigen recognized by a monoclonal antibody, Ki-1, which was raised by immunizing mice with a HL-derived cell line, L428
35 (Muta and Podack, Immunol Res (2013) 57: 151-158). CD30 antigen expression has been used to identify ALCL and Reed-Sternberg cells in Hodgkin's disease (Falini et al., Blood (1995) 85(1):1-14). With the wide expression in the lymphoma malignant cells, CD30 is therefore a potential target for developing both antibody-based immunotherapy and cellular therapies. Importantly, CD30 is not typically expressed on normal tissues under physiologic conditions, thus is notably absent on resting
40 mature or precursor B or T cells (Younes and Ansell, Semin Hematol (2016) 53: 186-189). Brentuximab vedotin, an antibody-drug conjugate that targets CD30 was initially approved for the

treatment of CD30-positive HL (Adcetris® US Package Insert 2018). Data from brentuximab vedotin trials support CD30 as a therapeutic target for the treatment of CD30-positive lymphoma.

Hodgkin lymphoma (HL) is an uncommon malignancy involving lymph nodes and the lymphatic
5 system. The incidence of HL is bimodal with most patients diagnosed between 15 and 30 years of age, followed by another peak in adults aged 55 years or older. In 2019 it is estimated there will be 8,110 new cases (3,540 in females and 4570 in males) in the United States and 1,000 deaths (410 female and 590 males) from this disease (American Cancer Society 2019). Based on 2012-2016 cases in National Cancer Institute's SEER database, the incidence rate for HL for the pediatric HL
10 patients in US is as follows: Age 1-4: 0.1; Age 5-9: 0.3; Age 10-14: 1.3; Age 15-19: 3.3 per 100,000 (SEER Cancer Statistics Review, 1975-2016]). The World Health Organization (WHO) classification divides HL into 2 main types: classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). In Western countries, cHL accounts for 95% and NLPHL accounts for 5% of all HL (National Comprehensive Cancer Network Guidelines 2019).

15

First-line chemotherapy for cHL patients with advanced disease is associated with cure rates between 70% and 75% (Karantanos et al., Blood Lymphat Cancer (2017) 7:37-52). Salvage chemotherapy followed by Autologous Stem Cell Transplant (ASCT) is commonly used in patients who relapse after primary therapy. Unfortunately, up to 50% of the cHL patients experience disease recurrence after
20 ASCT. The median overall survival of patients who relapse after ASCT is approximately two years (Alinari Blood (2016) 127:287-295). Despite aggressive combination chemotherapy, between 10% and 40% of patients do not achieve a response to salvage chemotherapy and there are no randomized clinical trial data supporting ASCT in non-responders. For patients who do not respond to salvage chemotherapy, relapse after ASCT or who are not candidates for this approach, the prognosis
25 continues to be grave and new treatment approaches are urgently needed (Keudell British Journal of Haematology (2019) 184:105–112).

While a majority of the pediatric population (children, adolescents, and young adults) will be cured with currently available therapy, a small fraction of patients may have refractory or relapsed disease and
30 require novel therapies that have an acceptable safety profile with improved efficacy benefit (Flerlage et al., Blood (2018) 132: 376-384; Kelly, Blood (2015) 126: 2452-2458; McClain and Kamdar, in UpToDate 2019; Moskowitz, ASCO Educational Book (2019) 477-486). HL patients treated with high dose chemotherapy during childhood commonly experience treatment-related long-term sequelae, such as cardiac, pulmonary, gonadal, and endocrine toxicity as well as second malignant neoplasms
35 (Castellino et al., Blood (2011) 117(6): 1806-1816).

In some embodiments, the cancer to be treated/prevented is an EBV-associated cancer. EBV infection is implicated in several cancers, as reviewed *e.g.* in Jha *et al.*, Front Microbiol. (2016) 7:1602, which is hereby incorporated by reference in its entirety. In some embodiments, the cancer is a cancer which is
40 caused or exacerbated by infection with EBV, a cancer for which infection with EBV is a risk factor and/or a cancer for which infection with EBV is positively associated with onset, development,

progression, severity or metastasis of the cancer. The cancer may be characterised by EBV infection, *e.g.* the cancer may comprise cells infected with EBV. Such cancers may be referred to as EBV-positive cancers.

5 EBV-associated cancers which may be treated/prevented in accordance with the present disclosure include B cell-associated cancers such as Burkitt's lymphoma, post-transplant lymphoproliferative disease (PTLD), central nervous system lymphoma (CNS lymphoma), Hodgkin's lymphoma, non-Hodgkin's lymphoma, and EBV-associated lymphomas associated with immunodeficiency (including *e.g.* EBV-positive lymphoma associated with X-linked lymphoproliferative disorder, EBV-positive
10 lymphoma associated with HIV infection/AIDS, and oral hairy leukoplakia), and epithelial cell-related cancers such as nasopharyngeal carcinoma (NPC) and gastric carcinoma (GC). In some embodiments, the cancer is selected from lymphoma (*e.g.* EBV-positive lymphoma), head and neck squamous cell carcinoma (HNSCC; *e.g.* EBV-positive HNSCC), nasopharyngeal carcinoma (NPC; *e.g.* EBV-positive NPC), and gastric carcinoma (GC; *e.g.* EBV-positive GC).
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In some embodiments, a CD30-positive cancer may be selected from: a solid cancer, a hematological cancer, a hematopoietic malignancy, Hodgkin's lymphoma (HL), anaplastic large cell lymphoma (ALCL), ALK-positive anaplastic T cell lymphoma, ALK-negative anaplastic T cell lymphoma, peripheral T cell lymphoma (*e.g.* PTCL-NOS), T cell leukemia, T cell lymphoma, cutaneous T cell
20 lymphoma (CTCL), NK-T cell lymphoma (*e.g.* extra-nodal NK-T cell lymphoma), non-Hodgkin's lymphoma (NHL), B cell non-Hodgkin's lymphoma, diffuse large B cell lymphoma (*e.g.* diffuse large B cell lymphoma-NOS), primary mediastinal B cell lymphoma, EBV-positive B cell lymphoma, EBV-positive diffuse large B cell lymphoma, advanced systemic mastocytosis, a germ cell tumor and testicular embryonal carcinoma.
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In some embodiments, the cancer is selected from: a CD30-positive cancer, an EBV-associated cancer, a hematological cancer, a myeloid hematologic malignancy, a hematopoietic malignancy a lymphoblastic hematologic malignancy, myelodysplastic syndrome, leukemia, T cell leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, lymphoma, Hodgkin's
30 lymphoma, non-Hodgkin's lymphoma, B cell non-Hodgkin's lymphoma, diffuse large B cell lymphoma, primary mediastinal B cell lymphoma, EBV-associated lymphoma, EBV-positive B cell lymphoma, EBV-positive diffuse large B cell lymphoma, EBV-positive lymphoma associated with X-linked lymphoproliferative disorder, EBV-positive lymphoma associated with HIV infection/AIDS, oral hairy leukoplakia, Burkitt's lymphoma, post-transplant lymphoproliferative disease, central nervous system
35 lymphoma, anaplastic large cell lymphoma, T cell lymphoma, ALK-positive anaplastic T cell lymphoma, ALK-negative anaplastic T cell lymphoma, peripheral T cell lymphoma, cutaneous T cell lymphoma, NK-T cell lymphoma, extra-nodal NK-T cell lymphoma, thymoma, multiple myeloma, a solid cancer, epithelial cell cancer, gastric cancer, gastric carcinoma, gastric adenocarcinoma, gastrointestinal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, head
40 and neck cancer, head and neck squamous cell carcinoma, oral cavity cancer, oropharyngeal cancer, oropharyngeal carcinoma, oral cancer, laryngeal cancer, nasopharyngeal carcinoma, oesophageal

cancer, colorectal cancer, colorectal carcinoma, colon cancer, colon carcinoma, cervical carcinoma, prostate cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, lung adenocarcinoma, squamous lung cell carcinoma, bladder cancer, urothelial carcinoma, skin cancer, melanoma, advanced melanoma, renal cell cancer, renal cell carcinoma, ovarian cancer, ovarian carcinoma, mesothelioma, breast cancer, brain cancer, glioblastoma, prostate cancer, pancreatic cancer, mastocytosis, advanced systemic mastocytosis, germ cell tumor or testicular embryonal carcinoma.

In some embodiments, the cancer may be a relapsed cancer. As used herein, a 'relapsed' cancer refers to a cancer which responded to a treatment (*e.g.* a first line therapy for the cancer), but which has subsequently re-emerged/progressed, *e.g.* after a period of remission. For example, a relapsed cancer may be a cancer whose growth/progression was inhibited by a treatment (*e.g.* a first line therapy for the cancer), and which has subsequently grown/progressed.

In some embodiments, the cancer may be a refractory cancer. As used herein, a 'refractory' cancer refers to a cancer which has not responded to a treatment (*e.g.* a first line therapy for the cancer). For example, a refractory cancer may be a cancer whose growth/progression was not inhibited by a treatment (*e.g.* a first line therapy for the cancer). In some embodiments a refractory cancer may be a cancer for which a subject receiving treatment for the cancer did not display a partial or complete response to the treatment.

In embodiments where the cancer is anaplastic large cell lymphoma, the cancer may be relapsed or refractory with respect to treatment with chemotherapy, brentuximab vedotin, or crizotinib. In embodiments where the cancer is peripheral T cell lymphoma, the cancer may be relapsed or refractory with respect to treatment with chemotherapy or brentuximab vedotin. In embodiments where the cancer is extranodal NK-T cell lymphoma, the cancer may be relapsed or refractory with respect to treatment with chemotherapy (with or without asparaginase) or brentuximab vedotin. In embodiments where the cancer is diffuse large B cell lymphoma, the cancer may be relapsed or refractory with respect to treatment with chemotherapy (with or without rituximab) or CD19 CAR-T therapy. In embodiments where the cancer is primary mediastinal B cell lymphoma, the cancer may be relapsed or refractory with respect to treatment with chemotherapy, immune checkpoint inhibitor (*e.g.* PD-1 inhibitor) or CD19 CAR-T therapy.

Treatment of a cancer in accordance with the methods of the present disclosure achieves one or more of the following treatment effects: reduces the number of cancer cells in the subject, reduces the size of a cancerous tumor/lesion in the subject, inhibits (*e.g.* prevents or slows) growth of cancer cells in the subject, inhibits (*e.g.* prevents or slows) growth of a cancerous tumor/lesion in the subject, inhibits (*e.g.* prevents or slows) the development/progression of a cancer (*e.g.* to a later stage, or metastasis), reduces the severity of symptoms of a cancer in the subject, increases survival of the subject (*e.g.* progression free survival or overall survival), reduces a correlate of the number or activity of cancer cells in the subject, and/or reduces cancer burden in the subject.

Subjects may be evaluated in accordance with the Revised Criteria for Response Assessment: The Lugano Classification (described *e.g.* in Cheson et al., J Clin Oncol (2014) 32: 3059-3068, incorporated by reference hereinabove) in order to determine their response to treatment. In some embodiments, treatment of a subject in accordance with the methods of the present disclosure achieves one of the following: complete response, partial response, or stable disease.

<u>Applications relating to treatment/prevention of alloreactive immune responses</u>

The CAR-expressing immune cells and compositions of the present disclosure can be used in methods involving allotransplantation, *e.g.* to treat/prevent a disease/condition in a subject. The CAR-expressing immune cells and compositions of the present disclosure are useful in methods to reduce/prevent alloreactive immune responses (particularly T cell-mediated alloreactive immune responses) and the deleterious consequences thereof.

Alloreactive T cells express CD30. Chan *et al.*, J Immunol (2002) 169(4):1784-91 identify CD30-expressing T cells as a subset of activated T cells (also expressing CD25 and CD45RO) having an important role in CD30 alloimmune responses. CD30 expression and the proliferation of CD30-expressing T cells increases in response to alloantigen. Chen *et al.*, Blood (2012) 120(3):691-6 identifies CD30 expression on CD8+ T cell subsets as a potential biomarker for GVHD, and propose CD30 as a therapeutic target for GVHD.

The utility of immune cells expressing CD30-specific CARs in methods to reduce/prevent alloreactive immune responses is described *e.g.* in WO 2021/222929 A1, which is hereby incorporated by reference in its entirety.

The CAR-expressing immune cells and compositions of the present disclosure are particularly useful in methods involving allotransplantation, and also in the processing/production of allotransplants.

In particular, the CAR-expressing immune cells and compositions are contemplated for use in the production and administration of “off-the-shelf” materials for use in therapeutic and prophylactic methods comprising administration of allogeneic material.

As explained hereinabove, CAR-expressing immune cells of the present disclosure are useful for the treatment/prevention of diseases/conditions by adoptive cell transfer. CAR-expressing immune cells of the present disclosure are less susceptible to T cell-mediated alloreactive immune responses of the recipient following adoptive transfer, and thus exhibit enhanced proliferation/survival in the recipient after transfer, and superior therapeutic/prophylactic effects.

The CAR-expressing immune cells and compositions of the present disclosure are also useful in methods comprising allotransplantation of allogeneic cells <u>other than</u> the CAR-expressing immune cells of the present disclosure. In particular, the CAR-expressing immune cells and compositions of

the present disclosure are useful for depleting allotransplants (populations of cells, tissues and organs) and subjects of alloreactive immune cells (*e.g.* alloreactive T cells).

In such methods the CAR-expressing immune cells and compositions are useful for conditioning of donor and/or recipient subjects, and/or treatment of the allotransplant to reduce/prevent an alloreactive immune response following allotransplantation.

Cells, tissues and organs to be allotransplanted include *e.g.* immune cells (*e.g.* adoptive cell transfer), the heart, lung, kidney, liver, pancreas, intestine, face, cornea, skin, hematopoietic stem cells (bone marrow), blood, hands, leg, penis, bone, uterus, thymus, islets of Langerhans, heart valve and ovary. Populations of cells, tissues or organs to be allotransplanted may be referred to as "allotransplants".

The disease/condition to be treated/prevented by the allotransplantation can be any disease/condition which would derive therapeutic or prophylactic benefit from the allotransplantation. In some embodiments, the disease/condition to be treated/prevented by allotransplantation may *e.g.* be a T cell dysfunctional disorder, a cancer, an infectious disease or an autoimmune disease.

A T cell dysfunctional disorder may be a disease/condition in which normal T cell function is impaired causing downregulation of the subject's immune response to pathogenic antigens, *e.g.* generated by infection by exogenous agents such as microorganisms, bacteria and viruses, or generated by the host in some disease states such as in some forms of cancer (*e.g.* in the form of tumor-associated antigens). The T cell dysfunctional disorder may comprise T cell exhaustion or T cell anergy. T cell exhaustion comprises a state in which CD8+ T cells fail to proliferate or exert T cell effector functions such as cytotoxicity and cytokine (*e.g.* IFNγ) secretion in response to antigen stimulation. Exhausted T cells may also be characterised by sustained expression of one or more markers of T cell exhaustion, *e.g.* PD-1, CTLA-4, LAG-3, TIM-3. The T cell dysfunctional disorder may manifest as an infection, or inability to mount an effective immune response against an infection. The infection may be chronic, persistent, latent or slow, and may be the result of bacterial, viral, fungal or parasitic infection. As such, treatment may be provided to patients having a bacterial, viral or fungal infection. Examples of bacterial infections include infection with Helicobacter pylori. Examples of viral infections include infection with HIV, hepatitis B or hepatitis C. The T cell dysfunctional disorder may be associated with a cancer, such as tumor immune escape. Many human tumors express tumor-associated antigens recognised by T cells and capable of inducing an immune response.

An infectious disease may be *e.g.* bacterial, viral, fungal, or parasitic infection. In some embodiments, it may be particularly desirable to treat chronic/persistent infections, *e.g.* where such infections are associated with T cell dysfunction or T cell exhaustion. It is well established that T cell exhaustion is a state of T cell dysfunction that arises during many chronic infections (including viral, bacterial and parasitic), as well as in cancer (Wherry Nature Immunology Vol.12, No.6, p492-499, June 2011). Examples of bacterial infections that may be treated include infection by Bacillus spp., Bordetella pertussis, Clostridium spp., Corynebacterium spp., Vibrio chloerae, Staphylococcus spp.,

Streptococcus spp. Escherichia, Klebsiella, Proteus, Yersinia, Erwina, Salmonella, Listeria sp, Helicobacter pylori, mycobacteria (*e.g.* Mycobacterium tuberculosis) and Pseudomonas aeruginosa. For example, the bacterial infection may be sepsis or tuberculosis. Examples of viral infections that may be treated include infection by influenza virus, measles virus, hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), lymphocytic choriomeningitis virus (LCMV), Herpes simplex virus and human papilloma virus (HPV). Examples of fungal infections that may be treated include infection by Alternaria sp, Aspergillus sp, Candida sp and Histoplasma sp. The fungal infection may be fungal sepsis or histoplasmosis. Examples of parasitic infections that may be treated include infection by Plasmodium species (*e.g.* Plasmodium falciparum, Plasmodium yoeli, Plasmodium ovale, Plasmodium vivax, or Plasmodium chabaudi chabaudi). The parasitic infection may be a disease such as malaria, leishmaniasis and toxoplasmosis.

In some embodiments, the disease/condition is an autoimmune disease. In such embodiments the treatment may be aimed at reducing the number of autoimmune effector cells. In some embodiments the autoimmune disease is selected from: diabetes mellitus type 1, celiac disease, Graves' disease, inflammatory bowel disease, multiple sclerosis, psoriasis, rheumatoid arthritis, and systemic lupus erythematosus.

The CAR-expressing immune cells and compositions of the present disclosure are also useful for the treatment/prevention of an alloreactive immune response, and diseases/conditions characterised by an alloreactive immune response.

Diseases and conditions characterised by an alloreactive immune response include diseases/conditions caused or exacerbated by alloreactive immune responses associated with allotransplantation. Such diseases/conditions include graft versus host disease (GVHD) and graft rejection, and are described in detail in Perkey and Maillard Annu Rev Pathol. (2018) 13:219-245, which is hereby incorporated by reference in its entirety.

Graft-versus-host disease (GVHD) can occur following allotransplantation of large numbers of donor immune cells, and involves reactivity of donor-derived immune cells against allogeneic recipient cells/tissues/organs. Graft rejection refers to the destruction of transplanted cells/tissue/organs by a recipient's immune system following transplantation. Where graft rejection is of an allotransplant, it may be referred to as allograft rejection.

The CAR-expressing immune cells and compositions of the present disclosure may be used to deplete alloreactive T cells in an allotransplant, which could otherwise lead to graft versus host disease (GVHD) in a recipient upon allotransplantation.

The CAR-expressing immune cells and compositions of the present disclosure may be used to deplete alloreactive T cells in a donor for an allotransplant (*e.g.* prior to harvesting/collecting the allotransplant), which could otherwise lead to GVHD in a recipient upon allotransplantation.

The CAR-expressing immune cells and compositions of the present disclosure may be used to deplete alloreactive T cells in the recipient for an allotransplant, which could otherwise cause/promote graft rejection.

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The present disclosure provides methods of treating/preventing graft-versus-host disease (GVHD) following allotransplantation, comprising administering a CAR-expressing immune cell or composition according to the present disclosure to a donor subject for an allotransplant. The present disclosure also provides methods of treating/preventing graft-versus-host disease (GVHD) following
10 allotransplantation, comprising contacting an allotransplant with a CAR-expressing immune cell or composition according to the present disclosure. The aim for such methods is to reduce/remove the ability of alloreactive immune cells in the allograft to mount an alloreactive immune response to cells, tissue and/or organs of the recipient for the allotransplant.

15 The present disclosure provides methods of treating/preventing graft rejection following allotransplantation, comprising administering a CAR-expressing immune cell or composition according to the present disclosure to a recipient subject for an allotransplant. The aim for such methods is to reduce/remove the ability of the receipt subject to mount an alloreactive immune response to the allotransplant. The CAR-expressing immune cells are useful to eliminate immune cells in the recipient
20 that would otherwise effect an alloreactive immune response against donor cells, tissue and/or organs.

The present disclosure provides methods comprising depleting an allotransplant of alloreactive immune cells (*e.g.* alloreactive T cells), comprising contacting an allotransplant (*e.g.* a population of cells, tissue or an organ to be transplanted) with a CAR-expressing immune cell or composition of the
25 present disclosure. The methods may comprise administering a CAR-expressing immune cell or composition of the present disclosure to a donor subject for the allotransplant. The aim for such methods is to reduce/remove the ability of alloreactive immune cells in the allograft to mount an alloreactive immune response to cells, tissue and/or organs of the recipient for the allotransplant.

30 In some embodiments the methods comprise one or more of:

obtaining/collecting a population of cells, tissue or organ from a subject;

contacting a population of cells, tissue or organ with a CAR-expressing immune cell or composition according to the present disclosure;

culturing a population of cells, tissue or organ *in vitro* or *ex vivo* in the presence of a CAR-
35 expressing immune cell according to the present disclosure;

harvesting/collecting a population of cells, tissue or organ depleted of alloreactive immune cells; and

transplanting/administering a population of cells, tissue or organ depleted of alloreactive immune cells to a subject.

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The present disclosure also provides methods comprising depleting a subject of alloreactive immune cells (*e.g.* alloreactive T cells), comprising administering a CAR-expressing immune cell or composition of the present disclosure to the subject. The subject may be a donor subject for an allotransplant, or may be an intended recipient subject for an allotransplant.

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In some embodiments the methods comprise one or more of:

administering a CAR-expressing immune cell or composition according to the present disclosure to a subject, in order to deplete alloreactive immune cells in the subject;

obtaining/collecting a population of cells, tissue or organ from a subject to which a CAR-
10 expressing immune cell or composition according to the present disclosure has been administered; and

transplanting/administering a population of cells, tissue or organ depleted of alloreactive immune cells to a subject.

15 In some embodiments the methods comprise one or more of:

administering a CAR-expressing immune cell or composition according to the present disclosure to a subject, in order to deplete alloreactive immune cells in the subject; and

transplanting/administering a population of cells, tissue or organ to a subject to which a CAR-expressing immune cell or composition according to the present disclosure have previously been
20 administered.

Depletion of alloreactive immune cells may result in *e.g.* a 2-fold, 10-fold, 100-fold, 1000-fold, 10000-fold or greater reduction in the quantity of alloreactive immune cells in the allotransplant or subject.

25 The methods may be performed *in vitro* or *ex vivo*, or *in vivo* in a subject. Method steps performed *in vitro* or *ex vivo* may comprise *in vitro* or *ex vivo* cell culture.

The methods may further comprise method steps for the production of CAR-expressing immune cells and compositions according to the present disclosure.

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In some embodiments, administration of a CAR-expressing immune cell or composition according to the present disclosure to a recipient subject for an allotransplantation and allotransplantation are performed simultaneously (*i.e.* at the same time, or within *e.g.* 1 hr, 2 hrs, 3 hrs, 4 hrs, 5 hrs, 6 hrs, 8 hrs, 12 hrs, 24 hrs, 36 hrs or 48 hrs).

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In some embodiments, administration of a CAR-expressing immune cell or composition according to the present disclosure to a recipient subject for an allotransplantation and allotransplantation are performed sequentially. The time interval between administration of a CAR-expressing immune cell or composition and allotransplantation may be any time interval, including hours, days, weeks, months,
40 or years. The CAR-expressing immune cell or composition may be administered to the recipient

subject before or after allotransplantation. The CAR-expressing immune cell or composition are preferably administered to the recipient subject prior to allotransplantation.

In some embodiments, administration of a CAR-expressing immune cell or composition according to the present disclosure to a donor subject for an allotransplantation and collection of the allotransplant (*i.e.* collection of the cells, tissue and/or an organ) from the subject are performed simultaneously (*i.e.* at the same time, or within *e.g.* 1 hr, 2 hrs, 3 hrs, 4 hrs, 5 hrs, 6 hrs, 8 hrs, 12 hrs, 24 hrs, 36 hrs or 48 hrs). In some embodiments administration of a CAR-expressing immune cell or composition according to the present disclosure to a donor subject for an allotransplantation and collection of the allotransplant (*i.e.* collection of the cells, tissue and/or an organ) from the subject are performed sequentially. The time interval between administration of a CAR-expressing immune cell or composition and collection of the allotransplant may be any time interval, including hours, days, weeks, months, or years. The CAR-expressing immune cell or composition may be administered to the donor subject before or after collection of the allotransplant. The CAR-expressing immune cell or composition are preferably administered to the donor subject prior to collection of the allotransplant.

In some embodiments, the methods comprise additional intervention to treat/prevent an alloreactive immune response, graft rejection and/or GVHD.

In some embodiments, the methods to treat/prevent alloreactivity, graft rejection and/or GVHD comprise administration of immunosuppressive and/or lymphodepletive therapy such as treatment with corticosteroids (*e.g.* prednisolone, hydrocortisone), calcineurin inhibitors (*e.g.* cyclosporin, tacrolimus) anti-proliferative agents (*e.g.* azathioprinem, mycophenolic acid) and/or mTOR inhibitors (*e.g.* sirolimus, everolimus).

In some embodiments, the methods to treat/prevent alloreactivity and/or graft rejection comprise antibody therapy, such as treatment with monoclonal anti-IL-2Rα receptor antibodies (*e.g.* basiliximab, daclizumab), anti-T cell antibodies (*e.g.* anti-thymocyte globulin, anti-lymphocyte globulin) and/or anti-CD20 antibodies (*e.g.* rituximab).

In some embodiments, the methods to treat/prevent alloreactivity and/or graft rejection comprise blood transfusion and/or bone marrow transplantation.

Where a method is disclosed herein, the present disclosure also provides the CAR-expressing immune cells and compositions of the present disclosure for use in such methods. Also provided is the use of the CAR-expressing immune cells or compositions of the present disclosure in the manufacture of products (*e.g.* medicaments) for use in such methods.

In some embodiments, the methods of various aspects of the present disclosure cause less depletion and/or increased survival of non-alloreactive immune cells as compared to methods employing immunosuppressive agent(s). For example, the present methods are useful for preserving/maintaining

the non-alloreactive immune cell compartment in a recipient subject for an allotransplant, or in an allotransplant.

In some embodiments of the methods of the present disclosure comprising allotransplantation, the present methods are associated with an increased number/proportion of non-alloreactive immune cells in the recipient subject for the allotransplant as compared to methods involving treatment with an immunosuppressive agent. In some embodiments of the methods of the present disclosure comprising adoptive transfer of allogeneic immune cells, the present methods are associated with an increased number/proportion of non-alloreactive immune cells in the recipient subject for the allogeneic immune cells as compared to methods involving treatment with an immunosuppressive agent.

In some embodiments of the methods of the present disclosure comprising allotransplantation, the present methods are associated with an increased number/proportion of non-alloreactive immune cells in the allotransplant as compared to methods involving treatment with an immunosuppressive agent.

The present disclosure also provides the CAR-expressing immune cell or composition of the present disclosure for use in a method of:

killing a cell expressing the target antigen for which the CAR is specific (*e.g.* a cell expressing CD30); and/or

killing an alloreactive immune cell (*e.g.* a T cell expressing CD30).

The present disclosure also provides the use of such CAR-expressing immune cells and compositions in such methods, and methods using the CAR-expressing immune cell and compositions to such ends.

Administration

Administration of the articles of the present disclosure is preferably in a ‘therapeutically-effective’ or ‘prophylactically-effective’ amount, this being sufficient to show therapeutic or prophylactic benefit to the subject. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease/condition and the particular article administered. Prescription of treatment, *e.g.* decisions on dosage *etc.*, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disease/disorder to be treated, the condition of the individual subject, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington’s ‘The Science and Practice of Pharmacy’ (ed. A. Adejare), 23rd Edition (2020), Academic Press.

Administration of the articles of the present disclosure may be topical, parenteral, systemic, intracavitary, intravenous, intra-arterial, intramuscular, intrathecal, intraocular, intravitreal, intraconjunctival, subretinal, suprachoroidal, subcutaneous, intradermal, intrathecal, oral, nasal or

transdermal. Administration may be by injection or infusion. Administration of the articles of the present disclosure may be intratumoral.

In some aspects and embodiments in accordance with the present disclosure there may be targeted
5 delivery of articles of the present disclosure, *i.e.* wherein the concentration of the relevant agent in the subject is increased in some parts of the body relative to other parts of the body. In some embodiments, the methods comprise intravenous, intra-arterial, intramuscular or subcutaneous administration and wherein the relevant article is formulated in a targeted agent delivery system. Suitable targeted delivery systems include, for example, nanoparticles, liposomes, micelles, beads,
10 polymers, metal particles, dendrimers, antibodies, aptamers, nanotubes or micro-sized silica rods. Such systems may comprise a magnetic element to direct the agent to the desired organ or tissue. Suitable nanocarriers and delivery systems will be apparent to one skilled in the art.

In some cases, the articles of the present disclosure are formulated for targeted delivery to specific
15 cells, a tissue, an organ and/or a tumor.

Further intervention

Administration may be alone or in combination with other treatments, either simultaneously or sequentially dependent upon the disease/condition to be treated. The antigen-binding molecule, CAR,
20 cell or composition described herein and another prophylactic/therapeutic agent may be administered simultaneously or sequentially.

In some embodiments, the methods comprise additional therapeutic or prophylactic intervention, *e.g.* for the treatment/prevention of a cancer. In some embodiments, the therapeutic or prophylactic
25 intervention is selected from chemotherapy, immunotherapy, radiotherapy, surgery, vaccination and/or hormone therapy. In some embodiments, the therapeutic or prophylactic intervention comprises leukapheresis. In some embodiments, the therapeutic or prophylactic intervention comprises a stem cell transplant.

30 Simultaneous administration refers to administration of the antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition and therapeutic agent together, for example as a pharmaceutical composition containing both agents (combined preparation), or immediately after each other and optionally via the same route of administration, *e.g.* to the same artery, vein or other blood vessel. Sequential administration refers to
35 administration of one of the antigen-binding molecule/composition or therapeutic agent followed after a given time interval by separate administration of the other agent. It is not required that the two agents are administered by the same route, although this is the case in some embodiments. The time interval may be any time interval.

40 In some embodiments, treatment of cancer further comprises chemotherapy and/or radiotherapy. Chemotherapy and radiotherapy respectively refer to treatment of a cancer with a drug or with ionising

radiation (*e.g.* radiotherapy using X-rays or γ-rays). The drug may be a chemical entity, *e.g.* small molecule pharmaceutical, antibiotic, DNA intercalator, protein inhibitor (*e.g.* kinase inhibitor), or a biological agent, *e.g.* antibody, antibody fragment, aptamer, nucleic acid (*e.g.* DNA, RNA), peptide, polypeptide, or protein. The drug may be formulated as a pharmaceutical composition or medicament. The formulation may comprise one or more drugs (*e.g.* one or more active agents) together with one or more pharmaceutically acceptable diluents, excipients or carriers.

Chemotherapy may involve administration of more than one drug. A drug may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

The chemotherapy may be administered by one or more routes of administration, *e.g.* parenteral, intravenous injection, oral, subcutaneous, intradermal or intratumoral.

The chemotherapy may be administered according to a treatment regime. The treatment regime may be a pre-determined timetable, plan, scheme or schedule of chemotherapy administration which may be prepared by a physician or medical practitioner and may be tailored to suit the patient requiring treatment. The treatment regime may indicate one or more of: the type of chemotherapy to administer to the patient; the dose of each drug or radiation; the time interval between administrations; the length of each treatment; the number and nature of any treatment holidays, if any *etc.* For a co-therapy a single treatment regime may be provided which indicates how each drug is to be administered.

Chemotherapeutic drugs may be selected from: Abemaciclib, Abiraterone Acetate, Abitrexate (Methotrexate), Abraxane (Paclitaxel Albumin-stabilized Nanoparticle Formulation), ABVD, ABVE, ABVE-PC, AC, Acalabrutinib, AC-T, Adcetris (Brentuximab Vedotin), ADE, Ado-Trastuzumab Emtansine, Adriamycin (Doxorubicin Hydrochloride), Afatinib Dimaleate, Afinitor (Everolimus), Akynzeo (Netupitant and Palonosetron Hydrochloride), Aldara (Imiquimod), Aldesleukin, Alecensa (Alectinib), Alectinib, Alemtuzumab, Alimta (Pemetrexed Disodium), Aliqopa (Copanlisib Hydrochloride), Alkeran for Injection (Melphalan Hydrochloride), Alkeran Tablets (Melphalan), Aloxi (Palonosetron Hydrochloride), Alunbrig (Brigatinib), Ambochlorin (Chlorambucil), Amboclorin (Chlorambucil), Amifostine, Aminolevulinic Acid, Anastrozole, Aprepitant, Aredia (Pamidronate Disodium), Arimidex (Anastrozole), Aromasin (Exemestane), Arranon (Nelarabine), Arsenic Trioxide, Arzerra (Ofatumumab), Asparaginase Erwinia chrysanthemi, Atezolizumab, Avastin (Bevacizumab), Avelumab, Axicabtagene Ciloleucel, Axitinib, Azacitidine, Bavencio (Avelumab), BEACOPP, Becenum (Carmustine), Beleodaq (Belinostat), Belinostat, Bendamustine Hydrochloride, BEP, Besponsa (Inotuzumab Ozogamicin) , Bevacizumab, Bexarotene, Bexxar (Tositumomab and Iodine I 131 Tositumomab), Bicalutamide, BiCNU (Carmustine), Bleomycin, Blinatumomab, Blincyto (Blinatumomab), Bortezomib, Bosulif (Bosutinib), Bosutinib, Brentuximab Vedotin, Brigatinib, BuMel, Busulfan, Busulfex (Busulfan), Cabazitaxel, Cabometyx (Cabozantinib-S-Malate), Cabozantinib-S-Malate, CAF, Calquence (Acalabrutinib), Campath (Alemtuzumab), Camptosar (Irinotecan Hydrochloride), Capecitabine, CAPOX, Carac (Fluorouracil--Topical), Carboplatin, CARBOPLATIN-

TAXOL, Carfilzomib, Carmubris (Carmustine), Carmustine, Carmustine Implant, Casodex (Bicalutamide), CEM, Ceritinib, Cerubidine (Daunorubicin Hydrochloride), Cervarix (Recombinant HPV Bivalent Vaccine), Cetuximab, CEV, Chlorambucil, CHLORAMBUCIL-PREDNISONE, CHOP, Cisplatin, Cladribine, Clafen (Cyclophosphamide), Clofarabine, Clofarex (Clofarabine), Clolar (Clofarabine), CMF, Cobimetinib, Cometriq (Cabozantinib-S-Malate), Copanlisib Hydrochloride, COPDAC, COPP, COPP-ABV, Cosmegen (Dactinomycin), Cotellic (Cobimetinib), Crizotinib, CVP, Cyclophosphamide, Cyfos (Ifosfamide), Cyramza (Ramucirumab), Cytarabine, Cytarabine Liposome, Cytosar-U (Cytarabine), Cytoxan (Cyclophosphamide), Dabrafenib, Dacarbazine, Dacogen (Decitabine), Dactinomycin, Daratumumab, Darzalex (Daratumumab), Dasatinib, Daunorubicin Hydrochloride, Daunorubicin Hydrochloride and Cytarabine Liposome, Decitabine, Defibrotide Sodium, Defitelio (Defibrotide Sodium), Degarelix, Denileukin Diftitox, Denosumab, DepoCyt (Cytarabine Liposome), Dexamethasone, Dexrazoxane Hydrochloride, Dinutuximab, Docetaxel, Doxil (Doxorubicin Hydrochloride Liposome), Doxorubicin Hydrochloride, Doxorubicin Hydrochloride Liposome, Dox-SL (Doxorubicin Hydrochloride Liposome), DTIC-Dome (Dacarbazine), Durvalumab, Efudex (Fluorouracil--Topical), Elitek (Rasburicase), Ellence (Epirubicin Hydrochloride), Elotuzumab, Eloxatin (Oxaliplatin), Eltrombopag Olamine, Emend (Aprepitant), Empliciti (Elotuzumab), Enasidenib Mesylate, Enzalutamide, Epirubicin Hydrochloride, EPOCH, Erbitux (Cetuximab), Eribulin Mesylate, Erivedge (Vismodegib), Erlotinib Hydrochloride, Erwinaze (Asparaginase Erwinia chrysanthemi), Ethyol (Amifostine), Etopophos (Etoposide Phosphate), Etoposide, Etoposide Phosphate, Evacet (Doxorubicin Hydrochloride Liposome), Everolimus, Evista (Raloxifene Hydrochloride), Evomela (Melphalan Hydrochloride), Exemestane, 5-FU (Fluorouracil Injection), 5-FU (Fluorouracil--Topical), Fareston (Toremifene), Farydak (Panobinostat), Faslodex (Fulvestrant), FEC, Femara (Letrozole), Filgrastim, Fludara (Fludarabine Phosphate), Fludarabine Phosphate, Fluoroplex (Fluorouracil--Topical), Fluorouracil Injection, Fluorouracil--Topical, Flutamide, Folex (Methotrexate), Folex PFS (Methotrexate), FOLFIRI, FOLFIRI-BEVACIZUMAB, FOLFIRI-CETUXIMAB, FOLFIRINOX, FOLFOX, Folotyn (Pralatrexate), FU-LV, Fulvestrant, Gardasil (Recombinant HPV Quadrivalent Vaccine), Gardasil 9 (Recombinant HPV Nonavalent Vaccine), Gazyva (Obinutuzumab), Gefitinib, Gemcitabine Hydrochloride, GEMCITABINE-CISPLATIN, GEMCITABINE-OXALIPLATIN, Gemtuzumab Ozogamicin, Gemzar (Gemcitabine Hydrochloride), Gilotrif (Afatinib Dimaleate), Gleevec (Imatinib Mesylate), Gliadel (Carmustine Implant), Gliadel wafer (Carmustine Implant), Glucarpidase, Goserelin Acetate, Halaven (Eribulin Mesylate), Hemangeol (Propranolol Hydrochloride), Herceptin (Trastuzumab), HPV Bivalent Vaccine, Recombinant, HPV Nonavalent Vaccine, Recombinant, HPV Quadrivalent Vaccine, Recombinant, Hycamtin (Topotecan Hydrochloride), Hydrea (Hydroxyurea), Hydroxyurea, Hyper-CVAD, Ibrance (Palbociclib), Ibritumomab Tiuxetan, Ibrutinib, ICE, Iclusig (Ponatinib Hydrochloride), Idamycin (Idarubicin Hydrochloride), Idarubicin Hydrochloride, Idelalisib, Idhifa (Enasidenib Mesylate), Ifex (Ifosfamide), Ifosfamide, Ifosfamidum (Ifosfamide), IL-2 (Aldesleukin), Imatinib Mesylate, Imbruvica (Ibrutinib), Imfinzi (Durvalumab), Imiquimod, Imlygic (Talimogene Laherparepvec), Inlyta (Axitinib), Inotuzumab Ozogamicin, Interferon Alfa-2b, Recombinant, Interleukin-2 (Aldesleukin), Intron A (Recombinant Interferon Alfa-2b), Iodine I 131 Tositumomab and Tositumomab, Ipilimumab, Iressa (Gefitinib), Irinotecan Hydrochloride, Irinotecan Hydrochloride Liposome, Istodax (Romidepsin), Ixabepilone, Ixazomib Citrate, Ixempra (Ixabepilone),

Jakafi (Ruxolitinib Phosphate), JEB, Jevtana (Cabazitaxel), Kadcyla (Ado-Trastuzumab Emtansine), Keoxifene (Raloxifene Hydrochloride), Kepivance (Palifermin), Keytruda (Pembrolizumab), Kisqali (Ribociclib), Kymriah (Tisagenlecleucel), Kyprolis (Carfilzomib), Lanreotide Acetate, Lapatinib Ditosylate, Lartruvo (Olaratumab), Lenalidomide, Lenvatinib Mesylate, Lenvima (Lenvatinib Mesylate),
5 Letrozole, Leucovorin Calcium, Leukeran (Chlorambucil), Leuprolide Acetate, Leustatin (Cladribine), Levulan (Aminolevulinic Acid), Linfolizin (Chlorambucil), LipoDox (Doxorubicin Hydrochloride Liposome), Lomustine, Lonsurf (Trifluridine and Tipiracil Hydrochloride), Lupron (Leuprolide Acetate), Lupron Depot (Leuprolide Acetate), Lupron Depot-Ped (Leuprolide Acetate), Lynparza (Olaparib), Marqibo (Vincristine Sulfate Liposome), Matulane (Procarbazine Hydrochloride), Mechlorethamine
10 Hydrochloride, Megestrol Acetate, Mekinist (Trametinib), Melphalan, Melphalan Hydrochloride, Mercaptopurine, Mesna, Mesnex (Mesna), Methazolastone (Temozolomide), Methotrexate, Methotrexate LPF (Methotrexate), Methylnaltrexone Bromide, Mexate (Methotrexate), Mexate-AQ (Methotrexate), Midostaurin, Mitomycin C, Mitoxantrone Hydrochloride, Mitozytrex (Mitomycin C), MOPP, Mozobil (Plerixafor), Mustargen (Mechlorethamine Hydrochloride), Mutamycin (Mitomycin C),
15 Myleran (Busulfan), Mylosar (Azacitidine), Mylotarg (Gemtuzumab Ozogamicin), Nanoparticle Paclitaxel (Paclitaxel Albumin-stabilized Nanoparticle Formulation), Navelbine (Vinorelbine Tartrate), Necitumumab, Nelarabine, Neosar (Cyclophosphamide), Neratinib Maleate, Nerlynx (Neratinib Maleate), Netupitant and Palonosetron Hydrochloride, Neulasta (Pegfilgrastim), Neupogen (Filgrastim), Nexavar (Sorafenib Tosylate), Nilandron (Nilutamide), Nilotinib, Nilutamide, Ninlaro
20 (Ixazomib Citrate), Niraparib Tosylate Monohydrate, Nivolumab, Nolvadex (Tamoxifen Citrate), Nplate (Romiplostim), Obinutuzumab, Odomzo (Sonidegib), OEPA, Ofatumumab, OFF, Olaparib, Olaratumab, Omacetaxine Mepesuccinate, Oncaspar (Pegaspargase), Ondansetron Hydrochloride, Onivyde (Irinotecan Hydrochloride Liposome), Ontak (Denileukin Diftitox), Opdivo (Nivolumab), OPPA, Osimertinib, Oxaliplatin, Paclitaxel, Paclitaxel Albumin-stabilized Nanoparticle Formulation, PAD,
25 Palbociclib, Palifermin, Palonosetron Hydrochloride, Palonosetron Hydrochloride and Netupitant, Pamidronate Disodium, Panitumumab, Panobinostat, Paraplat (Carboplatin), Paraplatin (Carboplatin), Pazopanib Hydrochloride, PCV, PEB, Pegaspargase, Pegfilgrastim, Peginterferon Alfa-2b, PEG-Intron (Peginterferon Alfa-2b), Pembrolizumab, Pemetrexed Disodium, Perjeta (Pertuzumab), Pertuzumab, Platinol (Cisplatin), Platinol-AQ (Cisplatin), Plerixafor, Pomalidomide, Pomalyst (Pomalidomide),
30 Ponatinib Hydrochloride, Portrazza (Necitumumab), Pralatrexate, Prednisone, Procarbazine Hydrochloride, Proleukin (Aldesleukin), Prolia (Denosumab), Promacta (Eltrombopag Olamine), Propranolol Hydrochloride, Provenge (Sipuleucel-T), Purinethol (Mercaptopurine), Purixan (Mercaptopurine), Radium 223 Dichloride, Raloxifene Hydrochloride, Ramucirumab, Rasburicase, R-CHOP, R-CVP, Recombinant Human Papillomavirus (HPV) Bivalent Vaccine, Recombinant Human
35 Papillomavirus (HPV) Nonavalent Vaccine, Recombinant Human Papillomavirus (HPV) Quadrivalent Vaccine, Recombinant Interferon Alfa-2b, Regorafenib, Relistor (Methylnaltrexone Bromide), R-EPOCH, Revlimid (Lenalidomide), Rheumatrex (Methotrexate), Ribociclib, R-ICE, Rituxan (Rituximab), Rituxan Hycela (Rituximab and Hyaluronidase Human), Rituximab, Rituximab and Hyaluronidase Human, Rolapitant Hydrochloride, Romidepsin, Romiplostim, Rubidomycin
40 (Daunorubicin Hydrochloride), Rubraca (Rucaparib Camsylate), Rucaparib Camsylate, Ruxolitinib Phosphate, Rydapt (Midostaurin), Sclerosol Intrapleural Aerosol (Talc), Siltuximab, Sipuleucel-T,

Somatuline Depot (Lanreotide Acetate), Sonidegib, Sorafenib Tosylate, Sprycel (Dasatinib), STANFORD V, Sterile Talc Powder (Talc), Steritalc (Talc), Stivarga (Regorafenib), Sunitinib Malate, Sutent (Sunitinib Malate), Sylatron (Peginterferon Alfa-2b), Sylvant (Siltuximab), Synribo (Omacetaxine Mepesuccinate), Tabloid (Thioguanine), TAC, Tafinlar (Dabrafenib), Tagrisso (Osimertinib), Talc, Talimogene Laherparepvec, Tamoxifen Citrate, Tarabine PFS (Cytarabine), Tarceva (Erlotinib Hydrochloride), Targretin (Bexarotene), Tasigna (Nilotinib), Taxol (Paclitaxel), Taxotere (Docetaxel), Tecentriq (Atezolizumab), Temodar (Temozolomide), Temozolomide, Temsirolimus, Thalidomide, Thalomid (Thalidomide), Thioguanine, Thiotepa, Tisagenlecleucel, Tolak (Fluorouracil--Topical), Topotecan Hydrochloride, Toremifene, Torisel (Temsirolimus), Tositumomab and Iodine I 131 Tositumomab, Totect (Dexrazoxane Hydrochloride), TPF, Trabectedin, Trametinib, Trastuzumab, Treanda (Bendamustine Hydrochloride), Trifluridine and Tipiracil Hydrochloride, Trisenox (Arsenic Trioxide), Tykerb (Lapatinib Ditosylate), Unituxin (Dinutuximab), Uridine Triacetate, VAC, Valrubicin, Valstar (Valrubicin), Vandetanib, VAMP, Varubi (Rolapitant Hydrochloride), Vectibix (Panitumumab), VeIP, Velban (Vinblastine Sulfate), Velcade (Bortezomib), Velsar (Vinblastine Sulfate), Vemurafenib, Venclexta (Venetoclax), Venetoclax, Verzenio (Abemaciclib), Viadur (Leuprolide Acetate), Vidaza (Azacitidine), Vinblastine Sulfate, Vincasar PFS (Vincristine Sulfate), Vincristine Sulfate, Vincristine Sulfate Liposome, Vinorelbine Tartrate, VIP, Vismodegib, Vistogard (Uridine Triacetate), Voraxaze (Glucarpidase), Vorinostat, Votrient (Pazopanib Hydrochloride), Vyxeos (Daunorubicin Hydrochloride and Cytarabine Liposome), Wellcovorin (Leucovorin Calcium), Xalkori (Crizotinib), Xeloda (Capecitabine), XELIRI, XELOX, Xgeva (Denosumab), Xofigo (Radium 223 Dichloride), Xtandi (Enzalutamide), Yervoy (Ipilimumab), Yescarta (Axicabtagene Ciloleucel), Yondelis (Trabectedin), Zaltrap (Ziv-Aflibercept), Zarxio (Filgrastim), Zejula (Niraparib Tosylate Monohydrate), Zelboraf (Vemurafenib), Zevalin (Ibritumomab Tiuxetan), Zinecard (Dexrazoxane Hydrochloride), Ziv-Aflibercept, Zofran (Ondansetron Hydrochloride), Zoladex (Goserelin Acetate), Zoledronic Acid, Zolinza (Vorinostat), Zometa (Zoledronic Acid), Zydelig (Idelalisib), Zykadia (Ceritinib) and Zytiga (Abiraterone Acetate).

In some embodiments, the treatment may comprise administration of a corticosteroid, *e.g.* dexamethasone and/or prednisone.

In some embodiments, a subject is administered lymphodepleting chemotherapy prior to administration of immune cells expressing/comprising a CAR described herein (or expressing/comprising nucleic acid encoding such a CAR).

That is, in some embodiments, methods of treating/preventing a disease/condition in accordance with the present disclosure comprise: (i) administering a lymphodepleting chemotherapy to a subject, and (ii) subsequently administering an immune cell expressing/comprising a CAR according to the present disclosure, or expressing/comprising a nucleic acid encoding a CAR according to the present disclosure.

As used herein, "lymphodepleting chemotherapy" refers to treatment with a chemotherapeutic agent which results in depletion of lymphocytes (*e.g.* T cells, B cells, NK cells, NKT cells or innate lymphoid cell (ILCs), or precursors thereof) within the subject to which the treatment is administered. A "lymphodepleting chemotherapeutic agent" refers to a chemotherapeutic agent which results in
5 depletion of lymphocytes.

Lymphodepleting chemotherapy and its use in methods of treatment by adoptive cell transfer are described *e.g.* in Klebanoff et al., Trends Immunol. (2005) 26(2):111-7 and Muranski et al., Nat Clin Pract Oncol. (2006) (12):668-81, both of which are hereby incorporated by reference in their entirety.
10 The aim of lymphodepleting chemotherapy is to deplete the recipient subject's endogenous lymphocyte population.

In the context of treatment of disease by adoptive transfer of immune cells, lymphodepleting chemotherapy is typically administered prior to adoptive cell transfer, to condition the recipient subject
15 to receive the adoptively transferred cells. Lymphodepleting chemotherapy is thought to promote the persistence and activity of adoptively transferred cells by creating a permissive environment, *e.g.* through elimination of cells expressing immunosuppressive cytokines, and creating the 'lymphoid space' required for expansion and activity of adoptively transferred lymphoid cells.

20 Chemotherapeutic agents commonly used in lymphodepleting chemotherapy include *e.g.* fludarabine, cyclophosphamide, bedamustine and pentostatin.

Multiple doses of the antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition may be provided. One or more, or each, of
25 the doses may be accompanied by simultaneous or sequential administration of another therapeutic agent.

Multiple doses may be separated by a predetermined time interval, which may be selected to be one of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29,
30 30, or 31 days, or 1, 2, 3, 4, 5, or 6 months. By way of example, doses may be given once every 7, 14, 21 or 28 days (plus or minus 3, 2, or 1 days).

In accordance with various aspects of the present disclosure, a method of treating and/or preventing a disease/condition may comprise one or more of the following: reducing the number/proportion of
35 CD30-expressing cells; inhibiting tumor growth (*e.g.* of a CD30+ tumor); reducing metastasis of a cancer (*e.g.* a CD30+ cancer); increasing survival of a subject having a cancer (*e.g.* a CD30+ cancer).

Methods of detection

The present disclosure also provides the articles of the present disclosure for use in methods for
40 detecting, localising or imaging CD30, or cells expressing CD30.

The antigen-binding molecules described herein may be used in methods that involve detecting binding of the antigen-binding molecule to CD30. Such methods may involve detection of the bound complex of the antigen-binding molecule and CD30. It will be appreciated that the CD30 may be CD30 expressed by a cell, *e.g.* in or at the cell surface of a cell expressing CD30.

As such, a method is provided, comprising contacting a sample containing, or suspected to contain, CD30, and detecting the formation of a complex of the antigen-binding molecule and CD30. Also provided is a method comprising contacting a sample containing, or suspected to contain, a cell expressing CD30, and detecting the formation of a complex of the antigen-binding molecule and a cell expressing CD30.

Suitable method formats are well known in the art, including immunoassays such as sandwich assays, *e.g.* ELISA. The methods may involve labelling the antigen-binding molecule, or target(s), or both, with a detectable moiety, *e.g.* a fluorescent label, phosphorescent label, luminescent label, immuno-detectable label, radiolabel, chemical, nucleic acid or enzymatic label as described herein. Detection techniques are well known to those of skill in the art and can be selected to correspond with the labelling agent.

Methods comprising detecting CD30, or cells expressing CD30, include methods for diagnosing/prognosing a disease/condition described herein.

Methods of this kind may be performed *in vitro* on a patient sample, or following processing of a patient sample. Once the sample is collected, the patient is not required to be present for the *in vitro* method to be performed, and therefore the method may be one which is not practised on the human or animal body. In some embodiments, the method is performed *in vivo*.

Such methods may involve detecting or quantifying CD30 and/or cells expressing CD30, *e.g.* in a patient sample. Where the method comprises quantifying the relevant factor, the method may further comprise comparing the determined amount against a standard or reference value as part of the diagnostic or prognostic evaluation. Other diagnostic/prognostic tests may be used in conjunction with those described herein to enhance the accuracy of the diagnosis or prognosis or to confirm a result obtained by using the tests described herein.

Detection in a sample may be used for the purpose of diagnosis of a disease/condition (*e.g.* a cancer), predisposition to a disease/condition, or for providing a prognosis (prognosticating) for a disease/condition, *e.g.* a disease/condition described herein. The diagnosis or prognosis may relate to an existing (previously diagnosed) disease/condition.

A sample may be taken from any tissue or bodily fluid. The sample may comprise or may be derived from: a quantity of blood; a quantity of serum derived from the individual's blood which may comprise the fluid portion of the blood obtained after removal of the fibrin clot and blood cells; a tissue sample or

biopsy; pleural fluid; cerebrospinal fluid (CSF); or cells isolated from said individual. In some embodiments, the sample may be obtained or derived from a tissue or tissues which are affected by the disease/condition (*e.g.* tissue or tissues in which symptoms of the disease manifest, or which are involved in the pathogenesis of the disease/condition).

A subject may be selected for diagnostic/prognostic evaluation based on the presence of symptoms indicative of a disease/condition described herein, or based on the subject being considered to be at risk of developing a disease/condition described herein.

The present disclosure also provides methods for selecting/stratifying a subject for treatment with a CD30-targeted agent. In some embodiments a subject is selected for treatment/prevention in accordance with the methods of the present disclosure, or is identified as a subject which would benefit from such treatment/prevention, based on detection/quantification of CD30, or cells expressing CD30, *e.g.* in a sample obtained from the individual.

Subjects

A subject in accordance with the various aspects of the present disclosure may be any animal or human. Therapeutic and prophylactic applications may be in human or animals (veterinary use).

The subject to be administered with an article of the present disclosure (*e.g.* in accordance with therapeutic or prophylactic intervention) may be a subject in need of such intervention. The subject is preferably mammalian, more preferably human. The subject may be a non-human mammal, but is more preferably human. The subject may be male or female. The subject may be a patient.

A subject may have (*e.g.* may have been diagnosed with) a disease or condition described herein, may be suspected of having such a disease/condition, or may be at risk of developing/contracting such a disease/condition. In embodiments according to the present disclosure, a subject may be selected for treatment according to the methods based on characterisation for one or more markers of such a disease/condition.

In some embodiments, a subject may be selected for therapeutic or prophylactic intervention as described herein based on the detection of cells/tissue expressing CD30, or of cells/tissue overexpressing CD30, *e.g.* in a sample obtained from the subject.

A subject may be an allogeneic subject with respect to an intervention in accordance with the present disclosure. A subject to be treated/prevented in accordance with the present disclosure may be genetically non-identical to the subject from which the CAR-expressing immune cells are derived. A subject to be treated/prevented in accordance with the present disclosure may be HLA mismatched with respect to the subject from which the CAR-expressing immune cells are derived. A subject to be treated/prevented in accordance with the present disclosure may be HLA matched with respect to the subject from which the CAR-expressing immune cells are derived.

The subject to which cells are administered in accordance with the present disclosure may be allogeneic/non-autologous with respect to the source from which the cells are/were derived. The subject to which cells are administered may be a different subject to the subject from which cells are/were obtained for the production of the cells to be administered. The subject to which the cells are administered may be genetically non-identical to the subject from which cells are/were obtained for the production of the cells to be administered.

The subject to which cells are administered may comprise MHC/HLA genes encoding MHC/HLA molecules which are non-identical to the MHC/HLA molecules encoded by the MHC/HLA genes of the subject from which cells are/were obtained for the production of the cells to be administered. The subject to which cells are administered may comprise MHC/HLA genes encoding MHC/HLA molecules which are identical to the MHC/HLA molecules encoded by the MHC/HLA genes of the subject from which cells are/were obtained for the production of the cells to be administered.

In some embodiments, the subject to which cells are administered is HLA matched with respect to the subject from which cells are/were obtained for the production of the cells to be administered. In some embodiments, the subject to which cells are administered is a near or complete HLA match with respect to the subject from which cells are/were obtained for the production of the cells to be administered.
In some embodiments, the subject is a ≥4/8 (*i.e.* 4/8, 5/8, 6/8, 7/8 or 8/8) match across HLA-A, -B, -C, and -DRB1. In some embodiments, the subject is a ≥5/10 (*i.e.* 5/10, 6/10, 7/10, 8/10, 9/10 or 10/10) match across HLA-A, -B, -C, -DRB1 and -DQB1. In some embodiments, the subject is a ≥6/12 (*i.e.* 6/12, 7/12, 8/12, 9/12, 10/12, 11/12 or 12/12) match across HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1. In some embodiments, the subject is an 8/8 match across HLA-A, -B, -C, and -DRB1. In some embodiments, the subject is a 10/10 match across HLA-A, -B, -C, -DRB1 and -DQB1. In some embodiments, the subject is a 12/12 match across HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1.

Kits

In some aspects of the present disclosure a kit of parts is provided. In some embodiments, the kit may have at least one container having a predetermined quantity of an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein.

In some embodiments, the kit may comprise materials for producing an antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition described herein.

The kit may provide the antigen-binding molecule, polypeptide, CAR, nucleic acid (or plurality thereof), expression vector (or plurality thereof), cell or composition together with instructions for administration to a patient in order to treat a specified disease/condition.

In some embodiments the kit may further comprise at least one container having a predetermined quantity of another therapeutic agent (*e.g.* as described herein). In such embodiments, the kit may also comprise a second medicament or pharmaceutical composition such that the two medicaments or
5 pharmaceutical compositions may be administered simultaneously or separately such that they provide a combined treatment for the specific disease or condition.

Kits according to the present disclosure may include instructions for use, *e.g.* in the form of an instruction booklet or leaflet. The instructions may include a protocol for performing any one or more of
10 the methods described herein.

Sequence identity

As used herein, 'sequence identity' refers to the percent of nucleotides/amino acid residues in a subject sequence that are identical to nucleotides/amino acid residues in a reference sequence, after
15 aligning the sequences and, if necessary, introducing gaps, to achieve the maximum percent sequence identity between the sequences. Pairwise and multiple sequence alignment for the purposes of determining percent sequence identity between two or more amino acid or nucleic acid sequences can be achieved in various ways known to a person of skill in the art, for instance, using publicly available computer software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-
20 960), T-coffee (Notredame *et al.* 2000, J. Mol. Biol. (2000) 302, 205-217), Kalign (Lassmann and Sonnhammer 2005, BMC Bioinformatics, 6(298)) and MAFFT (Katoh and Standley 2013, Molecular Biology and Evolution, 30(4) 772–780) software. When using such software, the default parameters, *e.g.* for gap penalty and extension penalty, are preferably used.

25 **Sequences**

SEQ ID NO:	DESCRIPTION	SEQUENCE
1	HRS3 VH (VH0)	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFK GKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS
2	HRS3, VH1, VH2, VH3, VH4, VH5 HC-CDR1	GYTFTTYT
3	HRS3, VH1, VH2, VH3, VH4, VH5 HC-CDR2	INPSSGCS
4	HRS3, VH1, VH2, VH3, VH4, VH5 HC-CDR3	ARRADYGNYEYTWFAY
5	HRS3 HC-FR1	QVQLQQSGAELARPGASVKMSCKAS
6	HRS3 HC-FR2	IHWVRRRPGHDLEWIGY
7	HRS3 HC-FR3	DYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYC
8	HRS3, VH1, VH2,	WGQGTTVTVSS

	VH3, VH4, VH5 HC-FR4	
9	HRS3 VL (VK0)	VIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIK
10	HRS3, VK1, VK2, VK3, VK4, VK5 LC-CDR1	QNVGTN
11	HRS3, VK1, VK2, VK3, VK4, VK5 LC-CDR2	SAS
12	HRS3, VK1, VK2, VK3, VK4, VK5 LC-CDR3	QQYHTYPLT
13	HRS3 LC-FR1	VIELTQSPKFMSTSVGDRVNVTYKAS
14	HRS3 LC-FR2	VAWFQQKPGQSPKVLIY
15	HRS3 LC-FR3	YRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFC
16	HRS3 LC-FR4	FGGGTKLEIK
17	VH1	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS
18	VH1 HC-FR1	QVQLQQSGAELAKPGASVKVSCKAS
19	VH1, VH2, VH3, VH4 HC-FR2	IHWVRRPPGKDLEWIGY
20	VH1 HC-FR3	DYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYC
21	VH2	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS
22	VH2, VH3, VH4, VH5 HC-FR1	QVQLVQSGSELKKPGASVKVSCKAS
23	VH2 HC-FR3	DYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYC
24	VH3	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS
25	VH3 HC-FR3	DYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYC
26	VH4	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS
27	VH4, VH5 HC-FR3	DYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYC
28	VH5	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS
29	VH5 HC-FR2	IHWVRQPPGKGLEWIGY
30	VK1	VIELTQSPSFLSASVGDRVNVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
31	VK1 LC-FR1	VIELTQSPSFLSASVGDRVNVTYKAS
32	VK1, VK2, VK3, VK4, VK5 LC-FR2	VAWFQQKPGKAPKVLIY
33	VK1 LC-FR3	YRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFC
34	VK1, VK2, VK3,	FGGGTKVEIK

	VK4, VK5 LC-FR4	
35	VK2	VIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
36	VK2, VK5 LC-FR1	VIELTQSPSFLSASVGDRVTVTYKAS
37	VK2 LC-FR3	YRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFC
38	VK3	DIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
39	VK3, VK4 LC-FR1	DIQLTQSPSFLSASVGDRVTVTYKAS
40	VK3 LC-FR3	YRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFC
41	VK4	DIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
42	VK4 LC-FR3	YRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFC
43	VK5	VIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
44	VK5 LC-FR3	YRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFC
45	VH1-5Con	QVQLX_1QSGX_2ELX_3KPGASVKVSCKASGYTFTTYTIHWVRX_4PPGKX_5LEWIGYINPSSGCSDYNQN FX_6GRX_7TITADKSTX_8TAYMELSSLX_9SEDX_{10}AVYYCARRADYGNYEYTWFAYWGQGTTVTVSS wherein X_1 = V or Q, X_2 = S or A, X_3 = K or A, X_4 = R or Q, X_5 = D or G, X_6 = K or Q, X_7 = T or V, X_8 = S or N, X_9 = R or T, X_{10} = T or S
46	VH1-5Con HC-FR1	QVQLX_1QSGX_2ELX_3KPGASVKVSCKAS wherein X_1 = V or Q, X_2 = S or A, X_3 = K or A
47	VH1-5Con, VH3-5Con HC-FR2	IHWVRX_4PPGKX_5LEWIGY wherein X_4 = R or Q, X_5 = D or G
48	VH1-5Con HC-FR3	DYNQNFX_6GRX_7TITADKSTX_8TAYMELSSLX_9SEDX_{10}AVYYC wherein X_6 = K or Q, X_7 = T or V, X_8 = S or N, X_9 = R or T, X_{10} = T or S
49	VK1-3Con	X_{11}IX_{12}LTQSPSFLSASVGDRVX_{13}VTX_{14}KASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRF X_{15}GSGSGTDFTLTISSLQX_{16}EDFAEYFCQQYHTYPLTFGGGTKVEIK wherein X_{11} = V or D, X_{12} = E or Q, X_{13} = T or N, X_{14} = Y or C, X_{15} = T or S, X_{16} = P or S
50	VK1-3Con LC-FR1	X_{11}IX_{12}LTQSPSFLSASVGDRVX_{13}VTX_{14}KAS wherein X_{11} = D or V, X_{12} = E or Q, X_{13} = T or N, X_{14} = Y or C
51	VK1-3Con LC-FR3	YRYSGVPDRFX_{15}GSGSGTDFTLTISSLQX_{16}EDFAEYFC wherein X_{15} = T or S, X_{16} = P or S
52	VH3-5Con	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRX_{17}PPGKX_{18}LEWIGYINPSSGCSDYNQNF X_{19}GRX_{20}TITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS wherein X_{17} = R or Q, X_{18} = D or G, X_{19} = Q or K, X_{20} = V or T
53	VH3-5Con HC-FR3	DYNQNFX_{19}GRX_{20}TITADKSTSTAYMELSSLRSEDTAVYYC

		wherein X_{19} = Q or K, X_{20} = V or T
54	VK2-3Con	X_{21}IX_{22}LTQSPSFLSASVGDRVTVTX_{23}KASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFX_{24}GSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK wherein X_{21} = V or D, X_{22} = E or Q, X_{23} = Y or C, X_{24} = T or S
55	VK2-3Con LC-FR1	X_{21}IX_{22}LTQSPSFLSASVGDRVTVTX_{23}KAS wherein X_{21} = V or D, X_{22} = E or Q, X_{22} = Y or C
56	VK2-3Con LC-FR3	YRYSGVPDRFX_{24}GSGSGTDFTLTISSLQPEDFAEYFC wherein X_{24} = T or S
57	G_4S	GGGGS
58	scFv linker	SGGGSGGGGSGGGGS
59	HRS3 scFv	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIK
60	VH1VK1 scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
61	VH1VK2 scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
62	VH1VK3 scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
63	VH1VK4 scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
64	VH1VK5 scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
65	VH2VK1 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
66	VH2VK2 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK

67	VH2VK3 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
68	VH2VK4 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
69	VH2VK5 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
70	VH3VK1 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
71	VH3VK2 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
72	VH3VK3 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
73	VH3VK4 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
74	VH3VK5 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
75	VH4VK1 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
76	VH4VK2 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
77	VH4VK3 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
78	VH4VK4 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ

		GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRES GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
79	VH4VK5 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRES GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
80	VH5VK1 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVNVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
81	VH5VK2 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
82	VH5VK3 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
83	VH5VK4 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRES GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
84	VH5VK5 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRES GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
85	VH1-5ConVK1-3Con scFv	QVQLX$_{1}$QSGX$_{2}$ELX$_{3}$KPGASVKVSCKASGYTFTTYTIHWVRX$_{4}$PPGKX$_{5}$LEWIGYINPSSGCSDYNQN FX$_{6}$GRX$_{7}$TITADKSTX$_{8}$TAYMELSSLX$_{9}$SEDX$_{10}$AVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSG GGSGGGGSGGGGSX$_{11}$IX$_{12}$LTQSPSFLSASVGDRVX$_{13}$VTX$_{14}$KASQNVGTNVAWFQQKPGKAPKVL IYSASYRYSGVPDRFX$_{15}$GSGSGTDFTLTISSLQX$_{16}$EDFAEYFCQQYHTYPLTFGGGTKVEIK wherein X_1 = V or Q, X_2 = S or A, X_3 = K or A, X_4 = R or Q, X_5 = D or G, X_6 = K or Q, X_7 = T or V, X_8 = S or N, X_9 = R or T, X_{10} = T or S, X_{11} = V or D, X_{12} = E or Q, X_{13} = T or N, X_{14} = Y or C, X_{15} = T or S, X_{16} = P or S
86	VH3-5ConVK2-3Con scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRX$_{17}$PPGKX$_{18}$LEWIGYINPSSGCSDYNQNF X$_{19}$GRX$_{20}$TITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGG SGGGGSGGGGSX$_{21}$IX$_{22}$LTQSPSFLSASVGDRVTVTX$_{23}$KASQNVGTNVAWFQQKPGKAPKVLIYSA SYRYSGVPDRFX$_{24}$GSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK wherein X_{17} = R or Q, X_{18} = D or G, X_{19} = Q or K, X_{20} = V or T, X_{21} = V or D, X_{22} = E or Q, X_{22} = Y or C, X_{24} = T or S
87	Human CD30 isoform 1 (UniProt: P28908-	MRVLLAALGLLFLGALRAFPQDRPFEDTCHGNPSHYYDKAVRRCCYRCPMGLFPTQQCPQRPTDC RKQCEPDYYLDEADRCTACVTCSRDDLVEKTPCAWNSSRVCECRPGMFCSTSAVNSCARCFFHS VCPAGMIVKFPGTAQKNTVCEPASPGVSPACASPENCKEPSSGTIPQAKPTPVSPATSSASTMPVR

	1, v1)	GGTRLAQEAASKLTRAPDSPSSVGRPSSDPGLSPTQPCPEGSGDCRKQCEPDYYLDEAGRCTACVSCSRDDLVEKTPCAWNSSRTCECRPGMICATSATNSCARCVPYPICAAETVTKPQDMAEKDTTFEAPPLGTQPDCNPTPENGEAPASTSPTQSLLVDSQASKTLPIPTSAPVALSSTGKPVLDAGPVLFWVILVLVVVVGSSAFLLCHRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK
88	Human CD30 isoform 2 (UniProt: P28908-2)	MSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK
89	Human CD30 isoform 3 (UniProt: P28908-3)	MFCSTSAVNSCARCFFHSVCPAGMIVKFPGTAQKNTVCEPASPGVSPACASPENCKEPSSGTIPQAKPTPVSPATSSASTMPVRGGTRLAQEAASKLTRAPDSPSSVGRPSSDPGLSPTQPCPEGSGDCRKQCEPDYYLDEAGRCTACVSCSRDDLVEKTPCAWNSSRTCECRPGMICATSATNSCARCVPYPICAAETVTKPQDMAEKDTTFEAPPLGTQPDCNPTPENGEAPASTSPTQSLLVDSQASKTLPIPTSAPVALSSTGKPVLDAGPVLFWVILVLVVVVGSSAFLLCHRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTLRSGASVTEPVAEERGLMSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK
90	Human CD30 signal peptide	MRVLLAALGLLFLGALRA
91	Mature human CD30 isoform 1	FPQDRPFEDTCHGNPSHYYDKAVRRCCYRCPMGLFPTQQCPQRPTDCRKQCEPDYYLDEADRCTACVTCSRDDLVEKTPCAWNSSRVCECRPGMFCSTSAVNSCARCFFHSVCPAGMIVKFPGTAQKNTVCEPASPGVSPACASPENCKEPSSGTIPQAKPTPVSPATSSASTMPVRGGTRLAQEAASKLTRAPDSPSSVGRPSSDPGLSPTQPCPEGSGDCRKQCEPDYYLDEAGRCTACVSCSRDDLVEKTPCAWNSSRTCECRPGMICATSATNSCARCVPYPICAAETVTKPQDMAEKDTTFEAPPLGTQPDCNPTPENGEAPASTSPTQSLLVDSQASKTLPIPTSAPVALSSTGKPVLDAGPVLFWVILVLVVVVGSSAFLLCHRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK
92	Human CD30 extracellular domain	FPQDRPFEDTCHGNPSHYYDKAVRRCCYRCPMGLFPTQQCPQRPTDCRKQCEPDYYLDEADRCTACVTCSRDDLVEKTPCAWNSSRVCECRPGMFCSTSAVNSCARCFFHSVCPAGMIVKFPGTAQKNTVCEPASPGVSPACASPENCKEPSSGTIPQAKPTPVSPATSSASTMPVRGGTRLAQEAASKLTRAPDSPSSVGRPSSDPGLSPTQPCPEGSGDCRKQCEPDYYLDEAGRCTACVSCSRDDLVEKTPCAWNSSRTCECRPGMICATSATNSCARCVPYPICAAETVTKPQDMAEKDTTFEAPPLGTQPDCNPTPENGEAPASTSPTQSLLVDSQASKTLPIPTSAPVALSSTGKPVLDAG
93	Human CD30 transmembrane domain	PVLFWVILVLVVVVGSSAFLL
94	Human CD30 cytoplasmic domain	CHRRACRKRIRQKLHLCYPVQTSQPKLELVDSRPRRSSTQLRSGASVTEPVAEERGLMSQPLMETCHSVGAAYLESLPLQDASPAGGPSSPRDLPEPRVSTEHTNNKIEKIYIMKADTVIVGTVKAELPEGRGLAGPAEPELEEELEADHTPHYPEQETEPPLGSCSDVMLSVEEEGKEDPLPTAASGK
95	HRS3 epitope	ATSSASTMPVRGGTRLAQEAASKLTRAPDSPSSVGRPSSDPGLSPTQPCPEGSGDCRKQCEPDYYLDEAGRCTACVSCSRDDLVEKTPCAWNSSRTCECRPGMICATSATNSCARCVPYPICAAETVTKPQDMAEKDTTFEAPPLGTQPDC

96	Human IgG1 constant region (IGHG1; UniProt:P01857-1, v1)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
97	CH1 IgG1 (positions 1-98 of P01857-1, v1)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
98	Hinge IgG1 (positions 99-110 of P01857-1, v1)	EPKSCDKTHTCP
99	CH2 IgG1 (positions 111-223 of P01857-1, v1)	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
100	CH3 IgG1 (positions 224-330 of P01857-1, v1)	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
101	Human IgG1 constant region G1m3 allotype (K214R, D356E and L358M (EU numbering) relative to P01857-1)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K
102	CH1 IgG1 G1m3 allotype	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
103	CH3 IgG1 G1m3 allotype	GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
104	Human IgG4 constant region (IGHG4; UniProt:P01861-1, v1)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
105	Human IgG1 CH2-CH3 region	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTK NQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
106	Human IgG1 G1m3 allotype CH2-CH3 region	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
107	Human IgG4 CH2-CH3 region	APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSV

		MHEALHNHYTQKSLSLSLGK
108	Cκ CL (IGKC; UniProt: P01834-1, v2)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
109	Cλ CL1 (IGLC1; UniProt: P0CG04-1, v1)	GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
110	Cλ CL2 (IGLC2; UniProt: P0DOY2-1, v1)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS
111	Cλ CL3 (IGLC3; UniProt: P0DOY3-1, v1)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS
112	Cλ CL6 (IGLC6; UniProt: P0CF74-1, v1)	GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVKVAWKADGSPVNTGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECS
113	Cλ CL7 (IGLC7; UniProt: A0M8Q6-1, v3)	GQPKAAPSVTLFPPSSEELQANKATLVCLVSDFNPGAVTVAWKADGSPVKVGVETTKPSKQSNNKYAASSYLSLTPEQWKSHRSYSCRVTHEGSTVEKTVAPAECS
114	VH1 IgG1 HC	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
115	VH2 IgG1 HC	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
116	VH3 IgG1 HC	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
117	VH4 IgG1 HC	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC

		KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
118	VH5 IgG1 HC	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
119	VH1-5Con IgG1 HC	QVQLX$_1$QSGX$_2$ELX$_3$KPGASVKVSCKASGYTFTTYTIHWVRX$_4$PPGKX$_5$LEWIGYINPSSGCSDYNQN FX$_6$GRX$_7$TITADKSTX$_8$TAYMELSSLX$_9$SEDX$_{10}$AVYYCARRADYGNYEYTWFAYWGQGTTVTVSSAS TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK wherein X_1 = V or Q, X_2 = S or A, X_3 = K or A, X_4 = R or Q, X_5 = D or G, X_6 = K or Q, X_7 = T or V, X_8 = S or N, X_9 = R or T, X_{10} = T or S
120	VH3-5Con IgG1 HC	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRX$_{17}$PPGKX$_{18}$LEWIGYINPSSGCSDYNQNF X$_{19}$GRX$_{20}$TITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK wherein X_{17} = R or Q, X_{18} = D or G, X_{19} = Q or K, X_{20} = V or T
121	VK1 LC	VIELTQSPSFLSASVGDRVNVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSG SGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
122	VK2 LC	VIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
123	VK3 LC	DIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
124	VK4 LC	DIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
125	VK5 LC	VIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSG

		SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
126	VK1-3Con LC	X_{11}IX_{12}LTQSPSFLSASVGDRVX_{13}VTX_{14}KASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRF X_{15}GSGSGTDFTLTISSLQX_{16}EDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY ACEVTHQGLSSPVTKSFNRGEC wherein X_{11} = V or D, X_{12} = E or Q, X_{13} = T or N, X_{14} = Y or C, X_{15} = T or S, X_{16} = P or S
127	VK2-3Con LC	X_{21}IX_{22}LTQSPSFLSASVGDRVTVTX_{23}KASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFX $_{23}$GSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC wherein X_{21} = V or D, X_{22} = E or Q, X_{23} = Y or C, X_{24} = T or S
128	Human IgG1 CH2-CH3 spacer	PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
129	Human IgG1 CH2-CH3 spacer variant	PCPAPPVAGPSVFLFPPKPKDTLMIARTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPK
130	Human IgG2 CH2-CH3 spacer	ESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDG VEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
131	OX40 spacer	ASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEV
132	41BB spacer	SPADLSPGASSVTPPAPAREPGHSP
133	CD96 spacer	FLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRG FNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKT
134	CD44 spacer	DVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATT
135	Human CD28 transmembrane domain	FWVLVVVGGVLACYSLLVTVAFIIFWV
136	Human CD3ζ transmembrane domain	LCYLLDGILFIYGVILTALFL
137	Human CD8α transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYCNHRN
138	Human CD28 intracellular domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
139	Human CD28	RSKRSRLLHSDYMNMTPRRPGPTRKHYQAYAAARDFAAYRS

	intracellular domain with mutated lck binding site	
140	Human 4-1BB intracellular domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
141	ITAM consensus	YXXL/I wherein X = any amino acid
142	Larger ITAM consensus	YXXL/I(X)$_{6-8}$YXXL/I wherein X = any amino acid
143	Human CD3ζ intracellular domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
144	Signal peptide 1	MDFQVQIFSFLLISASVIMS
145	Signal peptide 2	MALPVTALLLPLALLLHAARP
146	Signal peptide 3	MDFQVQIFSFLLISASVIMSRMA
147	CAR signalling domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
148	CAR transmembrane and intracellular domains	FWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
149	HRS3scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
150	HRS3scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

151	HRS3scFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
152	HRS3scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
153	HRS3scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
154	VH3VK3scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
155	VH3VK3scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
156	VH3VK3scFv-	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQ

	41BBspcr-CD28TMD-CD28ICD-CD3ζICD	NFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
157	VH3VK3scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
158	VH3VK3scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
159	VH4VK2scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
160	VH4VK2scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
161	VH4VK2scFv-	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ

	41BBspcr- CD28TMD- CD28ICD- CD3ζICD	GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGAS SVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRP GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR
162	VH4VK2scFv- CD96spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTD PPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGT DTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLA CYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
163	VH4VK2scFv- CD44spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSER SSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
164	VH4VK3scFv- IgG2spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPS CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREE QFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM NMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR
165	VH4VK3scFv- OX40spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICED RDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTV AFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR
166	VH4VK3scFv- 41BBspcr-	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG

	CD28TMD- CD28ICD- CD3ζICD	GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGAS SVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRP GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR
167	VH4VK3scFv- CD96spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTD PPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGT DTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLA CYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
168	VH4VK3scFv- CD44spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSER SSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
169	VH5VK2scFv- IgG2spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQ NFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS SGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPK VLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRS DPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY VDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVL ACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFS RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD KMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
170	VH5VK2scFv- OX40spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICED RDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTV AFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR
171	VH5VK2scFv- 41BBspcr- CD28TMD-	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS

	CD28ICD- CD3ζICD	GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGAS SVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRP GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR
172	VH5VK2scFv- CD96spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTD PPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGT DTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLA CYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADA PAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI GMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
173	VH5VK2scFv- CD44spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSER SSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHD GLYQGLSTATKDTYDALHMQALPPR
174	VH5VK3scFv- IgG2spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPS CPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREE QFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM NMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT YDALHMQALPPR
175	VH5VK3scFv- OX40spcr- CD28TMD- CD28ICD- CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICED RDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTV AFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQ NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERR RGKGHDGLYQGLSTATKDTYDALHMQALPPR
176	VH5VK3scFv- 41BBspcr- CD28TMD- CD28ICD-	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGAS

	CD3ζICD	SVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
177	VH5VK3scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
178	VH5VK3scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTYKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
179	HRS3 hIgG1 HC	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
180	HRS3 hK LC	VIELTQSPKFMSTSVGDRVNVTYKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
181	VK0Cys	VIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIK
182	VK1Cys	VIELTQSPSFLSASVGDRVNVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
183	VK2Cys	VIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
184	VK3Cys	DIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
185	VK4Cys	DIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
186	VK5Cys	VIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
187	VK0Cys LC-FR1	VIELTQSPKFMSTSVGDRVNVTCKAS

188	VK1Cys LC-FR1	VIELTQSPSFLSASVGDRVNVTCKAS
189	VK2Cys, VK5Cys LC-FR1	VIELTQSPSFLSASVGDRVTVTCKAS
190	VK3Cys, VK4Cys LC-FR1	DIQLTQSPSFLSASVGDRVTVTCKAS
191	HRS3Cys scFv	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIK
192	VH1VK1Cys scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
193	VH1VK2Cys scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
194	VH1VK3Cys scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
195	VH1VK4Cys scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
196	VH1VK5Cys scFv	QVQLQQSGAELAKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
197	VH2VK1Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
198	VH2VK2Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
199	VH2VK3Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
200	VH2VK4Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK

201	VH2VK5Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTNTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
202	VH3VK1Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
203	VH3VK2Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
204	VH3VK3Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
205	VH3VK4Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
206	VH3VK5Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
207	VH4VK1 scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVNVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
208	VH4VK2Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
209	VH4VK3Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
210	VH4VK4Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
211	VH4VK5Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
212	VH5VK1Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ

		GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVNVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIK
213	VH5VK2Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
214	VH5VK3Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYS GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
215	VH5VK4Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRES GVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
216	VH5VK5Cys scFv	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQ GRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRES GVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIK
217	VK1Cys LC	VIELTQSPSFLSASVGDRVNVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSG SGTDFTLTISSLQSEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
218	VK2Cys LC	VIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
219	VK3Cys LC	DIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGS GSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
220	VK4Cys LC	DIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFSGS GSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC
221	VK5Cys LC	VIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRESGVPDRFTGSG SGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ GLSSPVTKSFNRGEC
222	HRS3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFK GKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSG GGGSGGGGSVIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRY SGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPAESKYGPPCP SCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREE QFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTK

		NQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
223	HRS3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
224	HRS3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
225	HRS3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
226	HRS3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLQQSGAELARPGASVKMSCKASGYTFTTYTIHWVRRRPGHDLEWIGYINPSSGCSDYNQNFKGKTTLTADKSSNTAYMQLNSLTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
227	VH3VK3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV

		MHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
228	VH3VK3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
229	VH3VK3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
230	VH3VK3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
231	VH3VK3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFKGRTTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
232	VH4VK2CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYM

		NMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
233	VH4VK2CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
234	VH4VK2CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
235	VH4VK2CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
236	VH4VK2CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
237	VH4VK3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLD

		KRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
238	VH4VK3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
239	VH4VK3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
240	VH4VK3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
241	VH4VK3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRRPPGKDLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
242	VH5VK2CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT

		YDALHMQALPPR
243	VH5VK2CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
244	VH5VK2CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
245	VH5VK2CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
246	VH5VK2CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSVIELTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
247	VH5VK3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAESKYGPPCPSCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFQSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

248	VH5VK3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAASNSSDAICEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
249	VH5VK3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPASPADLSPGASSVTPPAPAREPGHSPKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
250	VH5VK3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPAFLLGSEISSTDPPLSVTESTLDTQPSPASSVSPARYPATSSVTLVDVSALRPNTTPQPSNSSMTTRGFNYPWTSSGTDTKKSVSRIPSETYSSSPSGAGSTLHDNVFTSTARAFSEVPTTANGSTKTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
251	VH5VK3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	QVQLVQSGSELKKPGASVKVSCKASGYTFTTYTIHWVRQPPGKGLEWIGYINPSSGCSDYNQNFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARRADYGNYEYTWFAYWGQGTTVTVSSSGGGSGGGGSGGGGSDIQLTQSPSFLSASVGDRVTVTCKASQNVGTNVAWFQQKPGKAPKVLIYSASYRYSGVPDRFSGSGSGTDFTLTISSLQPEDFAEYFCQQYHTYPLTFGGGTKVEIKRSDPADVSSGSSSERSSTSGGYIFYTFSTVHPIPDEDSPWITDSTDRIPATTKDPKFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
252	HRS3Cys hK LC	VIELTQSPKFMSTSVGDRVNVTCKASQNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTISNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Table A

Antibody	Column A			Column B		
	VH			VL		
	HC-CDR1	HC-CDR2	HC-CDR3	LC-CDR1	LC-CDR2	LC-CDR3
HRS3	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK1	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK2	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK3	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK4	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK5	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK1	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK2	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK3	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK4	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK5	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK1	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK2	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK3	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK4	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK5	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK1	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK2	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK3	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK4	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK5	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK1	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK2	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12

VH5VK3	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK4	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK5	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
HRS3 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK1 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK2 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK3 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK4 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1VK5 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK1 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK2 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK3 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK4 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH2VK5 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK1 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK2 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK3 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK4 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3VK5 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK1 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK2 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK3 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK4 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH4VK5 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK1 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK2 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK3 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH5VK4 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12

VH5VK5 Cys	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH1-5ConVK1-3Con	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12
VH3-5ConVK2-3Con	SEQ ID NO:2	SEQ ID NO:3	SEQ ID NO:4	SEQ ID NO:10	SEQ ID NO:11	SEQ ID NO:12

Table B

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Antibody	Column A				Column B			
	VH				VL			
	HC-FR1	HC-FR2	HC-FR3	HC-FR4	LC-FR1	LC-FR2	LC-FR3	LC-FR4
HRS3	SEQ ID NO:5	SEQ ID NO:6	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:13	SEQ ID NO:14	SEQ ID NO:15	SEQ ID NO:16
VH1VK1	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:31	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH1VK2	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH1VK3	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH1VK4	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH1VK5	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH2VK1	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:31	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH2VK2	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH2VK3	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH2VK4	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH2VK5	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH3VK1	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:31	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH3VK2	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH3VK3	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH3VK4	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH3VK5	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH4VK1	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:31	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH4VK2	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH4VK3	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34

VH4VK4	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH4VK5	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH5VK1	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:31	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH5VK2	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH5VK3	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH5VK4	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:39	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH5VK5	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:36	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
HRS3 Cys	SEQ ID NO:5	SEQ ID NO:6	SEQ ID NO:7	SEQ ID NO:8	SEQ ID NO:187	SEQ ID NO:14	SEQ ID NO:15	SEQ ID NO:16
VH1VK1 Cys	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:188	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH1VK2 Cys	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH1VK3 Cys	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH1VK4 Cys	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH1VK5 Cys	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:20	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH2VK1 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:188	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH2VK2 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH2VK3 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH2VK4 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH2VK5 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:23	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH3VK1 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:188	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH3VK2 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH3VK3 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH3VK4 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH3VK5 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:25	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH4VK1 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:188	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH4VK2 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH4VK3 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH4VK4 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH4VK5 Cys	SEQ ID NO:22	SEQ ID NO:19	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34

VH5VK1 Cys	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:188	SEQ ID NO:32	SEQ ID NO:33	SEQ ID NO:34
VH5VK2 Cys	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:37	SEQ ID NO:34
VH5VK3 Cys	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:40	SEQ ID NO:34
VH5VK4 Cys	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:190	SEQ ID NO:32	SEQ ID NO:42	SEQ ID NO:34
VH5VK5 Cys	SEQ ID NO:22	SEQ ID NO:29	SEQ ID NO:27	SEQ ID NO:8	SEQ ID NO:189	SEQ ID NO:32	SEQ ID NO:44	SEQ ID NO:34
VH1-5ConVK1-3Con	SEQ ID NO:46	SEQ ID NO:47	SEQ ID NO:48	SEQ ID NO:8	SEQ ID NO:50	SEQ ID NO:32	SEQ ID NO:51	SEQ ID NO:34
VH3-5ConVK2-3Con	SEQ ID NO:22	SEQ ID NO:47	SEQ ID NO:48	SEQ ID NO:8	SEQ ID NO:55	SEQ ID NO:32	SEQ ID NO:56	SEQ ID NO:34

Table C

	Column A	Column B
Antibody	**VH**	**VL**
HRS3	SEQ ID NO:1	SEQ ID NO:9
VH1VK1	SEQ ID NO:17	SEQ ID NO:30
VH1VK2	SEQ ID NO:17	SEQ ID NO:35
VH1VK3	SEQ ID NO:17	SEQ ID NO:38
VH1VK4	SEQ ID NO:17	SEQ ID NO:41
VH1VK5	SEQ ID NO:17	SEQ ID NO:43
VH2VK1	SEQ ID NO:21	SEQ ID NO:30
VH2VK2	SEQ ID NO:21	SEQ ID NO:35
VH2VK3	SEQ ID NO:21	SEQ ID NO:38
VH2VK4	SEQ ID NO:21	SEQ ID NO:41
VH2VK5	SEQ ID NO:21	SEQ ID NO:43
VH3VK1	SEQ ID NO:24	SEQ ID NO:30
VH3VK2	SEQ ID NO:24	SEQ ID NO:35
VH3VK3	SEQ ID NO:24	SEQ ID NO:38
VH3VK4	SEQ ID NO:24	SEQ ID NO:41
VH3VK5	SEQ ID NO:24	SEQ ID NO:43
VH4VK1	SEQ ID NO:26	SEQ ID NO:30
VH4VK2	SEQ ID NO:26	SEQ ID NO:35
VH4VK3	SEQ ID NO:26	SEQ ID NO:38
VH4VK4	SEQ ID NO:26	SEQ ID NO:41
VH4VK5	SEQ ID NO:26	SEQ ID NO:43
VH5VK1	SEQ ID NO:28	SEQ ID NO:30
VH5VK2	SEQ ID NO:28	SEQ ID NO:35
VH5VK3	SEQ ID NO:28	SEQ ID NO:38
VH5VK4	SEQ ID NO:28	SEQ ID NO:41
VH5VK5	SEQ ID NO:28	SEQ ID NO:43
HRS3 Cys	SEQ ID NO:1	SEQ ID NO:181
VH1VK1 Cys	SEQ ID NO:17	SEQ ID NO:182
VH1VK2 Cys	SEQ ID NO:17	SEQ ID NO:183
VH1VK3 Cys	SEQ ID NO:17	SEQ ID NO:184
VH1VK4 Cys	SEQ ID NO:17	SEQ ID NO:185
VH1VK5 Cys	SEQ ID NO:17	SEQ ID NO:186
VH2VK1 Cys	SEQ ID NO:21	SEQ ID NO:182
VH2VK2 Cys	SEQ ID NO:21	SEQ ID NO:183
VH2VK3 Cys	SEQ ID NO:21	SEQ ID NO:184
VH2VK4 Cys	SEQ ID NO:21	SEQ ID NO:185
VH2VK5 Cys	SEQ ID NO:21	SEQ ID NO:186
VH3VK1 Cys	SEQ ID NO:24	SEQ ID NO:182
VH3VK2 Cys	SEQ ID NO:24	SEQ ID NO:183

VH3VK3 Cys	SEQ ID NO:24	SEQ ID NO:184
VH3VK4 Cys	SEQ ID NO:24	SEQ ID NO:185
VH3VK5 Cys	SEQ ID NO:24	SEQ ID NO:186
VH4VK1 Cys	SEQ ID NO:26	SEQ ID NO:182
VH4VK2 Cys	SEQ ID NO:26	SEQ ID NO:183
VH4VK3 Cys	SEQ ID NO:26	SEQ ID NO:184
VH4VK4 Cys	SEQ ID NO:26	SEQ ID NO:185
VH4VK5 Cys	SEQ ID NO:26	SEQ ID NO:186
VH5VK1 Cys	SEQ ID NO:28	SEQ ID NO:182
VH5VK2 Cys	SEQ ID NO:28	SEQ ID NO:183
VH5VK3 Cys	SEQ ID NO:28	SEQ ID NO:184
VH5VK4 Cys	SEQ ID NO:28	SEQ ID NO:185
VH5VK5 Cys	SEQ ID NO:28	SEQ ID NO:186
VH1-5ConVK1-3Con	SEQ ID NO:45	SEQ ID NO:49
VH3-5ConVK2-3Con	SEQ ID NO:52	SEQ ID NO:54

Table D

	Column A	Column B
Name	**Heavy Chain**	**Light Chain**
HRS3 hIgG1	SEQ ID NO:179	SEQ ID NO:180
VH1VK1 hIgG1	SEQ ID NO:114	SEQ ID NO:121
VH1VK2 hIgG1	SEQ ID NO:114	SEQ ID NO:122
VH1VK3 hIgG1	SEQ ID NO:114	SEQ ID NO:123
VH1VK4 hIgG1	SEQ ID NO:114	SEQ ID NO:124
VH1VK5 hIgG1	SEQ ID NO:114	SEQ ID NO:125
VH2VK1 hIgG1	SEQ ID NO:115	SEQ ID NO:121
VH2VK2 hIgG1	SEQ ID NO:115	SEQ ID NO:122
VH2VK3 hIgG1	SEQ ID NO:115	SEQ ID NO:123
VH2VK4 hIgG1	SEQ ID NO:115	SEQ ID NO:124
VH2VK5 hIgG1	SEQ ID NO:115	SEQ ID NO:125
VH3VK1 hIgG1	SEQ ID NO:116	SEQ ID NO:121
VH3VK2 hIgG1	SEQ ID NO:116	SEQ ID NO:122
VH3VK3 hIgG1	SEQ ID NO:116	SEQ ID NO:123
VH3VK4 hIgG1	SEQ ID NO:116	SEQ ID NO:124
VH3VK5 hIgG1	SEQ ID NO:116	SEQ ID NO:125
VH4VK1 hIgG1	SEQ ID NO:117	SEQ ID NO:121
VH4VK2 hIgG1	SEQ ID NO:117	SEQ ID NO:122
VH4VK3 hIgG1	SEQ ID NO:117	SEQ ID NO:123
VH4VK4 hIgG1	SEQ ID NO:117	SEQ ID NO:124
VH4VK5 hIgG1	SEQ ID NO:117	SEQ ID NO:125
VH5VK1 hIgG1	SEQ ID NO:118	SEQ ID NO:121
VH5VK2 hIgG1	SEQ ID NO:118	SEQ ID NO:122
VH5VK3 hIgG1	SEQ ID NO:118	SEQ ID NO:123
VH5VK4 hIgG1	SEQ ID NO:118	SEQ ID NO:124
VH5VK5 hIgG1	SEQ ID NO:118	SEQ ID NO:125
HRS3 Cys hIgG1	SEQ ID NO:179	SEQ ID NO:252
VH1VK1 Cys hIgG1	SEQ ID NO:114	SEQ ID NO:217
VH1VK2 Cys hIgG1	SEQ ID NO:114	SEQ ID NO:218
VH1VK3 Cys hIgG1	SEQ ID NO:114	SEQ ID NO:219
VH1VK4 Cys hIgG1	SEQ ID NO:114	SEQ ID NO:220
VH1VK5 Cys hIgG1	SEQ ID NO:114	SEQ ID NO:221
VH2VK1 Cys hIgG1	SEQ ID NO:115	SEQ ID NO:217
VH2VK2 Cys hIgG1	SEQ ID NO:115	SEQ ID NO:218
VH2VK3 Cys hIgG1	SEQ ID NO:115	SEQ ID NO:219
VH2VK4 Cys hIgG1	SEQ ID NO:115	SEQ ID NO:220
VH2VK5 Cys hIgG1	SEQ ID NO:115	SEQ ID NO:221
VH3VK1 Cys hIgG1	SEQ ID NO:116	SEQ ID NO:217
VH3VK2 Cys hIgG1	SEQ ID NO:116	SEQ ID NO:218
VH3VK3 Cys hIgG1	SEQ ID NO:116	SEQ ID NO:219

VH3VK4 Cys hIgG1	SEQ ID NO:116	SEQ ID NO:220
VH3VK5 Cys hIgG1	SEQ ID NO:116	SEQ ID NO:221
VH4VK1 Cys hIgG1	SEQ ID NO:117	SEQ ID NO:217
VH4VK2 Cys hIgG1	SEQ ID NO:117	SEQ ID NO:218
VH4VK3 Cys hIgG1	SEQ ID NO:117	SEQ ID NO:219
VH4VK4 Cys hIgG1	SEQ ID NO:117	SEQ ID NO:220
VH4VK5 Cys hIgG1	SEQ ID NO:117	SEQ ID NO:221
VH5VK1 Cys hIgG1	SEQ ID NO:118	SEQ ID NO:217
VH5VK2 Cys hIgG1	SEQ ID NO:118	SEQ ID NO:218
VH5VK3 Cys hIgG1	SEQ ID NO:118	SEQ ID NO:219
VH5VK4 Cys hIgG1	SEQ ID NO:118	SEQ ID NO:220
VH5VK5 Cys hIgG1	SEQ ID NO:118	SEQ ID NO:221
VH1-5ConVK1-3Con IgG1	SEQ ID NO:119	SEQ ID NO:126
VH3-5ConVK2-3Con IgG1	SEQ ID NO:120	SEQ ID NO:127

***

The present disclosure includes the combination of the aspects and preferred features described except where such a combination is clearly impermissible or expressly avoided.

5 The section headings used herein are for organisational purposes only and are not to be construed as limiting the subject matter described.

Aspects and embodiments of the present disclosure will now be illustrated, by way of example, with reference to the accompanying figures. Further aspects and embodiments will be apparent to those
10 skilled in the art. All documents mentioned in this text are incorporated herein by reference.

Throughout this specification, including the claims which follow, unless the context requires otherwise, the word 'comprise,' and variations such as 'comprises' and 'comprising,' will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer
15 or step or group of integers or steps.

As used herein, an amino acid sequence, or a region of a polypeptide which 'corresponds' to a specified reference amino acid sequence or region of a polypeptide has at least 60%, *e.g.* one of at least 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence
20 identity to the amino acid sequence of the amino acid sequence/polypeptide/region. An amino acid sequence/region/position of a polypeptide/amino acid sequence which 'corresponds' to a specified reference amino acid sequence/region/position of a polypeptide/amino acid sequence can be identified by sequence alignment of the subject sequence to the reference sequence, *e.g.* using sequence alignment software such as ClustalOmega (Söding, J. 2005, Bioinformatics 21, 951-960).
25

It must be noted that, as used in the specification and the appended claims, the singular forms 'a,' 'an,' and 'the' include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from 'about' one particular value, and/or to 'about' another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular
30 value. Similarly, when values are expressed as approximations, by the use of the antecedent 'about,' it will be understood that the particular value forms another embodiment.

Where a nucleic acid sequence is disclosed herein, the reverse complement thereof is also expressly contemplated.
35

Methods described herein may preferably be performed *in vitro*. The term '*in vitro*' is intended to encompass procedures performed with cells in culture whereas the term '*in vivo*' is intended to encompass procedures with/on intact multi-cellular organisms.

Brief Description of the Figures

Embodiments and experiments illustrating the principles of the present disclosure will now be discussed with reference to the accompanying figures.

Figures 1A and 1B. Single cycle kinetic raw sensorgrams and fitted curves with a Langmuir 1:1 model for (**1A**) HRS-3 scFv (VH0VK0), (**1B**) representative variants that retained binding to CD30, and (**1C**) representative variants that do not bind to CD30.

Figures 2A and 2B. Dose dependent binding curves based on mean fluorescence of scFv-mFcs binding to (**2A**) CD30 expressing KM-H2 cells and (**2B**) CD30 negative Raji cells.

Figures 3A to 3C. Expression and efficacy of humanised CD30 CARs. (**3A**) Activated T cells were transduced at varying efficiencies to express the humanised CD30 CARs. (**3B** and **3C**) Humanised CD30 CARs were able to specifically kill CD30 expressing KM-H2 cells (**3B**) without significant non-specific cytotoxicity with control Raji cells (**3C**). Data presented are mean ± SD of at least 3 independent donors.

Figures 4A to 4C. Expression and efficacy of humanised VH3VK3 and VH5VK3 variants with cysteine mutations. (**4A**) Activated T cells were transduced at varying efficiencies to express the humanised CD30 CARs. (**4B**) Humanised CD30 CARs were able to specifically kill CD30 expressing KM-H2 cells an effectors to target ratio of 1:1. (**4C**) Introduction of Cys23 in light chain resulted in lower level of cytokine secretion in resting CAR T cells. Data presented are mean ± SD of 3 independent donors.

Figures 5A to 5C. Expression and functional assessment of CARs expressing novel 4-1BB spacer with humanised CD30 scFv. (**5A**) Expression of humanised CD30 CARs with the 4-1BB spacer on surface of transduced primary T cells. (**5B**) Cytotoxic efficacy of CAR T cells expressing CARs with 4-1BB spacer. (**5C**) Levels of secretion of GM-CSF and TNF-α by resting CAR T cells expressing humanised CARs with 4-1BB.

Figures 6A to 6E. Generation and characterisation of T cells bearing humanised CD30 CAR with the 4-1BB spacer. (**6A**) Fold expansion in culture of untransduced T cells and T cells expressing HRS3-41BB, VH3Vk3-Cys-41BB, or VH5Vk3-Cys-41BB. (**6B**) Transduction efficiency of the CD30CAR variants at day 11. (**6C**) Ratio of CD4 T cells to CD8 T cells in T cells transduced with the CD30CAR variants at day 11. Each color represents a unique donor that is consistent across panels A-C. (**6D**) CD30 expression detected by the BerH8 and BY88 clones on total T cells over the culture period to demonstrate CD30 masking in *cis* by the CAR. (**6E**) Proportions of Tim3, PD-1, LAG3 expressing cells and combinations thereof among CD4 and CD8 T cells in the day 11 final product.

Figures 7A to 7D. *In vitro* anti-tumor activity of T cells bearing humanised CD30 CAR with the 4-1BB spacer. (**7A**) Cytolysis of KM-H2 and HuT-78 cells were assessed using the xCELLigence Real-Time Cell Analysis system at 2:1 E:T ratio for 4 and 5 donors respectively. (**7B**) Serial killing assay set-up. See

Example 1. (**7C**) Serial killing cytolysis for 3 donors. (**7D**) Total T cell numbers at the end of each serial killing encounter.

Figures 8A to 8F. *In vitro* on-target, off-tumor activity of T cells bearing humanised CD30 CAR with the 4-1BB spacer. (**8A**) CD30 expression on HSPCs from 2 donors over 12 days of stimulation with 8 ng/mL FLT3L, SCF and TPO. (**8B**) CD30 expression on KM-H2 cells. (**8C**) CD30 expression on HSPC subsets after 2 days of stimulation. (**8D**) Impact of CD30 CAR T cell exposure on the survival of HSPC subsets. Two HSPC donors and two T cell donors were combined to obtain four co-culture combinations for **8D** to **8F**. (**8E**) Effect of CD30 CAR T cell exposure on the erythroid and myeloid developmental potential of HSPCs. (**8F**) Effect of priming CD30 CAR T cells with CD30-high KM-H2 targets on cytolysis of CD30-low HSPCs.

Figures 9A to 9G. *In vivo* persistency, efficacy, and toxicity of humanised CD30 CAR T cells in GM-CSF and IL-3-supplemented humanised mice xenografted with peripheral T cell lymphoma. (**9A**) Experiment set up of HuT-78 model. (**9B**) Tumor volume, tumor volume changes and endpoint tumor count. (**11C**) IVIS monitoring twice a week to track T cell biodistribution. (**9D**) Survival curves of mice following treatment. (**9E-9F**)) Immune cell subsets present in organs at endpoint. (**9G**) Cytokine levels in mice plasma at 8 days post treatment.

Figures 10A to 10F. *In vivo* persistency, efficacy, and toxicity of humanised CD30 CAR T cells in GM-CSF and IL-3-supplemented humanised mice xenografted with CD30high NALM-6. (**10A**) Experiment set up of NALM-6 model. (**10B**) Body weight changes in mice. (**10C**) IVIS monitoring twice a week to track NALM-6 disease (**10D-10E**) Immune cell subsets present in organs at endpoint. (**10F**) Cytokine levels in mice plasma at 11 days post treatment.

Figures 11A to 11C. Characterisation of EBVSTs transduced to express humanised CD30 CARs. (**11A**) Expression and transduction efficiency of **CD30** CARs in EBVSTs. (**11B**) Masking efficiency of CD30 CARs in EBVSTs. (**11C**) Cytotoxic efficacy and specificity of CD30 CAR EBVSTs with CD30+ KM-H2 and CD30- Daudi cells.

Examples

In the following Examples, the inventors describe the generation and characterisation of novel CD30-binding antigen-binding molecules, and novel CAR constructs employing such antigen-binding molecules as their antigen-binding domain.

Example 1: Materials and Methods

1.1 Humanisation of murine HRS-3 scFv using Composite Human Antibody™ Technology

Humanisation of the murine HRS-3 scFv was done using Abzena's Composite Human Antibody™ technology, combining germline humanisation (15) and deimmunisation (16) to generate variants with reduced immunogenicity. Briefly, structural models of the murine HRS-3 scFv were analysed to identify amino acid residues critical for the conformation of the scFv and binding to CD30. Suitable

sequence segments of human antibodies were then identified as building blocks that can be used to recreate the identical or similar complementarity determining regions (CDRs) as those of HRS-3, to best preserve binding specificity and affinity. A preliminary list of possible variants constructed from the assembly of these sequence segments was then analysed *in silico* for possible peptides binding
5 to human class II alleles (17). Finally, 5 heavy chain and 5 light chain variants were shortlisted that are either devoid of, or have significantly reduced numbers of T cell epitopes.

Molecule	**Name**	**Amino acid sequence**
[1]	VH0VK0 (HRS3) scFv	SEQ ID NO:59
[2]	VH1VK1 scFv	SEQ ID NO:60
[3]	VH1VK2 scFv	SEQ ID NO:61
[4]	VH1VK3 scFv	SEQ ID NO:62
[5]	VH1VK4 scFv	SEQ ID NO:63
[6]	VH1VK5 scFv	SEQ ID NO:64
[7]	VH2VK1 scFv	SEQ ID NO:65
[8]	VH2VK2 scFv	SEQ ID NO:66
[9]	VH2VK3 scFv	SEQ ID NO:67
[10]	VH2VK4 scFv	SEQ ID NO:68
[11]	VH2VK5 scFv	SEQ ID NO:69
[12]	VH3VK1 scFv	SEQ ID NO:70
[13]	VH3VK2 scFv	SEQ ID NO:71
[14]	VH3VK3 scFv	SEQ ID NO:72
[15]	VH3VK4 scFv	SEQ ID NO:73
[16]	VH3VK5 scFv	SEQ ID NO:74
[17]	VH4VK1 scFv	SEQ ID NO:75
[18]	VH4VK2 scFv	SEQ ID NO:76
[19]	VH4VK3 scFv	SEQ ID NO:77
[20]	VH4VK4 scFv	SEQ ID NO:78
[21]	VH4VK5 scFv	SEQ ID NO:79
[22]	VH5VK1 scFv	SEQ ID NO:80
[23]	VH5VK2 scFv	SEQ ID NO:81
[24]	VH5VK3 scFv	SEQ ID NO:82
[25]	VH5VK4 scFv	SEQ ID NO:83
[26]	VH5VK5 scFv	SEQ ID NO:84
[27]	VH0VK0 (HRS3) Cys scFv	SEQ ID NO:191
[28]	VH1VK1 Cys scFv	SEQ ID NO:192
[29]	VH1VK2 Cys scFv	SEQ ID NO:193
[30]	VH1VK3 Cys scFv	SEQ ID NO:194
[31]	VH1VK4 Cys scFv	SEQ ID NO:195
[32]	VH1VK5 Cys scFv	SEQ ID NO:196

[33]	VH2VK1 Cys scFv	SEQ ID NO:197
[34]	VH2VK2 Cys scFv	SEQ ID NO:198
[35]	VH2VK3 Cys scFv	SEQ ID NO:199
[36]	VH2VK4 Cys scFv	SEQ ID NO:200
[37]	VH2VK5 Cys scFv	SEQ ID NO:201
[38]	VH3VK1 Cys scFv	SEQ ID NO:202
[39]	VH3VK2 Cys scFv	SEQ ID NO:203
[40]	VH3VK3 Cys scFv	SEQ ID NO:204
[41]	VH3VK4 Cys scFv	SEQ ID NO:205
[42]	VH3VK5 Cys scFv	SEQ ID NO:206
[43]	VH4VK1 Cys scFv	SEQ ID NO:207
[44]	VH4VK2 Cys scFv	SEQ ID NO:208
[45]	VH4VK3 Cys scFv	SEQ ID NO:209
[46]	VH4VK4 Cys scFv	SEQ ID NO:210
[47]	VH4VK5 Cys scFv	SEQ ID NO:211
[48]	VH5VK1 Cys scFv	SEQ ID NO:212
[49]	VH5VK2 Cys scFv	SEQ ID NO:213
[50]	VH5VK3 Cys scFv	SEQ ID NO:214
[51]	VH5VK4 Cys scFv	SEQ ID NO:215
[52]	VH5VK5 Cys scFv	SEQ ID NO:216

1.2 Expression of recombinant humanised scFv-mFcs fusion proteins.

25 humanised scFvs representing the different combinations of the 5 heavy and 5 light chain variants were expressed as a fusion protein to mouse IgG2b-Fc. Small scale production was done by transient transfection of CHO cells using the TransIT-Pro cell reagent (MirusBio LLC, Madison USA). Briefly, 3×10^6 cells were aliquoted into 50 ml spin tubes in a final volume of 5 ml. Transfections were carried out using 1.5 µg of DNA per 1 $\times10^6$ cells. The DNA was diluted in 500 µl of OptiMEM, to which TransIT-PRO reagent added at a 1 to 1 ratio, reagent: DNA, followed by a 5 minute incubation at room temperature before addition to the cells. All transfections were carried out in FreeStyle CHO medium containing 8 mM L-Glutamine (ThermoFisher, Loughborough, UK) and 1x Hypoxanthine-Thymidine (ThermoFisher, Loughborough, UK). Cultures were harvested after 7 days and filtered using 0.2 µM filter systems (Corning, New York, US). The expressed scFv-mFc were then quantified on the Octet QK 384 using Protein A biosensors (Molecular Devices, Wokingham, Berkshire, UK). Large scale production of 5 shortlisted lead candidcates was done by transient transfection of CHO cells using the MaxCyte STX® electroporation system (MaxCyte Inc., Gaithersburg, USA). CHO supernatants were harvested 14 days post transfection, filtered using 0.2 µM filter systems (Corning, New York, US) to remove remaining cell debris and supplemented with 10xPBS to neutralise pH. The scFv-mFcs were then purified using an AKTA Pure instrument with a 1 mL Hitrap MabSelect PrismA column (Cytiva, Marlborough, USA). Purified scFv-mFcs were then

analysed on an analytical size-exclusion HPLC and further purified by size exclusion on the AKTA Pure instruction with a 16/600 Superdex 200 column, when necessary.

1.3 Binding assessment of scFv-mFcs

Binding of scFv-mFcs to recombinant CD30 protein was assessed by surface plasmon resonance using two different analysis protocols. Single cycle kinetic analysis was done to screen the initial 25 humanised scFv variants. Subsequently, multi-cycle kinetic analysis was done to compare the original HRS-3 scFv with the 5 lead humanised variants. All kinetic experiments were done on a Biacore T200 (Cytiva, Uppsala, Sweden). HBS-P+ supplemented with 1%BSA w/v (Cytiva, Marlborough, USA) was used as the running buffer and dilution buffer for the analyte and ligand. For the scFv-mFc in the filtered culture supernatants or purified lead scFv-mFc were captured at a flow rate of 10μL/min onto a series S mouse antibody capture sensor chip (Cytiva, Marlborough, USA) to an immobilisation level of around 140 response units (RU).

For the single cycle kinetic analysis, recombinant CD30 was injected at a flow rate of 30μL/min at concentrations between 37.5nM to 300nM in a four point, two-fold dilution series. Recombinant CD30 was allowed to associate for 210 seconds for each of the four injections of increasing concentrations without regeneration, and allowed to dissociate for 600 seconds after the last injection of antigen. A reference channel with no scFv-mFc captured was used to correct for bulk effect and non-specific binding while a blank run (no antigen flowed) was used to correct for surface stability. The double referenced sensorgrams were fitted with the Langmuir (1:1) binding model to obtain the association k_a, dissociation k_d and equilibrium constant K_D, and the closeness of fit was evaluated with the Chi square value.

For multi-cycle kinetic analysis, recombinant CD30 was injected at a flow rate of 40μL/min at concentrations between 25nM to 800nM in a six point, two-fold dilution series. For each concentration, recombinant CD30 was allowed to associate for 240 seconds and dissociate for 600 seconds. Regeneration was done with 10mM glycine (pH 1). A reference channel with no scFv-mFc captured was used to correct for bulk effect and non-specific binding while a blank run (no antigen flowed) was used to correct for surface stability. The double referenced sensorgrams were fitted with the Langmuir (1:1) binding model to obtain the association k_a, dissociation k_d and equilibrium constant K_D, and the closeness of fit was evaluated with the Chi square value.

Binding of scFv-mFcs to cell surface expressed CD30 on cell lines was assessed by flow cytometry. Briefly, samples and controls were prepared in 100μL of FACs buffer (PBS + 1% BSA + 0.1% sodium azide) in a 8-fold 7 point dilution series starting at 10μL/mL. $1x10^5$ CD30 expressing KM-H2 cells and CD30 negative Raji cells were pelleted and resuspended in the diluted samples and incubated at 4°C for 30min. Brentuximab (a human anti-CD30 antibody) was used a positive control for the assay. Cells were then washed twice with FACS buffer and resuspended in 100μL of either anti-human IgG PE (Thermofisher, Loughborough, UK) or anti-mouse IgG FITC (Thermofisher, Loughborough, UK) diluted at 1:200. Cells were incubated at 4°C for 30min, washed and

resuspended in 200μL of BD CellFix solution (BD Pharmingen, Berkshire, UK). Analysis was done on an Attune NxT Flow Cytometer (Thermofisher, Loughborough, UK).

1.4 Differential scanning fluorimetry or thermal shift assay

5 A commonly used measure of protein stability is the melting temperature at which it unfolds to a denatured state. This can be measured by measuring the increase in fluorescence of Sypro Orange dye upon binding to exposed hydrophobic regions of denatured proteins. Samples of the purified scFv-mFc proteins were prepared in PBS and Sypro Orange at a final concentration of 0.5mg/mL. 9μL of each sample were subjected to a thermal gradient of 24-95°C at a ramp rate of 0.3°C/min.
10 Samples were excited at 473nm and emission spectra from 250-720nm was recorded. Area under curve between 510-680nm was used to calculate the Tm. Data acquisition and analysis were all performed on the UNcle biostability platform (Unchained Labs, Pleasanton, USA).

1.5 Plasmid constructs and retrovirus production

15 Murine and humanised CD30 scFvs were cloned into a pSFG retrovirus vector upstream of either a wildtype IgG1 Fc, OX40 or 4-1BB derived spacer, followed by CD28 transmembrane domain, a CD28 and CD3ζ signalling domains. A truncated form of the CD30 molecule that consisted only of the CD30 extracellular domain was cloned into pSFG retrovirus vector.

Molecule	CAR domains	Amino acid sequence
[53]	HRS3scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:149
[54]	HRS3scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:150
[55]	HRS3scFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:151
[56]	HRS3scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:152
[57]	HRS3scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:153
[58]	VH3VK3scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:154
[59]	VH3VK3scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:155
[60]	VH3VK3scFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:156
[61]	VH3VK3scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:157
[62]	VH3VK3scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:158
[63]	VH4VK2scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:159
[64]	VH4VK2scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:160
[65]	VH4VK2scFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:161
[66]	VH4VK2scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:162
[67]	VH4VK2scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:163
[68]	VH4VK3scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:164
[69]	VH4VK3scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:165
[70]	VH4VK3scFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:166
[71]	VH4VK3scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:167
[72]	VH4VK3scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:168

[73]	VH5VK2scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:169
[74]	VH5VK2scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:170
[75]	VH5VK2scFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:171
[76]	VH5VK2scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:172
[77]	VH5VK2scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:173
[78]	VH5VK3scFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:174
[79]	VH5VK3scFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:175
[80]	VH5VK3scFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:176
[81]	VH5VK3scFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:177
[82]	VH5VK3scFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:178
[83]	HRS3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:222
[84]	HRS3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:223
[85]	HRS3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:224
[86]	HRS3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:225
[87]	HRS3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:226
[88]	VH3VK3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:227
[89]	VH3VK3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:228
[90]	VH3VK3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:229
[91]	VH3VK3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:230
[92]	VH3VK3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:231
[93]	VH4VK2CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:232
[94]	VH4VK2CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:233
[95]	VH4VK2CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:234
[96]	VH4VK2CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:235
[97]	VH4VK2CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:236
[98]	VH4VK3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:237
[99]	VH4VK3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:238
[100]	VH4VK3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:239
[101]	VH4VK3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:240
[102]	VH4VK3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:241
[103]	VH5VK2CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:242
[104]	VH5VK2CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:243
[105]	VH5VK2CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:244
[106]	VH5VK2CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:245
[107]	VH5VK2CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:246
[108]	VH5VK3CysscFv-IgG2spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:247
[109]	VH5VK3CysscFv-OX40spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:248
[110]	VH5VK3CysscFv-41BBspcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:249
[111]	VH5VK3CysscFv-CD96spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:250
[112]	VH5VK3CysscFv-CD44spcr-CD28TMD-CD28ICD-CD3ζICD	SEQ ID NO:251

Retroviruses carrying the CD30 CARs or truncated CD30 were produced in HEK293VG or RD114 packaging cell line (BioVec Pharma, Québec, Canada) by transient transfection with the pSFG vector using PEIpro transfection reagent (Polyplus, Illkirch, FRANCE). Medium containing retroviruses were harvested at 48h and 72h post transfection and concentrated 10-fold using RetroX Concentrator (Takara Bio, Kusatsu, Shiga, Japan). The retroviruses were either used immediately or snap frozen and stored at -80°C.

The stable RD114 retrovirus packaging cell line that produces high titers of GFP-Firefly Luciferase (GFP-FFluc) virus particles was a kind gift from Dr Masataka Suzuki (Baylor Center for Gene Therapy Baylor College of Medicine).

1.6 Donors

Enriched leukapheresis products, collected from consented healthy donors by Spectra Optia® Apheresis System CMNC collection protocol and frozen in ACD-A anticoagulant, was purchased from HemaCare (Northridge, California, U.S.A.). The frozen leukopaks were thawed and PBMCs were extracted by gradient centrifugation using Ficoll-Paque PLUS (Cytiva, MA, U.S.A.). The PBMCs were either used immediately for experiments or frozen in smaller aliquots of 30-50 x 10^6 cells per cryovial in CryoStor® CS10 Cell Freezing Medium (STEMCELL Technologies, Cambridge, Massachusetts, U.S.A.).

Cord blood CD34+ cells, isolated from cord blood mononuclear cells via positive immunomagnetic separation from consented donors, were purchased from Lonza (Walkersville, Maryland, U.S.A). Frozen vials of up to 1 x 10^6 cells were thawed and CD34+ cells were either used immediately for experiments or frozen in aliquots of 5 x 10^4 cells per cryovial in CryoStor® CS10 Cell Freezing Medium (STEMCELL Technologies, Cambridge, Massachusetts, U.S.A.).

1.7 Production of CAR T cells

For activated T cells (ATC) transduction, PBMC were thawed and plated onto cell culture plates pre-coated with anti-CD3/CD28, and cultured in 10% FBS, 45% Advanced RPMI and 45% Click's media with 5% CO2 at 37°C to generate ATCs. Two days after culture, IL-7 and IL-15 were added into cell culture. On the third day of culture, retrovirus containing CD30.CAR with the indicated scFv and spacer was transduced into ATCs by spinfection. Retrovirus was washed off 24 hours later and ATCs were cultured with occasional media change to replenish the IL-7 and IL-15. For cells to be used in *in vivo* solid tumor models, ATCs were additionally transduced on the fourth day of culture with a retroviral vector encoding GFP-Firefly Luciferase (GFP-FFluc) to enable tracking of ATCs by IVIS in live mice. On day 11 post-transduction, ATCs were either frozen down using CryoStor following manufacturer's manual or injected into mice. For humanised mouse studies, ATCs were generated from CD34- cord blood cells instead of PBMCs, and later infused into humanised mice reconstituted with CD34+ cord blood cells from the same donor.

For Epstein Barr virus specific T cells transduction, CD45RA depletion of PBMCs (RAD-PBMCs, optional) was performed by negative selection using CD45RA MACS Beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Whole PBMCs or RAD-PBMCs were cultured 1×10^6 cells/well with viral peptides consisting of overlapping peptide libraries (15-mers overlapping by 11 amino acids) from JPT Technologies (Berlin, Germany). Five days later, cells were transduced with humanised CD30 CAR constructs using RetroNectin (Takara Bio, Kusatsu, Shiga, Japan), according to the description above. T cells were then stimulated with irradiated co-stimulatory cells expressing markers such as CD80, CD86, 4-1BB four days post transduction. Seven-eight days later, VSTs were harvested, frozen or used for cell assays.

1.8 Transduction efficiency and phenotyping by flow cytometry and antibody staining

Flow cytometry in this study was performed with the Aurora cytometer (Cytek Biosciences) or with the FACSymphony A3 cell analyzer (BD Biosciences). Up to 200k T-cells were stained with Live/Dead™ NIR viability dye (Thermo Fisher) and assessed for surface presentation of epitopes using fluorescently labeled monoclonal antibodies to CD3 (clone SK7, BD Biosciences), CD4 (clone SK3, BD Biosciences), CD8 (clone SK1, BioLegend), CD56 (clone B159, BD Biosciences), CD19 (clone SJ25C1, BioLegend), CD30 (clone BerH8, BD Biosciences; clone BY88, BioLegend), PD-1 (clone EH12.1, BD Biosciences), Tim3 (clone 7D3, BD Biosciences), and LAG3 (clone 11C3C65, BioLegend). CD30 CAR expression was measured with recombinant human CD30 protein fused with a His tag (10777-H08H, Sino Biological) followed by PE-conjugated anti-His (clone J095G46, BioLegend), or with biotinylated recombinant human CD30 protein (ACROBiosystems, CD0-H82E6) followed by PE-conjugated streptavidin (BD Biosciences, 554061), or with FITC-labeled recombinant human CD30 protein (ACROBiosystems, CD0-HF2H3). Flow data were analyzed and gated in FlowJo v10.8.1 for Windows.

1.9 Cytotoxicity Assay

Prior to assessment of CAR potency via cytotoxicity assay, the cell media is changed to 2% assay media containing RPMI and 2% FBS. Cytotoxicity assay was carried out using the xCelligence Real-Time Cell Analysis System with 5% CO_2 at 37°C (Agilent). Target cells will be added onto PET plates tethered with anti-CD40 antibody following manufacturer's manual (Aligent). 24 hours after target cell adherence, CAR T cells will be plated into the PET plates at CAR T: target cell ratio of 0.2:1, 1:1 or 5:1. The PET plates will return into the xCelligence system and cytotoxicity will be monitored for 48 hours.

1.10 Serial Killing Potency Assay

KM-H2 cells were used as the target cell line with an initial E:T ratio of 1:2, with 50,000 effector and 100,000 target cells per well of a 96-well plate for Encounter 1. Three sets of this set-up were prepared. After 48h, cells from 2 sets were harvested and added to 2 new sets of 100,000 KM-H2 target cells per well to set up Encounter 2. After a further 48h, cells from 1 set were harvested and added to a new set of 100,000 KM-H2 target cells per well for Encounter 3. To distinguish target cells from the different encounters, KM-H2 cells for Encounters 2 and 3 were labelled with the

lipophilic membrane dyes PKH67 and PKH26 (Sigma-Aldrich) respectively. 48h after each encounter, 1 set of cells was stained with Live/Dead™ NIR viability dye (Thermo Fisher) and fluorescently labelled monoclonal antibodies to CD3 (clone UCHT1, BD Biosciences), CD4 (clone SK3, BD Biosciences), CD8 (clone RPA-T8, BD Biosciences), CD40 (clone 5C3, BioLegend), and CD30 (clone BerH8, BD Biosciences) to enumerate the target cell populations remaining at each round of 48h culture. See Figure 7B for schematic diagram of assay set-up. Cytolysis was calculated as (KM-H2 count in target-only control well – KM-H2 count in assay well) / KM-H2 count in target-only control well.

1.11 Cytokine Release Assay

Prior to assessment of cytokine release, the cell media is changed to 2% assay media containing RPMI and 2% FBS. Target cells will be added onto 96-well flat-bottom plates. CAR T cells will be plated on top of the target cells at CAR T: target cell ratio 1:1. The plates will be incubated at 5% CO2 at 37°C for 24 hours. After 24 hours, the cell-free media will be collect by centrifugation at 500 g for 5 mins. The cytokine released in the media, such as TNFα, GMCSF and IFNγ will be assayed by ELISA kits from BioLegends.

1.12 Luminex Multiplex Assay

Milliplex map human high sensitivity T cell panel premixed 13-plex (Millipore, HSTCMAG28SPMX13) was used to determine cytokine levels in co-cultures or plasma by FLEXMAP 3D® (Luminex). Analysis was performed using Bio-plex Manager software (Bio-Rad)

1.13 *In vitro* safety assays with hematopoietic stem and progenitor cells (HSPCs)

CD34+ HSPCs were stimulated with 10 ng/mL each of Flt3-ligand (FLT3L), stem cell factor (SCF) and thrombopoietin (TPO) (all from Miltenyi Biotec) for the specified time period at 5,000 – 20,000 cells per 200µL per well of a 96-well U-bottom plate.

In co-cultures of CD34+ HSPCs and T cells, CD34+ cells were isolated by depletion of T cells using magnetic beads conjugated to an anti-CD3 antibody (Miltenyi Biotec). HSPC subsets were analyzed by flow cytometry after staining with Live/Dead™ NIR viability dye (Thermo Fisher) and fluorescently labelled monoclonal antibodies to CD34 (clone 561, BD Biosciences), CD133 (clone 7, BioLegend), CD45RA (clone HI100, BD Biosciences), CD38 (clone HIT2, BD Biosciences), CD10 (clone HI10a, BD Biosciences) following the gating strategy described in Hombach et al (18).

Erythroid and myeloid developmental potential were assessed using the StemMACS™ HSC-CFU Assay kit (Miltenyi Biotec, 130-125-042). Cells were labelled with fluorescently conjugated antibodies to CD14, CD15 and CD235a as part of the StemMACS kit antibody cocktail, and colony types were identified as per the manufacturer's protocol. Colony types include colony forming units for granulocytes (CFU-G) and macrophages (CFU-M), burst forming units for erythrocytes (BFU-E). For more primitive progenitors, CFU-GM give rise to both granulocytes and macrophages, while CFU-GEMM differentiate into all three cell populations.

1.14 IL-3/GM-CSF-supplemented humanised mice

To generate humanised mice, NSG pups were irradiated and reconstituted with 1x10^5 CD34$^+$ cells from HLA-typed cord blood donors. On week 16, human IL-3/GM-CSF encoding plasmids were delivered via hydrodynamic tail vein injection to mice with more than 20% of hCD45 reconstitution, to support myeloid cells reconstitution. Cheek bleeds were performed on humanised mice prior to experiments, for assessment of baseline human cytokine levels in serum.

1.15 Peripheral T cell lymphoma mouse model

1x10^6 of HuT-78 were injected subcutaneously into the right flank of humanised mice. On day 4 or 6, mice were injected intravenously with 1x10^6 or 4x10^6 of CAR and luciferase double transduced ATCs T cells as specified in figures. For the CD30CAR spacer studies IL-2 and mouse FcR block injection were administered in a regimen similar to the NK/T lymphoma model. Cheek bleeds were done performed at 6 days post treatment to assess the cytokine levels in the plasma 6 days post ATCs treatment. For the CD30CAR scFv studies, an additional cheek bleed was done at 6 days post treatment. T cell biodistribution was evaluated using the IVIS imaging system (Perkin Elmer). Region of interest (ROI) was drawn over the mice and measured the average radiance (p/s/cm2/sr) was quantified using Living Image®4.7.4 software. Mice were sacrificed and blood, spleen, liver, and tumor were collected for endpoint flow cytometry analysis. For the CD30CAR scFv studies, bone marrow and lung were harvested as well

1.16 CD30 positive NALM-6 systemic tumor model

Nalm6-CD30high expressing GFP-FFluc was generated by transducing Nalm6 with retroviral vectors encoding truncated CD30 and GFP-FFluc. Nalm6 expressing both CD30 and GFP was sorted twice to generate >99% double positive clones. 2x10^6 of Nalm6-CD30high cells stably expressing GFP-Firefly Luciferase (GFP-FFluc) were injected intravenously into humanised mice. Tumor engraftment was monitored using IVIS imaging system (Perkin Elmer) once a week. Mice were cheek bled 9 days before T cell treatment to assess cytokine levels in plasma post tumor engraftment. For the CD30CAR spacer studies (, mice were injected intra-peritoneally with 35mg human IVIG (Sigma-Aldrich, I4506) and in some treatment groups with 8μg/g mouse FcR block (Bio X Cell, Cat#:BE0307) for 2 days before ATC treatment. At 20 or 21 days post NALM-6 injection, 5x10^6 T cells were administered to mice via retroorbital injection. Body weight and temperature were monitored daily and tumor burden was tracked by IVIS twice a week. Cheek bleeds were performed at 2 and 7 days post treatment for the CD30CAR spacer studies, and at 3 and 6 days post treatment for the CD30CAR scFv studies. At the experiment end point, mice were sacrificed and blood, spleen, liver and bone marrow were collected for flow cytometry analysis.

Example 2: Results

2.1 Design, screening, and identification of lead humanised variants of HRS-3

Based on the structural analysis and *in silico* analysis of HRS-3 (VH0 and VK0) of potential peptide sequences binding to human MHC class II allele, five heavy chains and five light chains were

designed for gene synthesis and expression. Analysis of the sequences by Abzena's proprietary *in silico* iTope™ (17) software, looking at overlapping 9mer peptides and their possible interactions with 35 MHC class II allotypes and scored with peptide scores between 0 and 1. The potential MHC Class II epitopes are further defined by the software as promiscuous high affinity peptides that bind
5 >50% of alleles with a binding score of >0.6 whilst promiscuous moderate affinity peptides bind >50% of alleles with a binding score >0.55 (without a majority >0.6). The iTope™ scores are summarised in Table 1, and the five heavy and five light chain variants are thus named VH1 to VH5 and VK1 to VK5 respectively, in order of reducing iTope™ score and thus reducing immunogenicity.

Heavy Chain						
Potential MHC Class II epitope	VH0	VH1	VH2	VH3	VH4	VH5
Number of Promiscuous High	7	4	3	3	2	2
Number of Promiscuous Moderate	8	7	6	6	6	4
Light Chain						
Potential MHC Class II epitope	VK0	VK1	VK2	VK3	VK4	VK5
Number of Promiscuous High	5	3	2	2	1	1
Number of Promiscuous Moderate	6	2	3	3	4	4

10

Table 1. Calculated iTope™ scores for humanised heavy (VH1-VH5) and light chain variants (VK1-VK5) of HRS-3 (VH0, VK0).

Recombinant scFvs comprising of the 25 possible combinations of the heavy and light chain variants
15 were then expressed as fusion proteins to a mouse IgG2b-Fc. All but 1 (VH1VK2) scFv-mFc was able to be expressed and the 24 variants were subsequently assessed for CD30 binding by single cycle kinetic analysis by surface plasmon resonance (Figure 1). Variants containing either VK4 or VK5 appeared to have abolished ability to recognise and bind CD30. The remaining 14 variants had similar binding kinetics as the original HRS-3 (VH0VK0) (Table 2)
20

scFv	**k_a (1/Ms)**	**k_d (1/s)**	**K_D (M)**	**K_D relative to HRS3 (VH0VK0)**	**R_{Max}**	**Chi^2 (RU^2)**
VH0VK0	1.50E+04	5.05E-04	**3.36E-08**	1.00	14.5	0.217
VH1VK1	1.36E+04	7.69E-04	**5.67E-08**	1.69	14	0.0464
VH1VK2	**Did not express**					
VH1VK3	1.43E+04	8.80E-04	**6.14E-08**	1.83	9.2	0.0273
VH1VK4	**Did not bind**					
VH1VK5	**Did not bind**					
VH2VK1	1.73E+04	9.33E-04	**5.40E-08**	1.61	6.5	0.0198

VH2VK2	1.31E+04	1.01E-03	**7.76E-08**	2.31	8.4	0.0299
VH2VK3	1.37E+04	9.12E-04	**6.65E-08**	1.98	8.6	0.024
VH2VK4	**Did not bind**					
VH2VK5	**Did not bind**					
VH3VK1	1.81E+04	1.20E-03	**6.67E-08**	1.99	5.6	0.0245
VH3VK2	1.43E+04	1.13E-03	**7.91E-08**	2.35	6.2	0.0282
VH3VK3	1.61E+04	1.06E-03	**6.61E-08**	1.97	6.8	0.0266
VH3VK4	**Did not bind**					
VH3VK5	**Did not bind**					
VH4VK1	1.72E+04	8.82E-04	**5.12E-08**	1.52	10.9	0.0216
VH4VK2	1.67E+04	8.40E-04	**5.04E-08**	1.50	9.2	0.0209
VH4VK3	1.65E+04	8.43E-04	**5.10E-08**	1.52	10.8	0.0204
VH4VK4	**Did not bind**					
VH4VK5	**Did not bind**					
VH5VK1	2.14E+04	6.27E-04	**2.93E-08**	0.87	12.4	0.0271
VH5VK2	1.66E+04	4.68E-04	**2.81E-08**	0.84	12.1	0.0206
VH5VK3	1.90E+04	6.00E-04	**3.16E-08**	0.94	12.4	0.0219
VH5VK4	**Did not bind**					
VH5VK5	**Did not bind**					

Table 2. Single cycle kinetic parameters for binding of HRS-3 (VH0VK0) and humanised variants to recombinant CD30. Relative K_D is the ratio of the K_D to that of VH0VK0.

5 Taking into considerations the binding affinity and iTope™ scores, the 5 lead candidates chosen for further characterisation were VH3VK3, VH4VK2, VH4VK3, VH5VK2, VH5VK3. Large scale production of the 5 lead scFv-mFcs was carried out and multi-cycle kinetic analysis on done on the purified parental HRS-3-mFc and the 5 lead variants. Multi-cycle kinetic parameters determined by SPR confirmed that the humanised leads have similar affinity to the parental HRS-3 scFv (Table 3).

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scFv	**k_a (1/Ms)**	**k_d (1/s)**	**K_D (M)**	**Relative K_D**	**R_{Max}**	**Chi^2 (RU^2)**
VH0VK0	9.47E+03	1.12E-03	**1.18E-07**	1.00	26.4	0.0405
VH3VK3	7.97E+03	3.87E-03	**4.86E-07**	4.12	16.7	0.0391
VH4VK2	1.50E+04	4.62E-03	**3.08E-07**	2.61	20.3	0.0392
VH4VK3	1.27E+04	3.23E-03	**2.55E-07**	2.16	22.5	0.172
VH5VK2	1.20E+04	1.75E-03	**1.46E-07**	1.24	23.5	0.164
VH5VK3	1.21E+04	1.66E-03	**1.37E-07**	1.16	29.9	0.222

Table 3. Multi-cycle kinetic parameters of original HRS-3 (VH0VK0) and the 5 lead humanised variants binding to recombinant CD30. Relative K_D is the ratio of the K_D of the humanised variant to that of VH0VK0.

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Next, the thermal stability of the humanised variants and that of the parental HRS-3 scFv-mFc was assessed by a thermal shift assay using the UNcle biostability platform. The melting temperature (Tm), i.e. temperature at which the scFv-mFc unfolds to a denatured state was measured by fluorescence. Tm of the parental HRS-3 scFv-mFc was measured to be 52.9°C. The humanised
5 clones demonstrated similar if not improved stability with Tm ranging from 52.7°C to 56.9°C (Table 4). Introducing a cysteine residue into the light chain at position 23 relative to SEQ ID NO:9 (Cys23) is predicted to introduce an intra-chain disulfide bond. Modification of VH0VK0 in this manner resulted in a markedly increase in stability, with a measured Tm of 65.4°C. Notably, humanisation did notably increase stability as well in variants VH3VK3 and VH4VK3, even in the absence of
10 Cys23 (Table 4)

scFv-mFc	Average Tm (°C)
VH0VK0-Cys	65.4
VH0VK0	52.9
VH3VK3	56.9
VH4VK2	55.5
VH4VK3	56.1
VH5VK2	52.7
VH5VK3	53.7

Table 4. Measured Tm values for parental HRS-3 (VH0VK0) and the 5 lead humanised variants by thermal shift assay.

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Lastly, binding of the humanised variants and that of the parental HRS-3 scFv-mFc to cell expressed CD30 was assessed by flow cytometry. All scFv-mFcs showed dose dependent binding to the CD30 expressing KM-H2 cells. Importantly no non-specific binding to CD30 negative Raji cells was observed in the parental HRS-3 scFv-mFc nor the humanised variants (Figure 2).

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2.2 Expression and functional cytotoxicity of humanised CD30 CARs

The 5 lead humanised HRS-3 variants were cloned into a retroviral vector pSFG as a CAR bearing a wildtype CH2CH3 spacer domain, CD28 derived transmembrane and intracellular domain, and a CD3ζ intracellular signalling domain. Primary PBMCs were activated on CD3 and CD28 coated
25 plates and transduced with retrovirus particles bearing the CD30 CAR transgenes. Efficiency of transduction was assessed by flow cytometry 6 days post transduction using labelled recombinant CD30. The humanised CD30 CARs demonstrated lower transduction efficiencies compared to the parental HRS-3 (Figure 3A). A cytotoxicity assay was set up to determine the efficiency of these humanised CARs to recognise and kill CD30 expressing KM-H2 target cells. Cytotoxicity was
30 determined at an effector to target ratio of 1:1 CAR expressing T cells to $CD30^+$ KM-H2 cells and $CD30^-$ Raji cells (Figures 3B and 3C). The humanised variants demonstrated variable killing efficacies as compared to the parental CAR. Notably the variants VH3VK3 and VH5VK3 had comparable killing potency as HRS-3.

Stability of the parental HRS3 scFv improved when the Cys23 was introduced into the light chain (Table 4), and so Cys23 was similarly introduced into the VH3VK3 and VH5VK3 humanised variants. The new VH3VK3-Cys, VH5VK3-Cys exhibited similar transduction efficiency and cytotoxicity as the parental HRS-3 CAR (Figures 4A and 4B). Importantly, the stability of the scFv will affect the level of tonic signalling in resting CAR T cells in the absence of antigen stimulation. This can be measured by the secretion of cytokines typical of activated T cells, e.g., GM-CSF, interferon-γ and TNFα. The Introduction of Cys23 in the light chain of both VH3VK3 and VH5VK3 resulted in lower levels of secretion of GM-CSF and TNFα thus suggesting improved stability of the CAR resulting in lower non-specific activation the CAR T cells (Figure 4C).

2.3 Novel OX40 and 41-BB derived spacers retain *in vitro* killing efficacy with lower cytokine production

A novel 4-1BB spacer was cloned into the humanised VH3VK3 and VH5VK3 CARs with and without the Cys23 mutation. The humanised CD30 CARs with the 4-1BB spacers were found to be well expressed in the transduced primary T cells (Figure 5A) and similarly elicited potent cytotoxicity with CD30 expressing KM-H2 cells at a 1:1 effector to target ratio (Figure 5B). Resting transduced cell expressing the humanised CARs with the 4-1BB spacers also preserved their low tonic signalling in the absence of antigen stimulation (Figure 5C).

2.4 Generation and characterisation of T cells bearing humanised CD30 CAR with the 4-1BB spacer

Next the inventors evaluated the phenotype and function of T cells transduced with CAR constructs comprising the 4-1BB spacer domain and the VH3Vk3-Cys or VH5Vk3-Cys antigen-binding domain. Untransduced or CD30 CAR ATCs were generated from PBMCs (4 donors) and from cord blood-derived CD34- cells (3 donors) for use in experiments involving mice humanised with the same cord blood donors. VH3Vk3-Cys-41BB and VH5Vk3-Cys-41BB CD30 CAR ATCs expanded as well as, if not better than HRS3-41BB CD30 CAR ATCs (Figure 6A). The CD30 CAR variants had high transduction efficiencies of up to 98% (Figure 6B). Where there was variation among the constructs, HRS3-41BB CD30 CAR T cells had slightly lower frequencies of CAR+ cells. Most donors generated relatively more CD4 than CD8 T cells, with CD4/CD8 ratios largely equivalent among the CD30 CAR variants (Figure 6C).

Upon activation, T cells upregulate CD30, which may bind CD30 CAR-expressing cells and activate them, possibly resulting in fratricide. CD30 CAR binding to CD30 in *cis* may help to protect cells from inadvertently activating their CD30 CAR-bearing brethren, and thus from fratricide as well. To evaluate the extent of CD30 masking by the CAR molecule, the inventors employed two distinct commercially available anti-CD30 clones, BerH8 and BY88. BerH8 is thought to bind to a similar site of CD30 as the parental HRS3 scFv, as CD30 expression is virtually undetectable by BerH8 once T cells are transduced with HRS3-IgG1. In contrast, staining CD30 with BY88 yields detectable populations in both untransduced and CD30 CAR T cells. Before transduction (day 0), CD30 expression detected by both BerH8 and BY88 were equivalent on ATCs, and remained similar on untransduced T cells over the course of expansion (Figure 6D). In CD30CAR transduced cells,

CD30 detection by BerH8 was undetectable as early as day 3 post-transduction. On the other hand, staining using BY-88 revealed that cell surface CD30 expression often spiked early on and gradually declined to under 10% in most conditions at the harvest time point (day 11), demonstrating that all of the scFv constructs effectively masked CD30 in *cis*.

To evaluate the extent of activation and/or exhaustion of the CD30CAR-expressing cells, the inventors stained cells with antibodies to PD-1, Tim3 and LAG3 and analyzed the proportions of cells expressing one or a combination of these proteins in 3 donors (Figure 6E). CD4 T cells primarily comprised Tim3 single-positive cells, with a small proportion (~5-20%) of cells expressing both Tim3 and PD-1, or none of the markers. CD8 T cells were similarly largely Tim3 single-positive, particularly for donor 5056. The remaining cells consisted of cells bearing none of the markers, and Tim3-LAG3 double expressors. Within each donor, the frequencies of these populations were comparable among the different CD30CAR variants. The fraction of cells expressing all three proteins was very low, suggesting that cells were not terminally exhausted at the end of the expansion process.

2.5 *In vitro* anti-tumor activity of T cells bearing humanised CD30 CARs

The inventors assessed the cytolytic function of T cells transduced with the humanised CD30CAR variants by co-culturing T cells at a 2:1 E:T ratio with two target tumor cell lines, KM-H2 and HuT-78, both of which express high levels of CD30. All CD30CAR variants achieved close to 100% cytolysis of KM-H2 targets by 60h in three of the four tested donors (Figure 7A). With HuT-78 as target cells, there was more heterogeneity in the killing capacity of the CD30CAR variants, both in terms of maximum cytolysis and in the kinetics of cytolysis. Most constructs attained at least 60% cytolysis of HuT-78 cells by 60h.

Effective tumor clearance *in vivo* is predicated on the ability of CAR T cells to maintain killing activity throughout successive rounds of target encounters. To model such a scenario *in vitro*, a flow cytometry-based serial killing assay was established in which CAR T cells underwent three consecutive target encounters, with each encounter lasting 48h (Figure 7B; see Example 1.10). To distinguish target cells from the different encounters, KM-H2 were labelled cells for Encounters 2 and 3 with the lipophilic membrane dyes PKH67 and PKH26 dyes respectively. The HRS3 and VH3Vk3-Cys CD30CAR variants exhibited good serial killing potency in the three donors tested, while VH5Vk3-Cys maintained cytolytic function through three successive rounds of target encounter only in one of the donors, 8584 (Figure 7C).

T cell expansion accompanied target cytolysis, with HRS3 and VH3Vk3-Cys cells proliferating well in all three donors (Figure 7D), whereas VH5Vk3-Cys cells multiplied only in donor 8584. HRS3-bearing cells increased steadily in numbers with each successive target encounter. VH3Vk3-Cys cells exhibited a peak in cell count at the end of Encounter 2, but declined following Encounter 3 with no apparent impact on killing potency.

2.6 *In vitro* on-target, off-tumor activity of T cells bearing humanised CD30 CAR with the 4-1BB spacer

Aside from being expressed on malignant lymphoid cells and on activated lymphocytes, CD30 can also be upregulated on hematopoietic stem and progenitor cells (HSPC) upon cytokine stimulation, albeit at lower levels than lymphoma cells (21). This presents a potential safety concern should CD30CAR T cells inadvertently attack CD30-expressing HSPCs in recipients. While one group observed minimal killing of HSPCs by HRS3scFv CAR T cells (21), the inventors wanted to ensure the same was true of our humanised CD30CAR variants.

The inventors first assessed the CD30 expression on cord blood CD34+ cells from two donors following the same cytokine stimulation protocol employed by Hombach *et al.* CD30 expression peaked at 2-3 days of stimulation for both donors following culture in 10 ng/mL of FLT3L, SCF and TPO (Figure 8A), but did not reach the high levels on KM-H2 cells (Figure 8B). CD30 surface expression is reportedly heterogeneous on HSPC subsets (23), and this was also found to be true for the two donors assayed (Figure 8C). With 2 days of stimulation, CD30 was upregulated on multipotent progenitors (MPPs) and lymphoid-primed multipotents (LMPPs), and to a lesser extent on multi-lymphoid primed progenitors (MLPs), but not on erythro-myeloid primed progenitors (EMPs). These data are consistent with the trends reported by Hombach *et al.* (23). HSPCs from 2 days post cytokine stimulation were thus used to capture the population at its peak of CD30 expression.

To determine if the CD30CAR variants had any impact on the survival of progenitor populations, CD34+ HSPCs were exposed to effector cells for 24h, then isolated the HSPCs for further culture over 9 days in the same cytokine cocktail of 10 ng/mL FLT3L, SCF and TPO before analyzing the cells by flow cytometry (Figure 8D; see Example 1). Encounter with any of the CD30CAR variants altered the progenitor population composition compared to untransduced T cells, suggesting there was some cytolysis of progenitors, especially the LMPP subset. In turn, the EMP subset experienced a reciprocal increase as a proportion of all progenitor cells. Humanisation of the CD30CAR appeared to confer a benefit, in that the reduction in LMPP frequencies tended to be more pronounced with the murine CD30CAR HRS3. Nevertheless, none of the populations was completely ablated. Furthermore, the most primitive MPP subset was not adversely affected, suggesting that even if some of the more differentiated progeny are killed, MPPs are still present to replenish these compartments.

Since alterations in the progenitor composition following co-culture with the CD30CAR variants were observed, the inventors next examined if these changes were functionally meaningful in terms of erythroid and myeloid developmental potential. Following co-culture with effector cells for 24h, a multi-well plate-based colony forming assay was set up using the isolated CD34+ HSPCs (24; Figure 8E; see Example 1). After 12 days of culture, cells were harvested and analyzed by flow cytometry for the presence of erythrocytes, granulocytes and monocytes to determine the original progenitor from which they arose on a per well basis. No differences in colony frequencies were observed among the CD30CAR variants for both HSPC donors when exposed to the T cell donor

0165. However, with T cells derived from the second donor 8584, the VH3Vk3-Cys and VH5Vk3-Cys humanised CD30CAR variants resulted in lower proportions of CFU-G in comparison to HRS3 upon co-culture with HSPC donor 41878. When cultured with the second HSPC donor 41817, VH5Vk3-Cys cells gave rise to more CFU-M than HRS3. These effects were not consistent across donors, but since there was no complete elimination of any colony type, the HRS3 and humanised CD30CAR variants do not appear to have a major functional impact on CD30-expressing HSPCs.

While the CD30CAR variants did not drastically affect HSPCs directly, the possibility that CD30CAR effectors might exhibit elevated cytolysis of HSPCs following encounter with CD30-high tumor cells was explored. T cells bearing the CD30CAR variants were first primed by co-culturing them with KM-H2 target cells for 24h, then added in 2 day-stimulated HSPCs (Figure 8F). After a further 24h of culture, cells were harvested and analyzed by flow cytometry. HSPCs emerged unscathed with all CD30CAR variants for both T cell donors, while KM-H2-directed cytolysis remained high and comparable to that when only KM-H2 cells and T cells were co-incubated for 48h. For reasons unknown, untransduced T cells from donor 0165 had high background killing of KM-H2 targets, while still sparing HSPCs. Prior exposure to CD30-high target cells thus did not appear to result in collateral damage of CD30-low HSPCs, even when the effectors outnumbered the HSPCs at an E:T ratio of 1:0.4.

<u>2.7 *In vivo* efficacy and safety of T cells bearing humanised CD30 CAR with the 4-1BB spacer in a peripheral T cell lymphoma model</u>

It was imperative in our next experiments to evaluate efficacy and safety of our CD30 CAR T cells in an *in vivo* model that contains critical components of the human immune system such as myeloid, lymphoid and natural killer cells. To this end, these candidates were evaluated in GM-CSF, IL-3 supplemented humanised mice which were subcutaneously xenografted with HuT-78 cells. Mice were randomised to receive either untransduced T cells, HRS3 or the humanised CD30CAR variants, stratified based on tumor volume, human CD45 and CD33 reconstitution levels and body weight. All infused T cells were also transduced with GFP-Luciferase (see Example 1) to enable *in vivo* cell tracking and discrimination from endogenous human T cells (Figure 9A).

Measurement of HuT-78 tumors indicated tumor growth and fold change was similar in mice from all 4 treatment groups. Our earlier experience suggests that HuT78 tumor size can increase following treatment as a result of CAR T cell infiltration and human immune cell responses, similar to the experience with immune checkpoint inhibitors (27, 28). Tumor size monitoring may therefore be a less accurate indicator of response. Indeed, endpoint flow cytometric analysis of flank tumors revealed significantly lower numbers of live HuT-78 cells in mice treated with any CD30 CAR T cells, with no appreciable difference observed between the different variants (Figure 9B). Tumor control was accompanied by IVIS detection of high numbers of HRS3, VH3Vk3-Cys and VH5Vk3-Cys humanised CD30CAR. CAR T cells in the dorsal and ventral plane as well as tumors of mice (Figure 9C). In contrast, bioluminescence signals from untransduced T cells were barely detectable in mice. Mice treated with VH5Vk3-Cys humanised CD30CAR. CAR T cells experienced a sudden

deterioration between 8 to 11 days after treatment with 4 of 8 mice succumbing quickly. Mice that were treated with untransduced T cells, HRS3 or VH3Vk3-Cys humanised CD30CAR. CAR T cells remained well till experiment endpoint (Figure 9D). The inventors probed for possible differences in human immune cell composition. Human CD3+, CD4+ and CD8+ T cell populations were noted to
5 be similar in mice from all treatment arms (Figure 9E). Other immune cell subsets including CD19+ B cells, CD56+ NK cells, CD33+ monocyte and subsets based on CD14 and CD16 expression, CD15+ neutrophils in various organs were noted to be also grossly normal across the different treatment groups. There were a few exceptions where the frequencies of CD19+ B cell in the liver, CD33+ CD14- monocytes in the lung, CD15+ neutrophils in the liver and lung in CD30 CAR T cells
10 treated mice was different compared to untransduced T cells (Figure 9F). These differences are thought to represent compensatory immune cell responses to tumor cell elimination. It should be highlighted that there was no observed ablation of any immune cell subsets in mice which received either of the three CD30 CAR T cell variants. Most importantly, human immune cell frequencies in mice which received the new humanised CD30CAR variants were similar to mice treated with HRS3
15 CD30 CAR T cells, which has been demonstrated in human clinical trials to have an excellent safety profile (29).

Luminex® analysis of mouse plasma drawn at Day 7 post treatment revealed that compared to untransduced T cells, inflammatory cytokines (IFN-γ, GM-CSF, IL-8, IL-6, TNF-α and IL-2) were
20 generally increased in mice from all 3 CD30 CAR T cells treatment groups. Several inflammatory cytokines were found to be highly elevated in 2 mice from the VH5Vk3-Cys humanised CD30CAR. CAR T cell treatment group (Figure 9G, in circles), both which eventually died at day 8 and 11 post treatment. It is noteworthy that similar levels of these cytokines were detected in mice which received HRS-3 and VH3Vk3-Cys humanised CD30CAR T cells and these mice remained well till
25 termination of experiment. Altogether, these data demonstrates that VH3Vk3-Cys humanised CD30CAR. CAR T cells have a good safety profile comparable to murine HRS3 CD30CAR. CAR T cells.

2.8 *In vivo* efficacy and safety of humanised CD30 CAR T cells in a model with high tumor burden
30 High tumor load is a known risk factor for the occurrence of cytokine release syndrome (CRS) associated with CAR T cell therapy in patients with haematological malignancies (20, 21). Myeloid cells including monocytes and macrophages have been delineated to be key players in the development of CRS and neurotoxicity after CAR-T cell therapy (22, 23).

35 With this in mind, the inventors sought to examine safety of T cells bearing humanised CD30 CAR in humanised NSG mice xenografted with truncated CD30-transduced NALM-6 cells. In order to support the stable engraftment of myeloid lineages, the mice utilised in this model were supplemented with human GM-CSF and interleukin-3 (IL-3) after human chimerism of >20% has been achieved in the peripheral blood (see Example 1). Disease progression was monitored by
40 bioluminescence imaging of CD30 + NALM-6 cells transduced with firefly luciferase. Treatment was administered when systemic tumor burden in mice was high (Figure 10A). Mice were randomised to

receive either untransduced T cells, HRS3 or the humanised CD30CAR variants, stratified based on tumor volume, human CD45 and CD33 reconstitution levels and body weight.

Body weight changes were similar in mice across all treatment groups (Figure 10B). Treatment with VH3Vk3-Cys and VH5Vk3-Cys humanised CD30 CAR T cells significantly improved tumor control in mice compared to untransduced T cells. In addition, anti-tumor responses in VH3Vk3-Cys and VH5Vk3-Cys humanised CD30 CAR T cells treated mice appeared more durable compared to HRS-3 CD30 CAR T cells (Figure 10C). An early death in a mouse treated with VH3Vk3-Cys humanised CD30 CAR T cells occurred 2 days post treatment and is believed to be precipitated by the incidental presence of NALM-6 in a major vessel rather than progressive disease (Figure 10C). While it was not possible to discern infused T cells from endogenous human T cells, CD3+, CD4+ and CD8+ T cell populations were similar between all treatment arms (Figure 10D). Immune cell subsets including CD19+ B cells, CD56+ NK cells (CD16 bright and dim), CD33+ monocyte and subsets based on CD14 and CD16 expression, CD66+ neutrophils were noted to be grossly normal in various organs across the different treatment. There were a few exceptions where the frequencies of CD19+ B cell in the spleen, CD33+ monocytes in the liver and CD33+ subsets in the lung in CD30 CAR T cells treated mice was different compared to untransduced T cells (Figure 10E). These differences likely represent compensatory immune cell responses in the various organs to NALM-6 cell killing. There was no observed ablation of any subsets in mice which received CD30 CAR T cells. Most importantly, human immune cell frequencies in mice which received the new humanised CD30CAR variants were similar to mice treated with HRS3 CD30 CAR T cells, which has been demonstrated in human clinical trials to have an excellent safety profile (27). Luminex® analysis of mouse serum at Day 11 post treatment levels showed while that inflammatory cytokines (IFN-γ, GM-CSF, IL-8, IL-6, TNF-α and IL-2) were higher in mice from CD30 CAR T cells treatment groups compared to untransduced T cells (Figure 10F), they were not at levels associated with the occurrence of CRS.

Altogether, these data demonstrates that VH3Vk3-Cys and VH5Vk3-Cys humanised CD30CAR. CAR T cells have a good safety profile comparable to murine HRS3 CD30CAR. CAR T cells in mice carrying high tumor load.

2.9 Expression and functional characterisation of humanised variant VH3VK3 and VH5VK3 on EBVSTs

Lastly, the functionality of humanised CARs with the 4-1BB expressed by EBV specific T cells (EBVSTs) was investigated. Whole PBMCs were depleted of CD45RA positive cells by a negative selection step using CD45RA MACs beads. The depleted cells (RAD-PBMCs) were then cultured with EBV peptide libraries and expanded cells were then transduced to express the original parental HRS-3, or the humanised variants VH3VK3, VH5VK3 CARs. Expression and transduction efficiency of the CARs were comparable in EBVSTs (Figure 11A). Masking of CD30 molecules expressed by the CAR T cells in *cis* by the CD30 CAR will affect the degree of fratricide and hence expansion of the CD30 CAR T cells. Efficiency of CD30 masking by the CD30 CAR was assessed by flow using the pair of CD30 specific antibodies BY88 and BerH8. BY88 does not share the same epitope as

HRS-3 and thus the level of staining with BY88 will be indicative of total CD30 expression on the CAR T cells. BerH8 binds to the same epitope as HRS-3 and the humanised variants, thus the lack of BerH8 binding to the CAR T cells will indicate the masking of CD30 by the expressed CARs. All three CARs showed similar efficiency of CD30 masking (Figure 11B). Lastly, cytotoxicity of target cells was assessed with CD30+ KM-H2 cells and CD30^{-} Daudi cells (Figure 11C). The humanised CARs demonstrated similar killing efficacy as the original HRS-3 CAR with similarly low non-specificity.

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Claims

1. An antigen-binding molecule, optionally isolated, which binds to CD30, wherein the antigen-binding molecule comprises a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:45, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:49;

wherein the VH region does not consist of the amino acid sequence of SEQ ID NO:1, and wherein the VH region does not consist of the amino acid sequence of SEQ ID NO:9.

2. The antigen-binding molecule according to claim 1, wherein the antigen-binding molecule comprises:

a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:52, 17, 21, 24, 26 or 28; and

a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:54, 30, 35, 38, 41, 43, 182, 183, 184, 185 or 186.

3. The antigen-binding molecule according to claim 1 or claim 2, wherein the antigen-binding molecule comprises:

(i) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:52, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:54; or

(ii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:17, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:30; or

(iii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:21, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:35; or

(iv) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:24, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:38; or

(v) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:26, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:41; or

(vi) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:28, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:43; or

(vii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:17, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:182; or

(viii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:21, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:183; or

(ix) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:24, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:184; or

5 (x) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:26, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:185; or

(xi) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:28, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:186.

10

4. The antigen-binding molecule according to any one of claims 1 to 3, wherein the VL region comprises a cysteine residue at the position corresponding to position 23 of SEQ ID NO:9.

5. An antigen-binding molecule, optionally isolated, which binds to CD30, wherein the antigen-binding
15 molecule comprises:

(i) a heavy chain variable (VH) region incorporating the following CDRs:

HC-CDR1 having the amino acid sequence of SEQ ID NO:2

HC-CDR2 having the amino acid sequence of SEQ ID NO:3

HC-CDR3 having the amino acid sequence of SEQ ID NO:4; and

20 (ii) a light chain variable (VL) region incorporating the following CDRs:

LC-CDR1 having the amino acid sequence of SEQ ID NO:10

LC-CDR2 having the amino acid sequence of SEQ ID NO:11

LC-CDR3 having the amino acid sequence of SEQ ID NO:12; and

wherein the VL region comprises a cysteine residue at the position corresponding to position 23
25 of SEQ ID NO:9.

6. The antigen-binding molecule according to claim 6, wherein the antigen-binding molecule comprises:

a VH region having an amino acid sequence having at least 70% amino acid sequence identity to any one of SEQ ID NOs:1, 52, 17, 21, 24, 26 or 28; and

30 a VL region having an amino acid sequence having at least 70% amino acid sequence identity to any one of SEQ ID NOs:181, 54, 182, 183, 184, 185 or 186.

7. The antigen-binding molecule according to claim 5 or claim 6, wherein the antigen-binding molecule comprises:

35 (i) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:1, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:181; or

(ii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:52, and a VL region having an amino acid sequence having at least 70% amino
40 acid sequence identity to SEQ ID NO:54; or

(iii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:17, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:182; or

(iv) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:21, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:183; or

(v) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:24, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:184; or

(vi) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:26, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:185; or

(vii) a VH region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:28, and a VL region having an amino acid sequence having at least 70% amino acid sequence identity to SEQ ID NO:186.

8. The antigen-binding molecule according to any one of claims 1 to 7, wherein the antigen-binding molecule is or comprises a single chain variable fragment (scFv) comprising the VH region and the VL region.

9. The antigen-binding molecule according to any one of claims 1 to 8, wherein the antigen-binding molecule is a multispecific antigen-binding molecule, and wherein the antigen-binding molecule further comprises an antigen-binding domain that binds to an antigen other than CD30.

10. A chimeric antigen receptor (CAR) comprising an antigen-binding molecule according to any one of claims 1 to 9.

11. The CAR according to claim 11, wherein the CAR comprises, or consists of, an amino acid sequence having at least 70% sequence identity to the amino acid sequence of SEQ ID NO:149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250 or 251.

12. A nucleic acid, or a plurality of nucleic acids, optionally isolated, encoding an antigen-binding molecule according to any one of claims 1 to 9, or a CAR according to claim 10 or claim 11.

13. An expression vector, or a plurality of expression vectors, comprising a nucleic acid or a plurality of nucleic acids according to claim 12.

14. A cell comprising an antigen-binding molecule according to any one of claims 1 to 9, a CAR according to claim 10 or claim 11, a nucleic acid or a plurality of nucleic acids according to claim 12, or an expression vector or a plurality of expression vectors according to claim 13.

5 15. The cell according to claim 14, wherein the cell is an immune cell, optionally wherein the immune cell is a T cell.

16. The cell according to claim 14 or claim 15, wherein the cell is a virus-specific T cell, optionally an Epstein Barr Virus (EBV)-specific T cell.

10

17. A method comprising culturing a cell according to any one of claims 14 to 16 under conditions suitable for expression of an antigen-binding molecule or CAR by the cell.

18. A composition comprising an antigen-binding molecule according to any one of claims 1 to 9, a CAR
15 according to claim 10 or claim 11, a nucleic acid or a plurality of nucleic acids according to claim 11, an expression vector or a plurality of expression vectors according to claim 12, or a cell according to any one of claims 14 to 16, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

19. An antigen-binding molecule according to any one of claims 1 to 9, a CAR according to claim 10 or
20 claim 11, a nucleic acid or a plurality of nucleic acids according to claim 11, an expression vector or a plurality of expression vectors according to claim 12, a cell according to any one of claims 14 to 16, or a composition according to claim 18, for use in a method of medical treatment or prophylaxis.

20. An antigen-binding molecule according to any one of claims 1 to 9, a CAR according to claim 10 or
25 claim 11, a nucleic acid or a plurality of nucleic acids according to claim 11, an expression vector or a plurality of expression vectors according to claim 12, a cell according to any one of claims 14 to 16, or a composition according to claim 18, for use in the treatment or prevention of a cancer.

21. The antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or
30 plurality of expression vectors, cell or composition for use according to claim 20, wherein the cancer is selected from the group consisting of: a CD30-positive cancer, an EBV-associated cancer, a hematological cancer, a myeloid hematologic malignancy, a hematopoietic malignancy a lymphoblastic hematologic malignancy, myelodysplastic syndrome, leukemia, T cell leukemia, acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, lymphoma, Hodgkin's lymphoma, non-
35 Hodgkin's lymphoma, B cell non-Hodgkin's lymphoma, diffuse large B cell lymphoma, primary mediastinal B cell lymphoma, EBV-associated lymphoma, EBV-positive B cell lymphoma, EBV-positive diffuse large B cell lymphoma, EBV-positive lymphoma associated with X-linked lymphoproliferative disorder, EBV-positive lymphoma associated with HIV infection/AIDS, oral hairy leukoplakia, Burkitt's lymphoma, post-transplant lymphoproliferative disease, central nervous system lymphoma, anaplastic large cell
40 lymphoma, T cell lymphoma, ALK-positive anaplastic T cell lymphoma, ALK-negative anaplastic T cell lymphoma, peripheral T cell lymphoma, cutaneous T cell lymphoma, NK-T cell lymphoma, extra-nodal

NK-T cell lymphoma, thymoma, multiple myeloma, a solid cancer, epithelial cell cancer, gastric cancer, gastric carcinoma, gastric adenocarcinoma, gastrointestinal adenocarcinoma, liver cancer, hepatocellular carcinoma, cholangiocarcinoma, head and neck cancer, head and neck squamous cell carcinoma, oral cavity cancer, oropharyngeal cancer, oropharyngeal carcinoma, oral cancer, laryngeal cancer, nasopharyngeal carcinoma, oesophageal cancer, colorectal cancer, colorectal carcinoma, colon cancer, colon carcinoma, cervical carcinoma, prostate cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, lung adenocarcinoma, squamous lung cell carcinoma, bladder cancer, urothelial carcinoma, skin cancer, melanoma, advanced melanoma, renal cell cancer, renal cell carcinoma, ovarian cancer, ovarian carcinoma, mesothelioma, breast cancer, brain cancer, glioblastoma, prostate cancer, pancreatic cancer, mastocytosis, advanced systemic mastocytosis, germ cell tumor or testicular embryonal carcinoma.

22. An antigen-binding molecule according to any one of claims 1 to 9, a CAR according to claim 10 or claim 11, a nucleic acid or a plurality of nucleic acids according to claim 11, an expression vector or a plurality of expression vectors according to claim 12, a cell according to any one of claims 14 to 16, or a composition according to claim 18, for use in the treatment or prevention of a disease or condition characterised by an alloreactive immune response.

23. The antigen-binding molecule, CAR, nucleic acid or plurality of nucleic acids, expression vector or plurality of expression vectors, cell or composition for use according to claim 22, wherein the disease or condition characterised by an alloreactive immune response is selected from the group consisting of: a disease or condition associated with allotransplantation, graft versus host disease (GVHD) or graft rejection.

24. Use of an antigen-binding molecule according to any one of claims 1 to 9, a CAR according to claim 10 or claim 11, a nucleic acid or a plurality of nucleic acids according to claim 11, an expression vector or a plurality of expression vectors according to claim 12, a cell according to any one of claims 14 to 16, or a composition according to claim 18 to deplete or increase killing of cells expressing CD30.

25. An *in vitro* complex, optionally isolated, comprising an antigen-binding molecule according to any one of claims 1 to 9 or a CAR according to claim 10 or claim 11 bound to CD30.

26. A method for detecting CD30 in a sample, comprising contacting a sample containing, or suspected to contain, CD30 with an antigen-binding molecule according to any one of claims 1 to 9, and detecting the formation of a complex of the antigen-binding molecule with CD30.

27. A method of selecting or stratifying a subject for treatment with a CD30-targeted agent, the method comprising contacting, *in vitro*, a sample from the subject with an antigen-binding molecule according to any one of claims 1 to 9 and detecting the formation of a complex of the antigen-binding molecule with CD30.

28. Use of an antigen-binding molecule according to any one of claims 1 to 9 as an *in vitro* or *in vivo* diagnostic or prognostic agent.

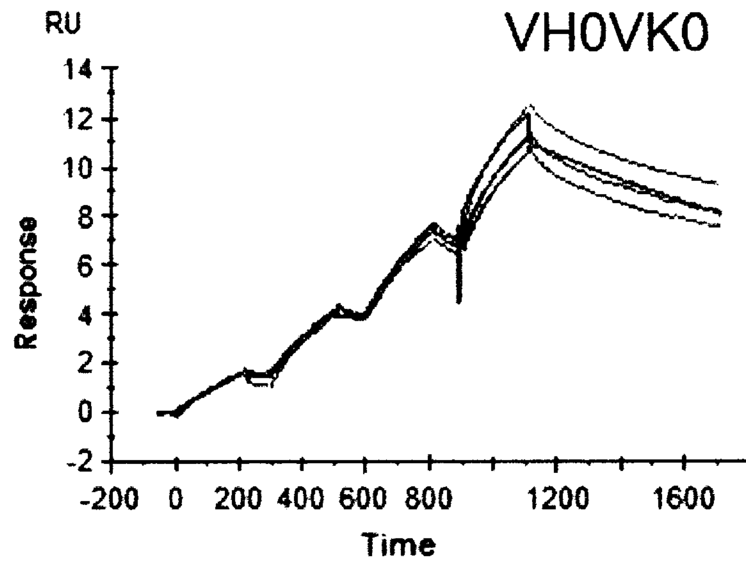


Figure 1A

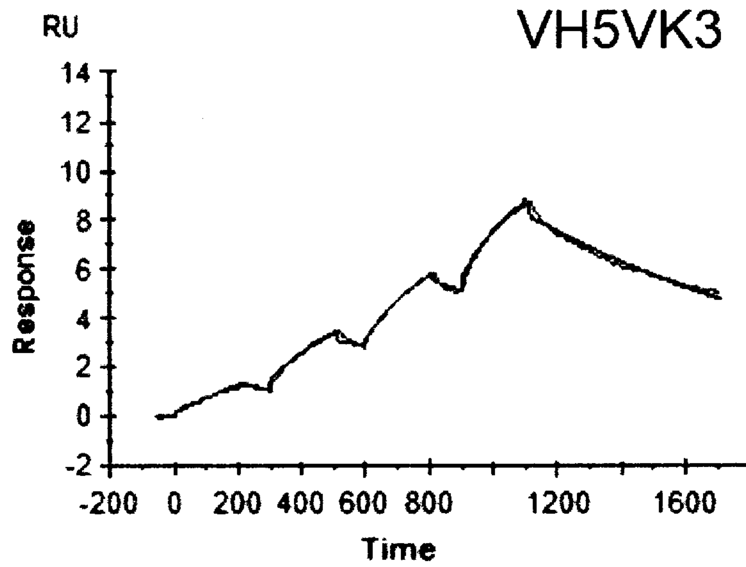
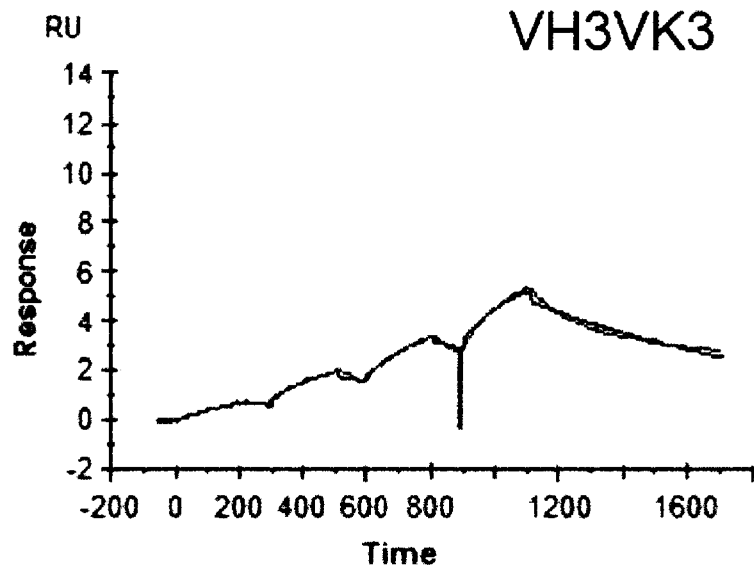


Figure 1B



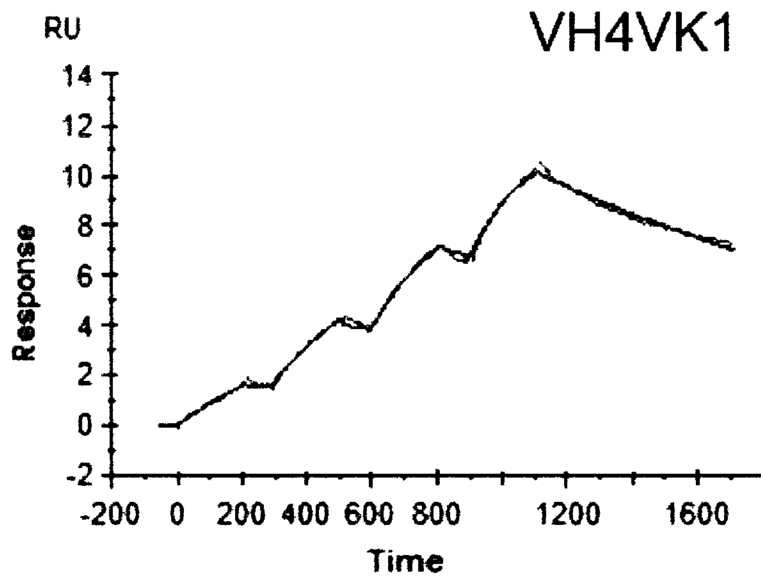
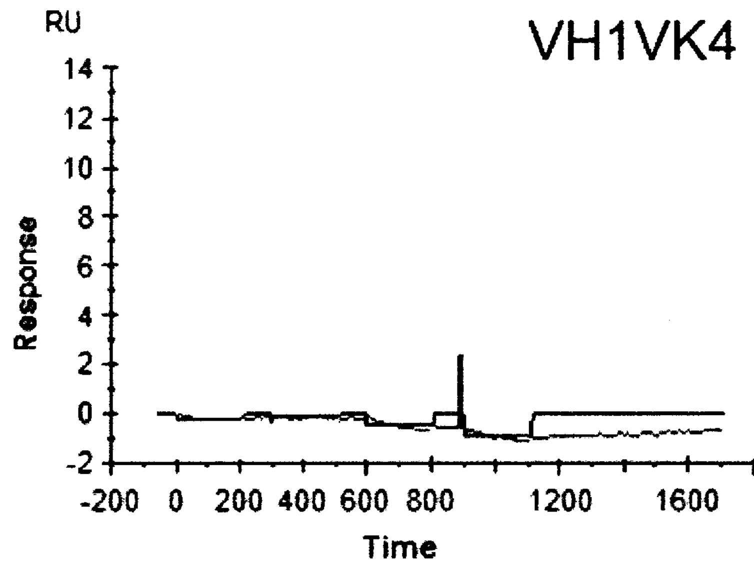


Figure 1B (Cont.)



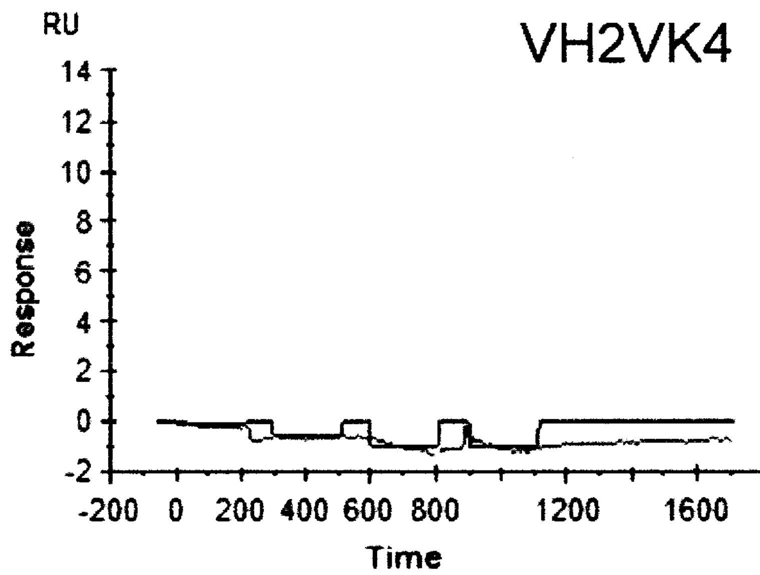
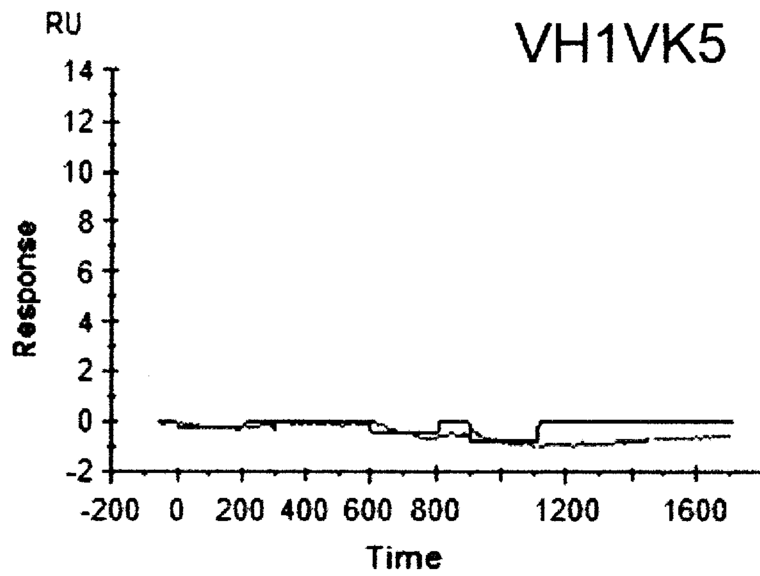
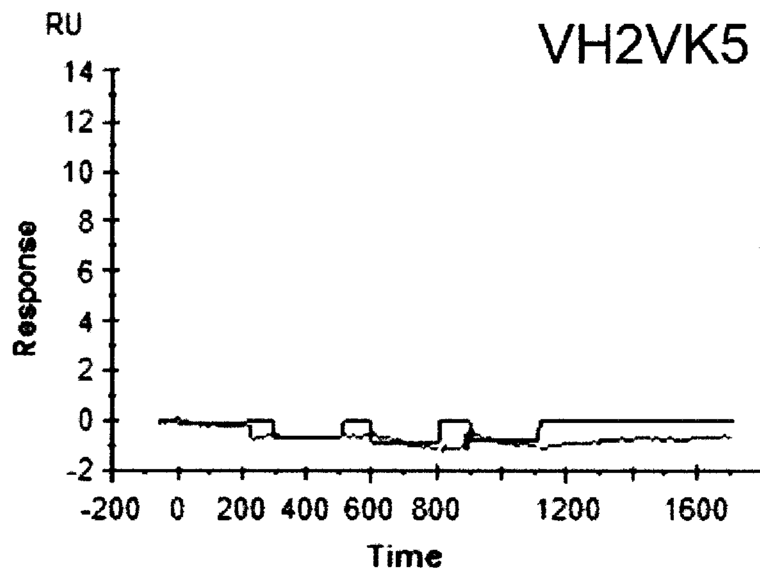


Figure 1C





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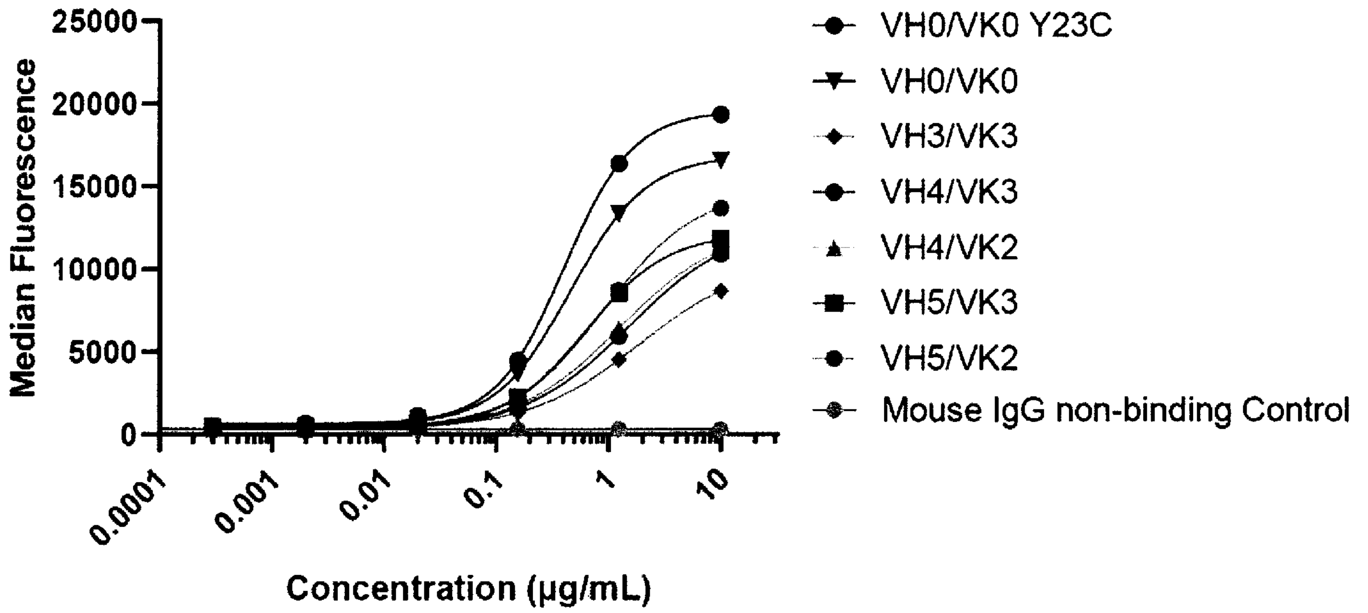


Figure 2A

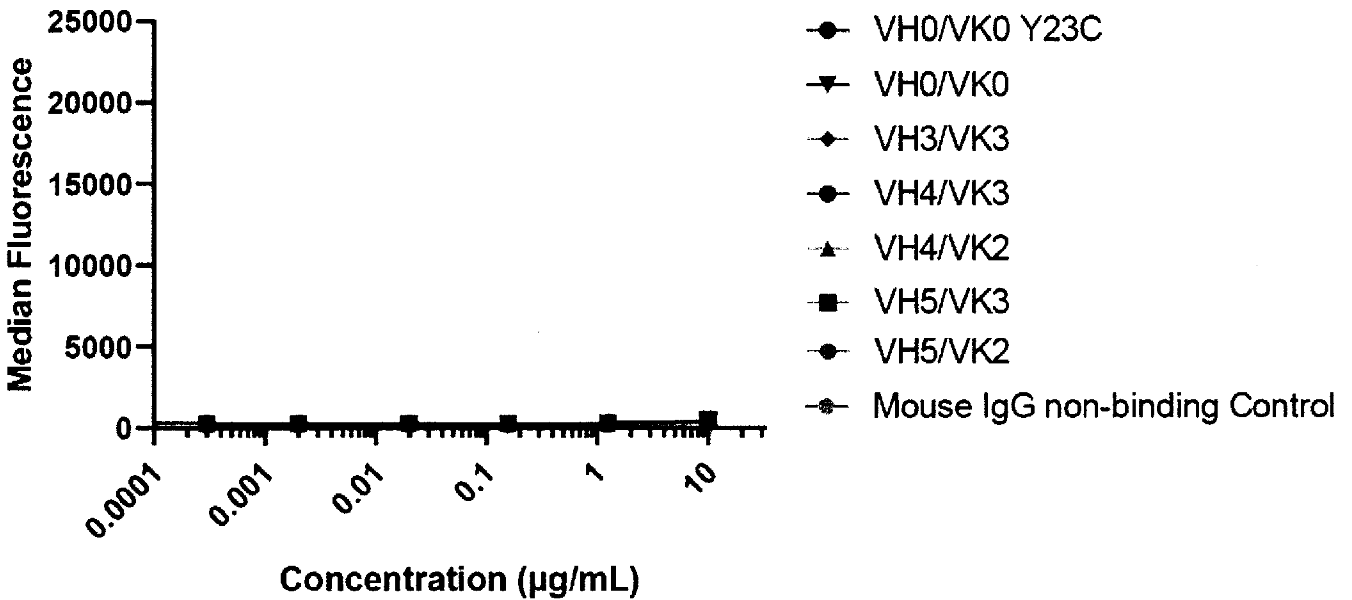


Figure 2B

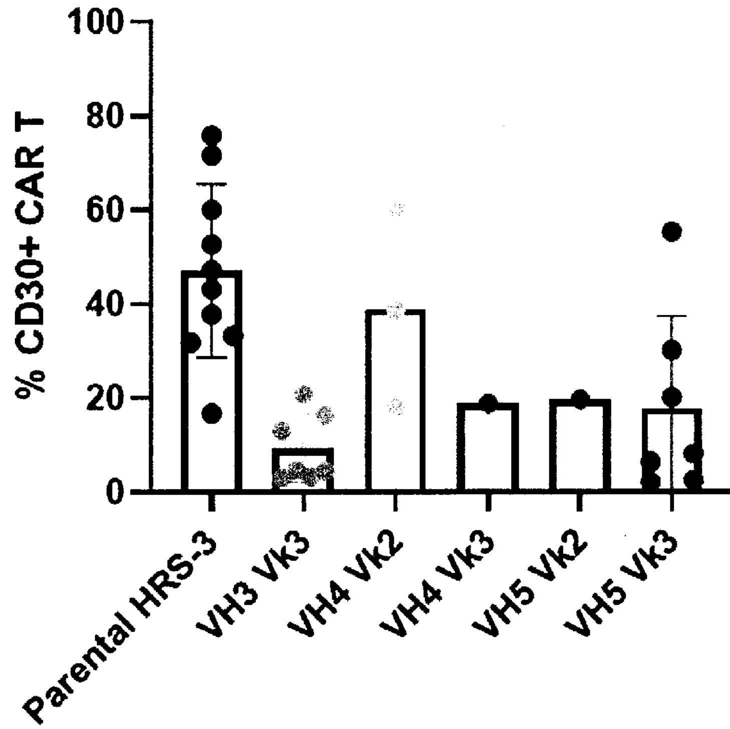


Figure 3A

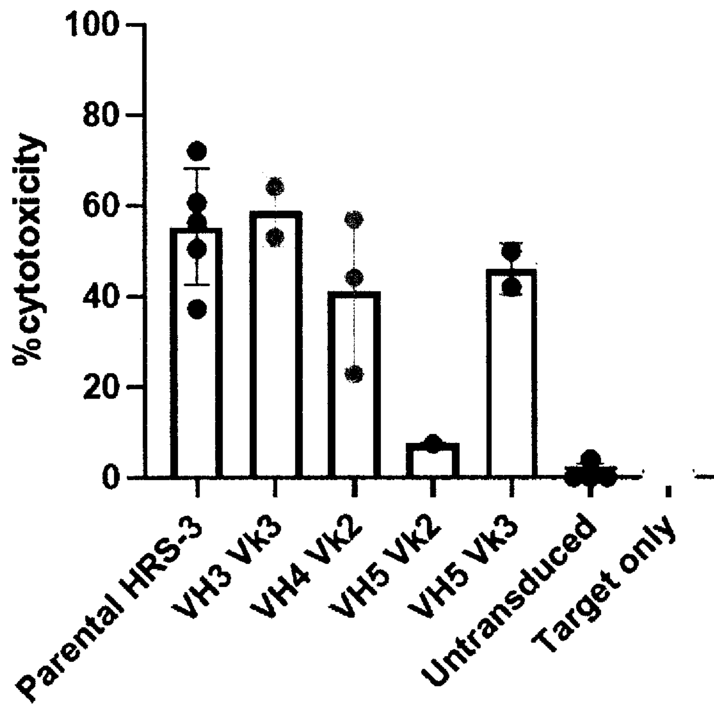


Figure 3B

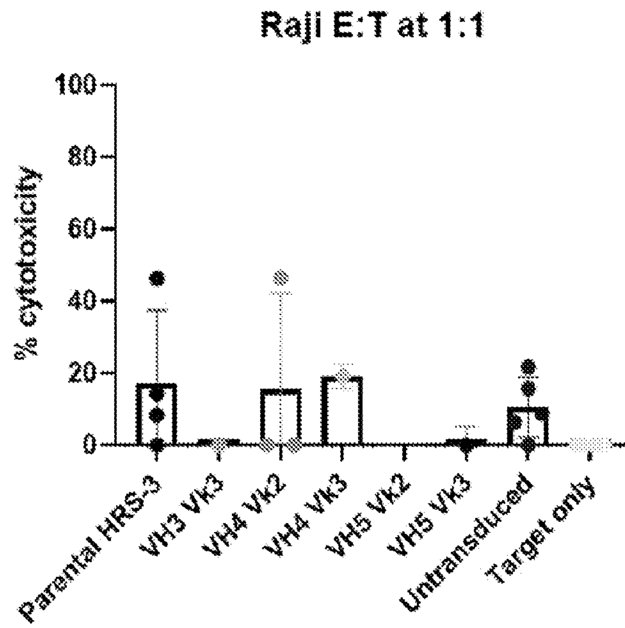


Figure 3C

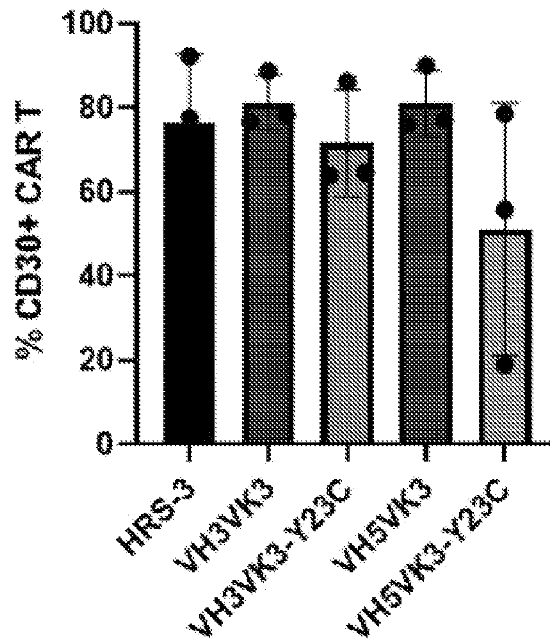


Figure 4A

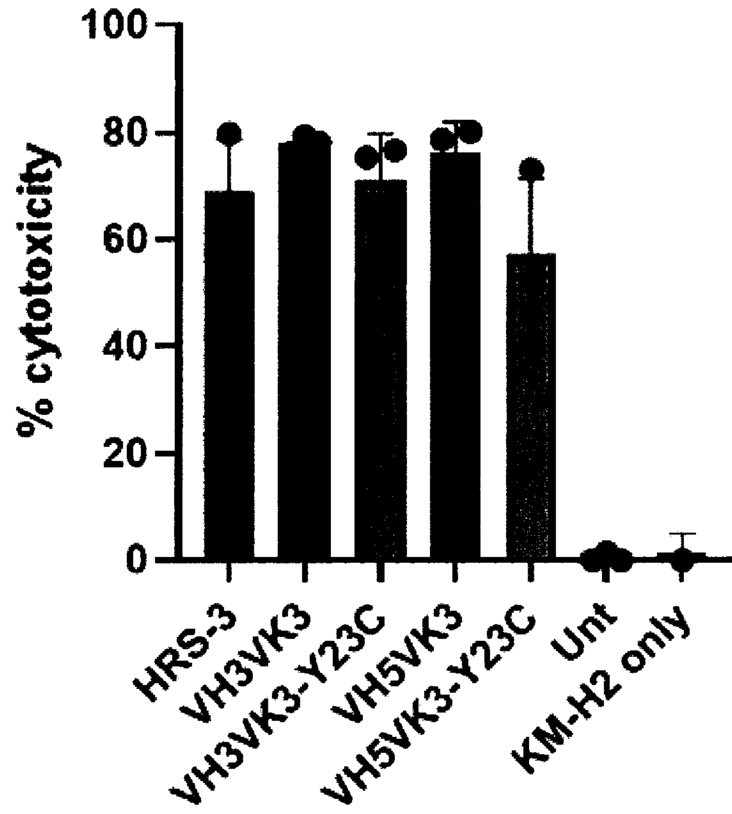


Figure 4B

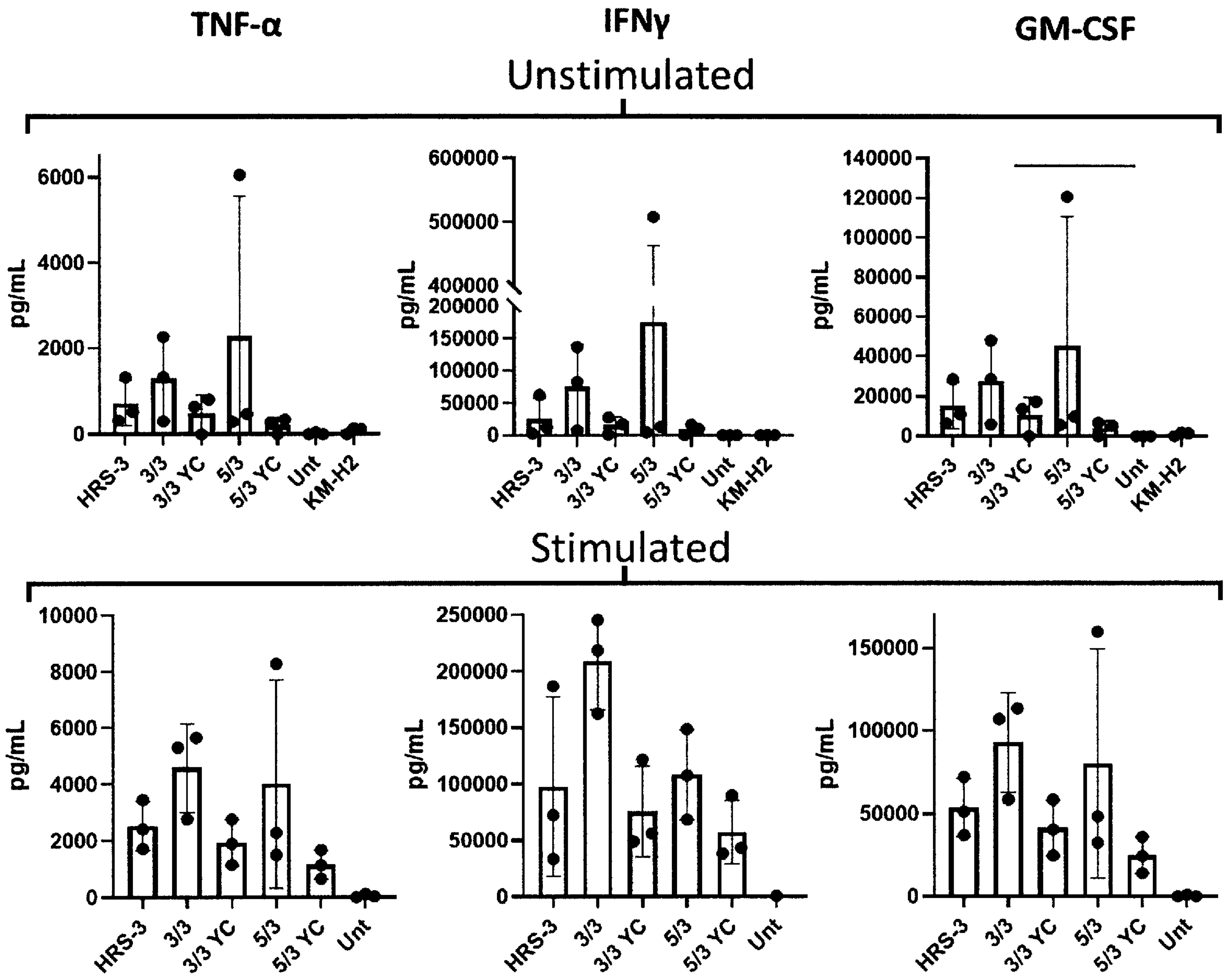


Figure 4C

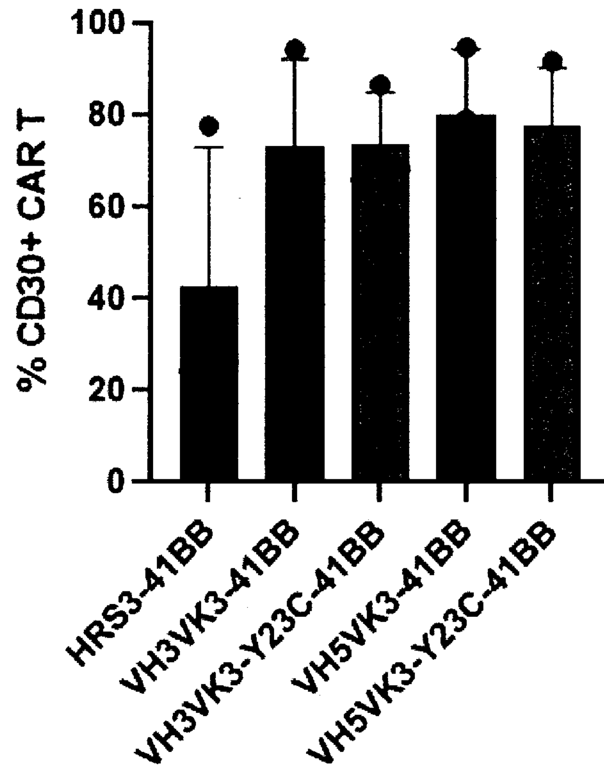


Figure 5A

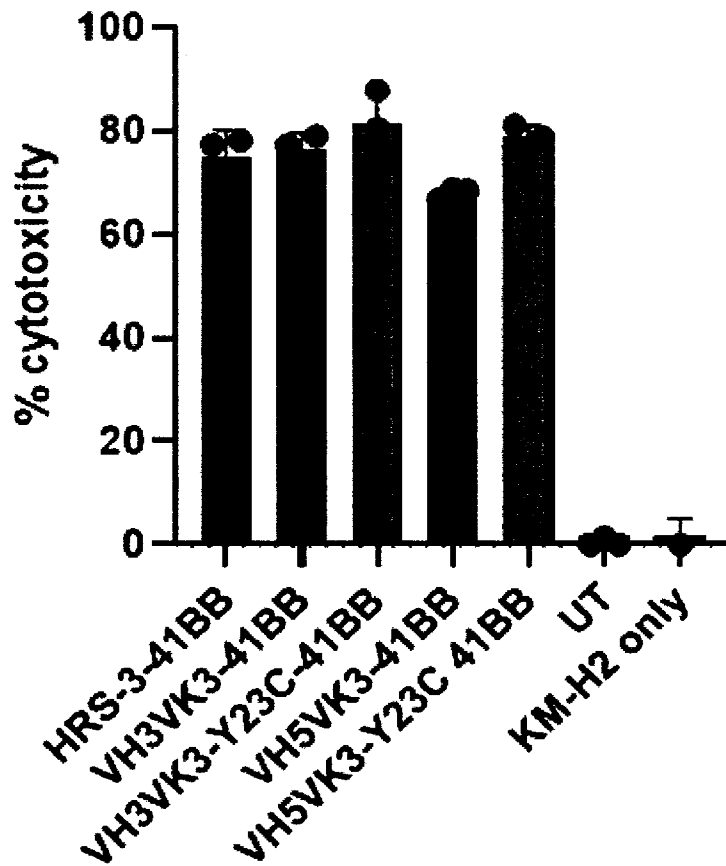


Figure 5B

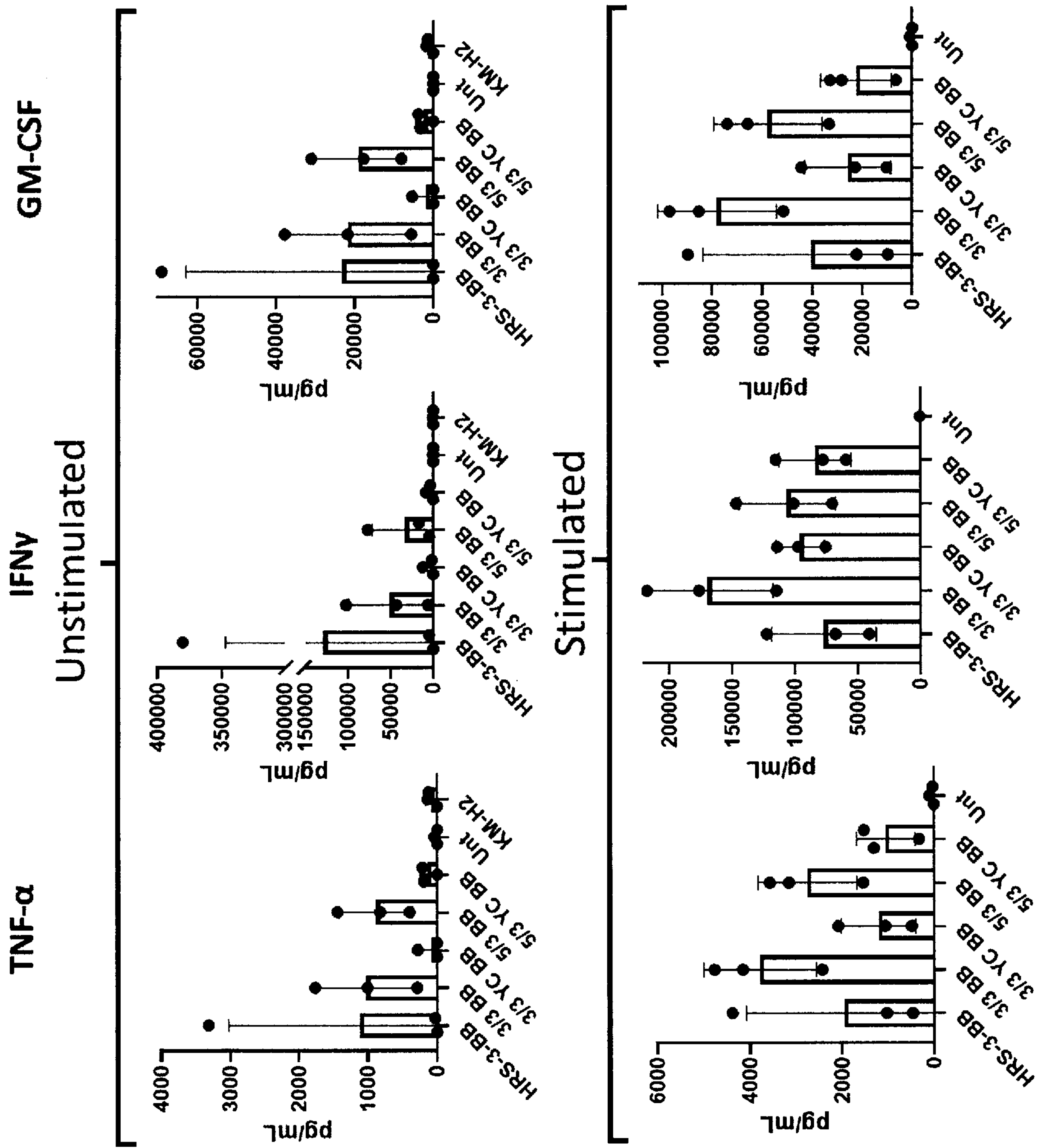


Figure 5C

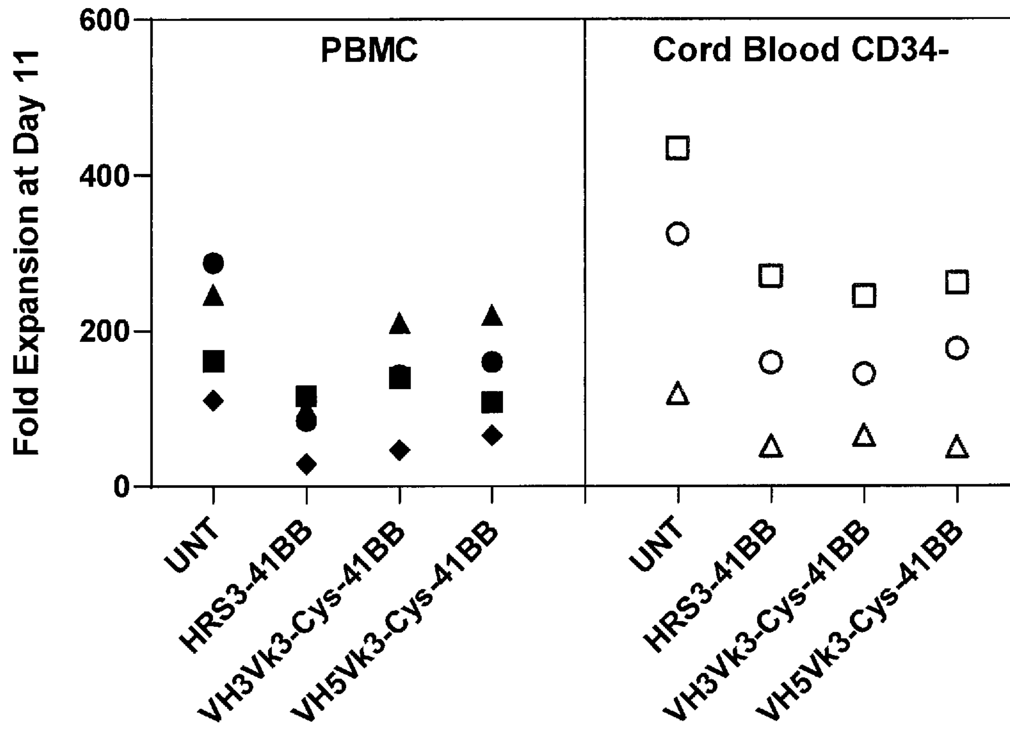


Figure 6A

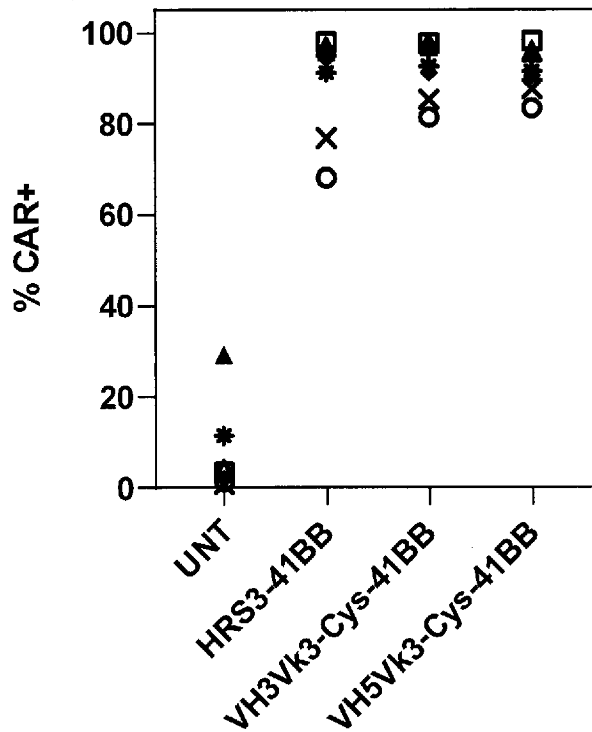


Figure 6B

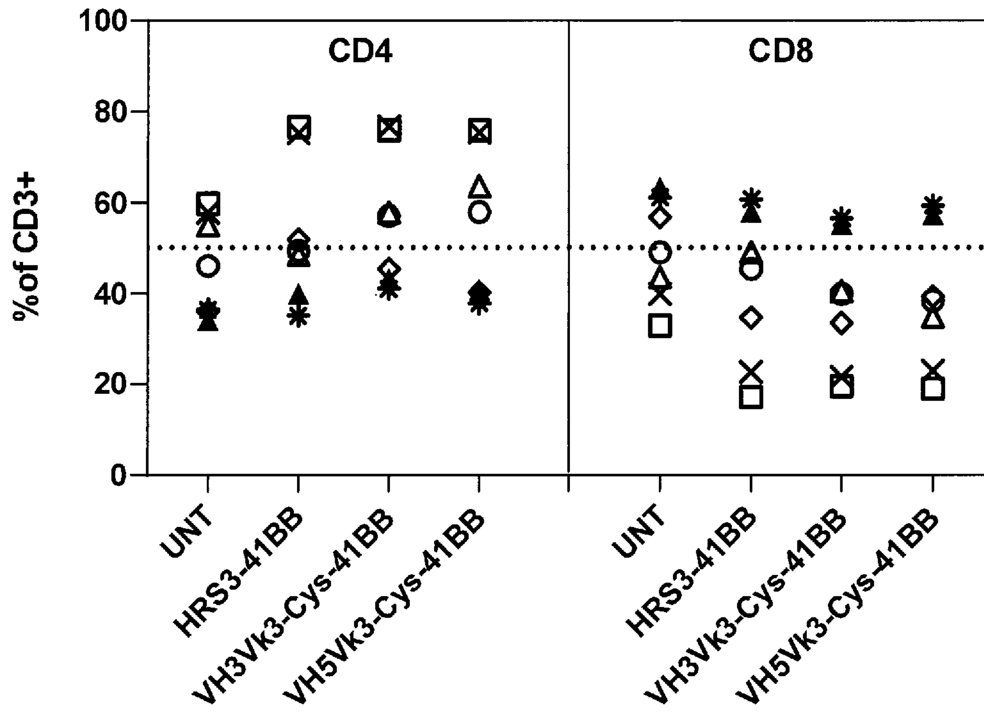


Figure 6C

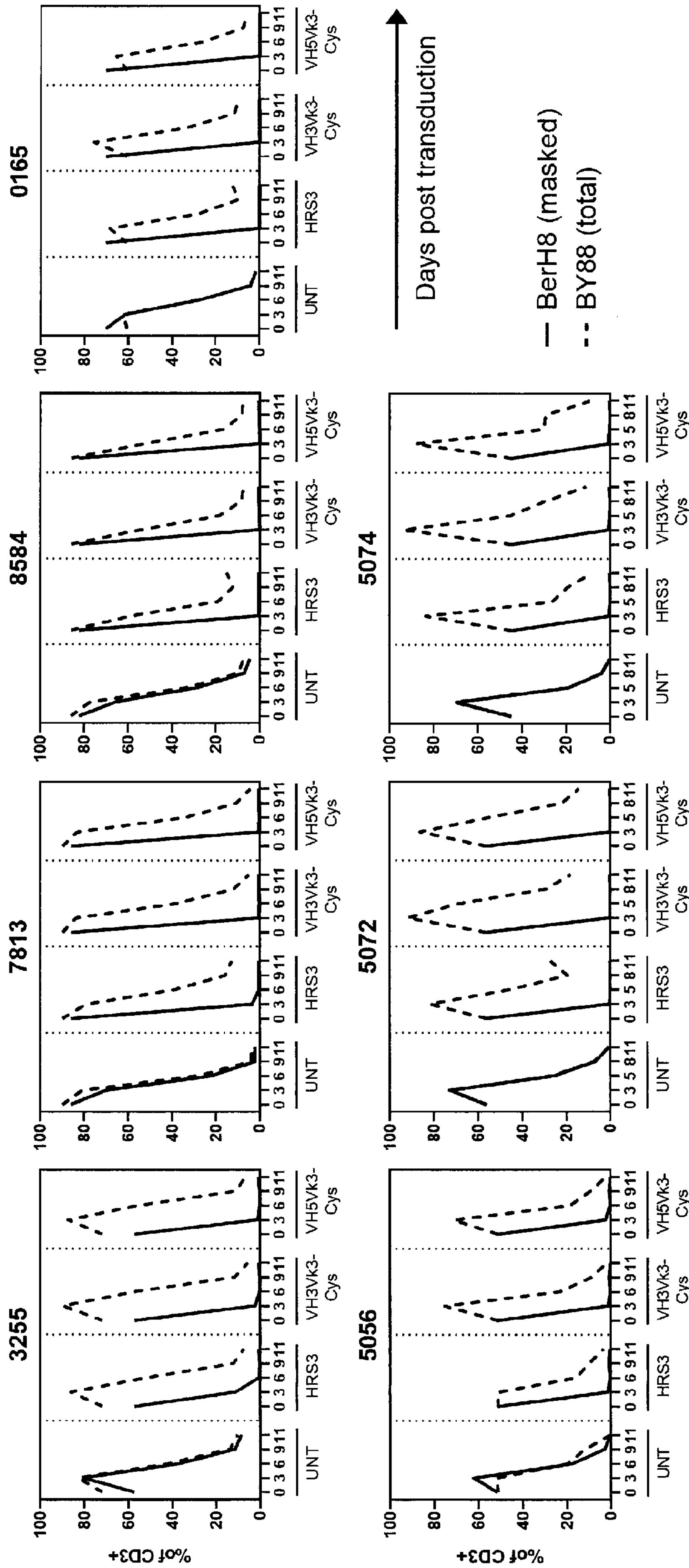


Figure 6D

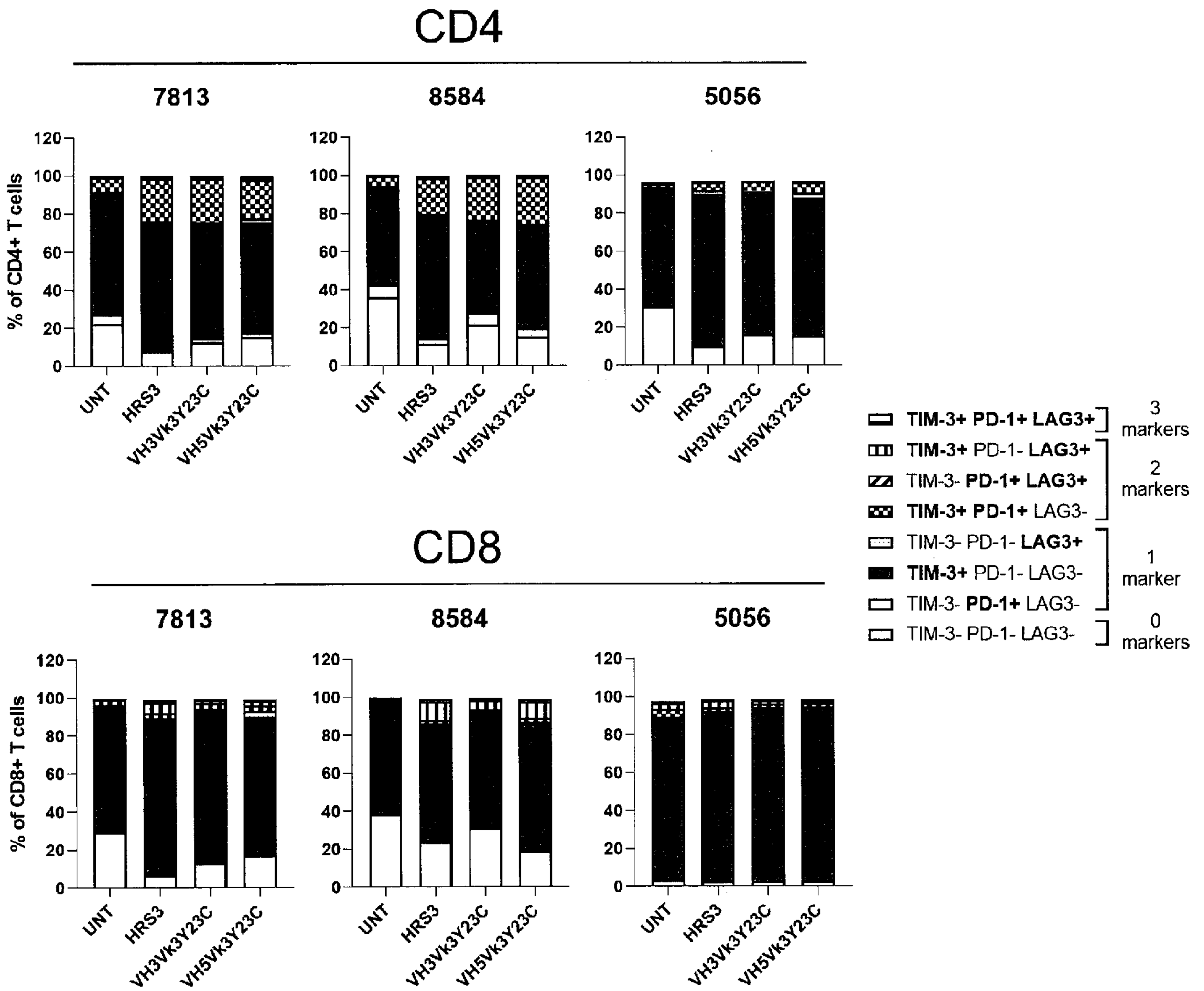


Figure 6E

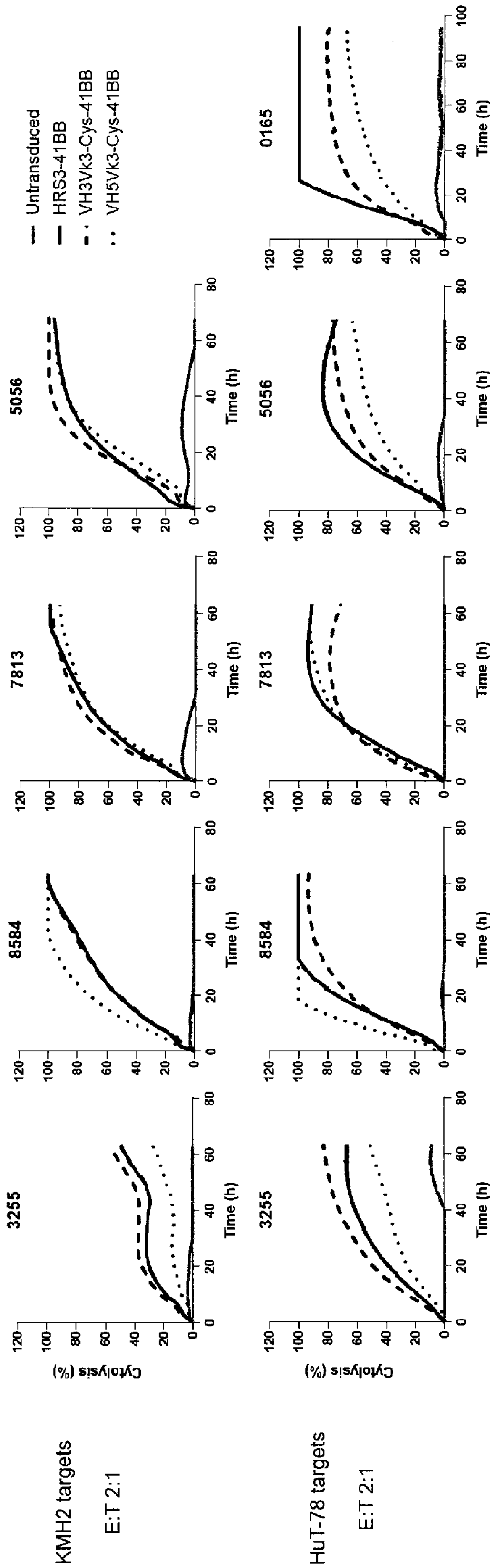


Figure 7A

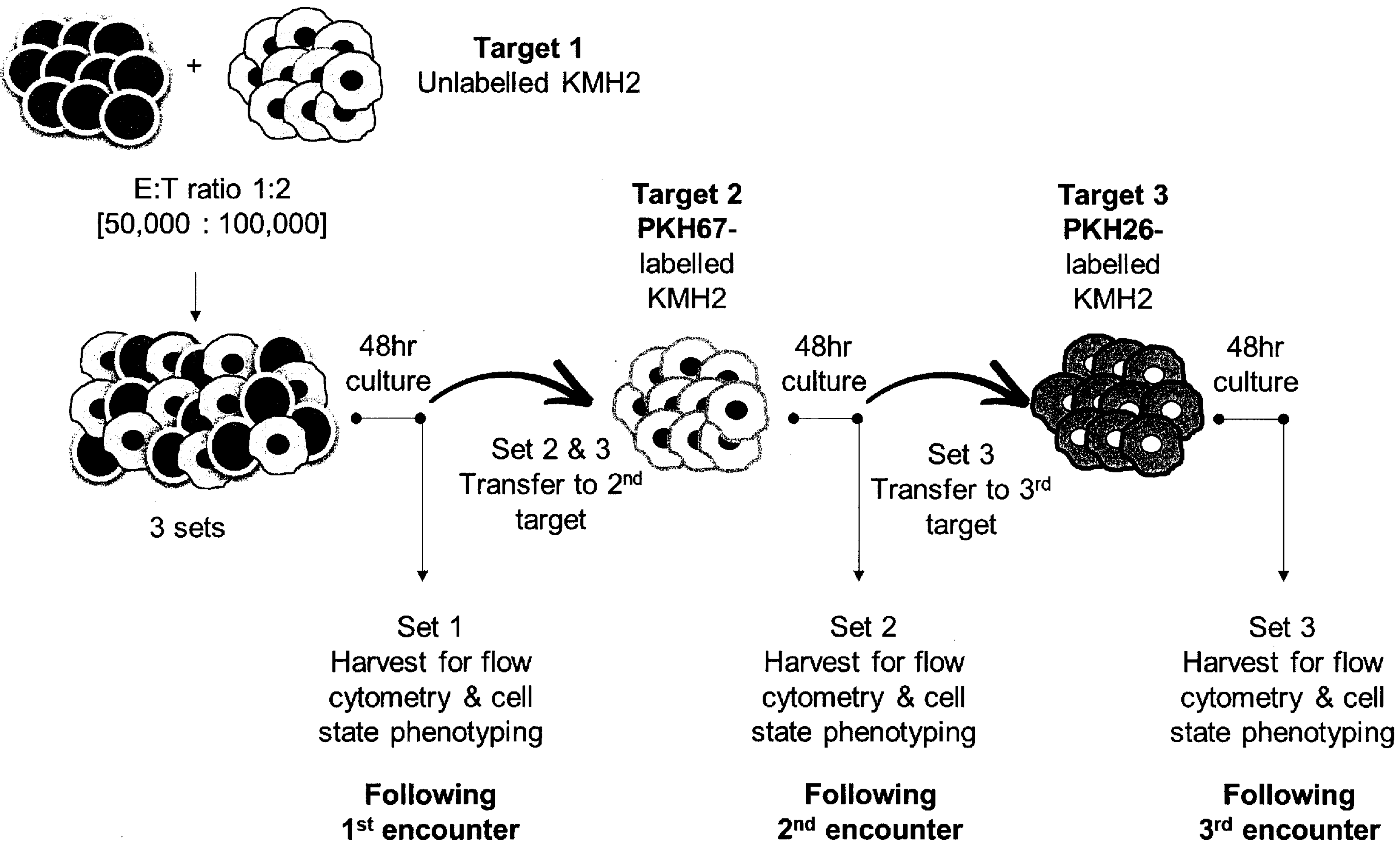


Figure 7B

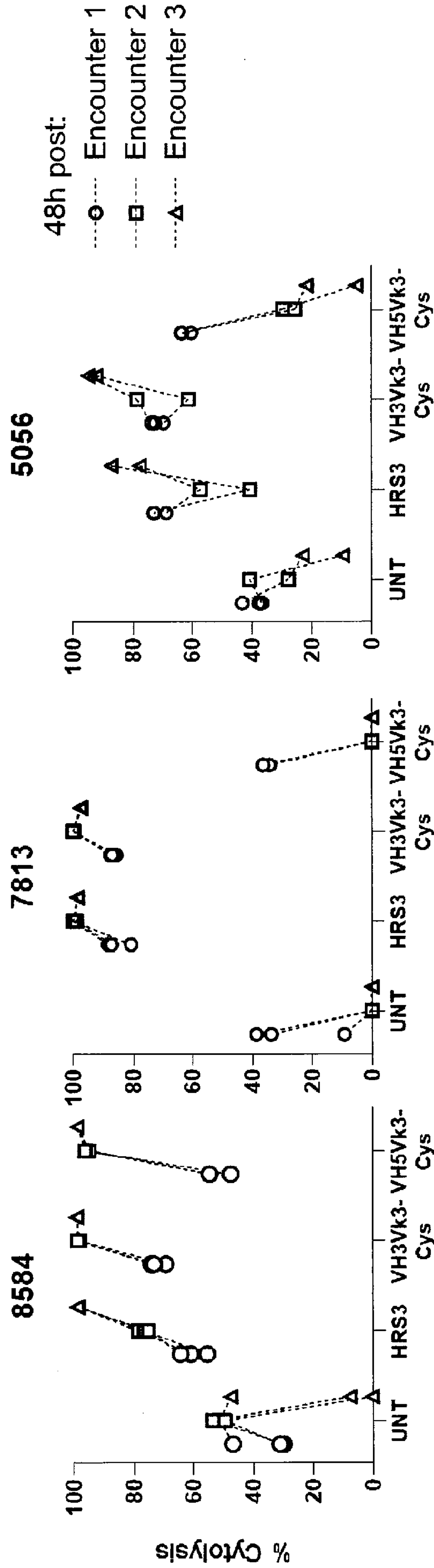


Figure 7C

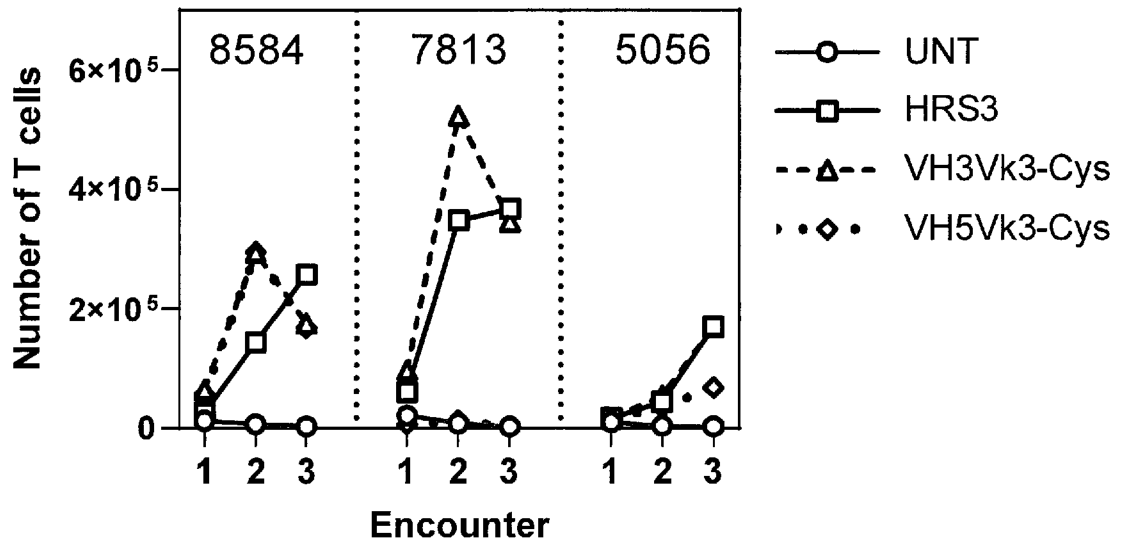


Figure 7D

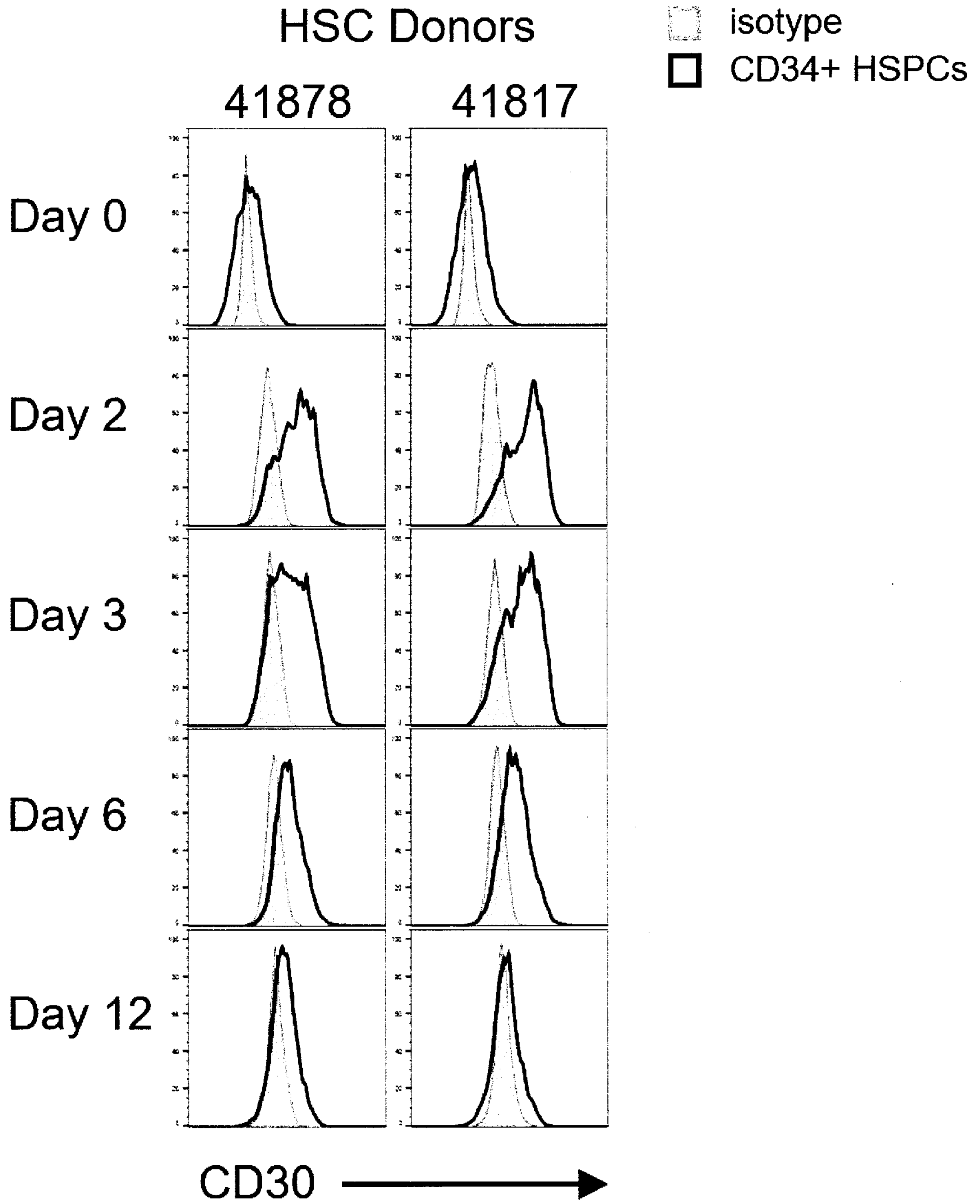


Figure 8A

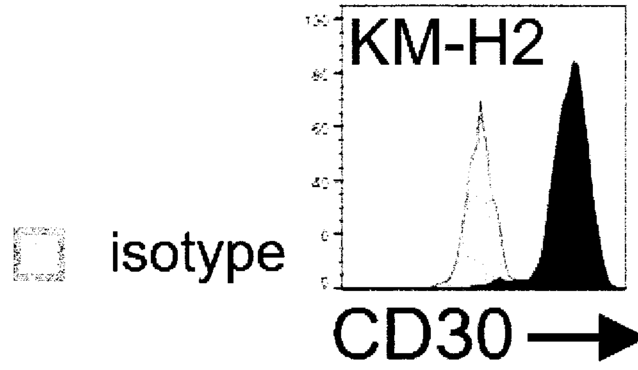


Figure 8B

Day 2 41878 41817

MPP

EMP

LMPP

MLP

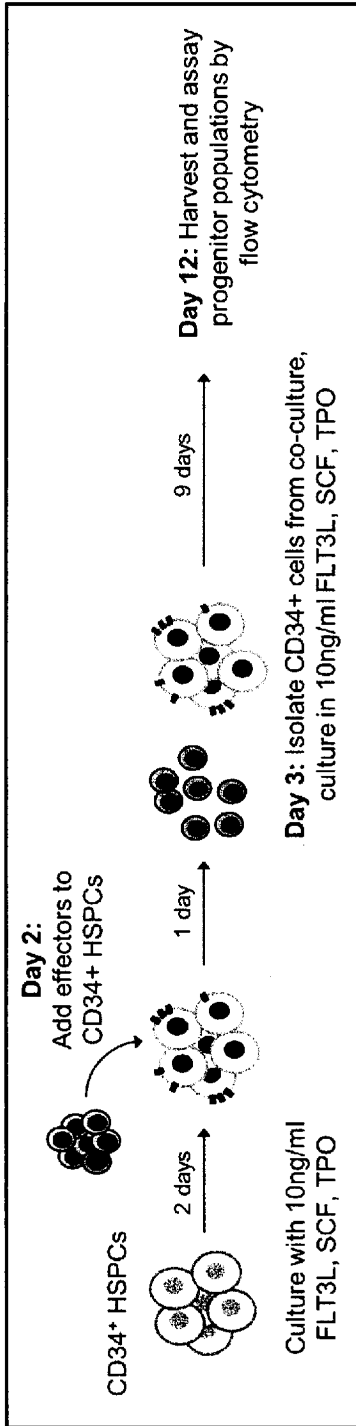
CD30

isotype

CD34+ HSPCs

HSPC subset

Figure 8C



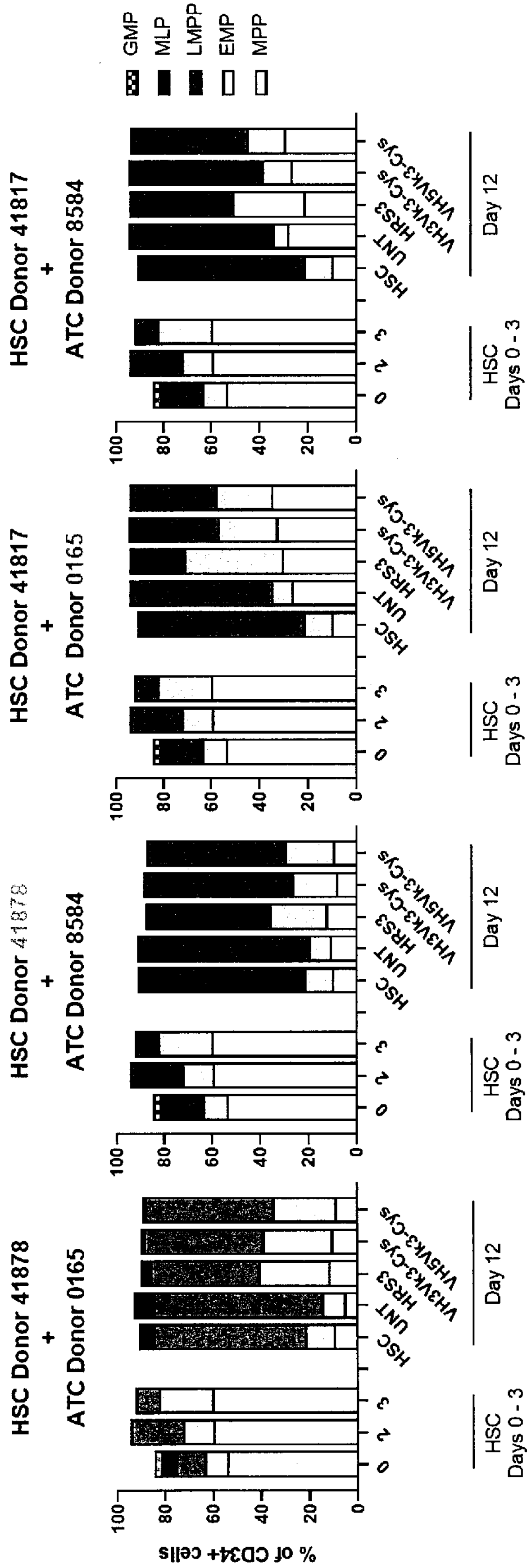
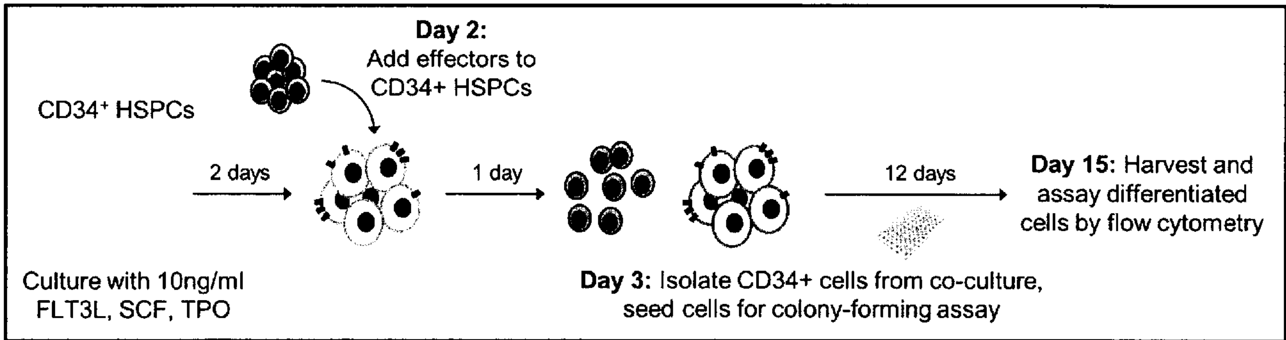


Figure 8D



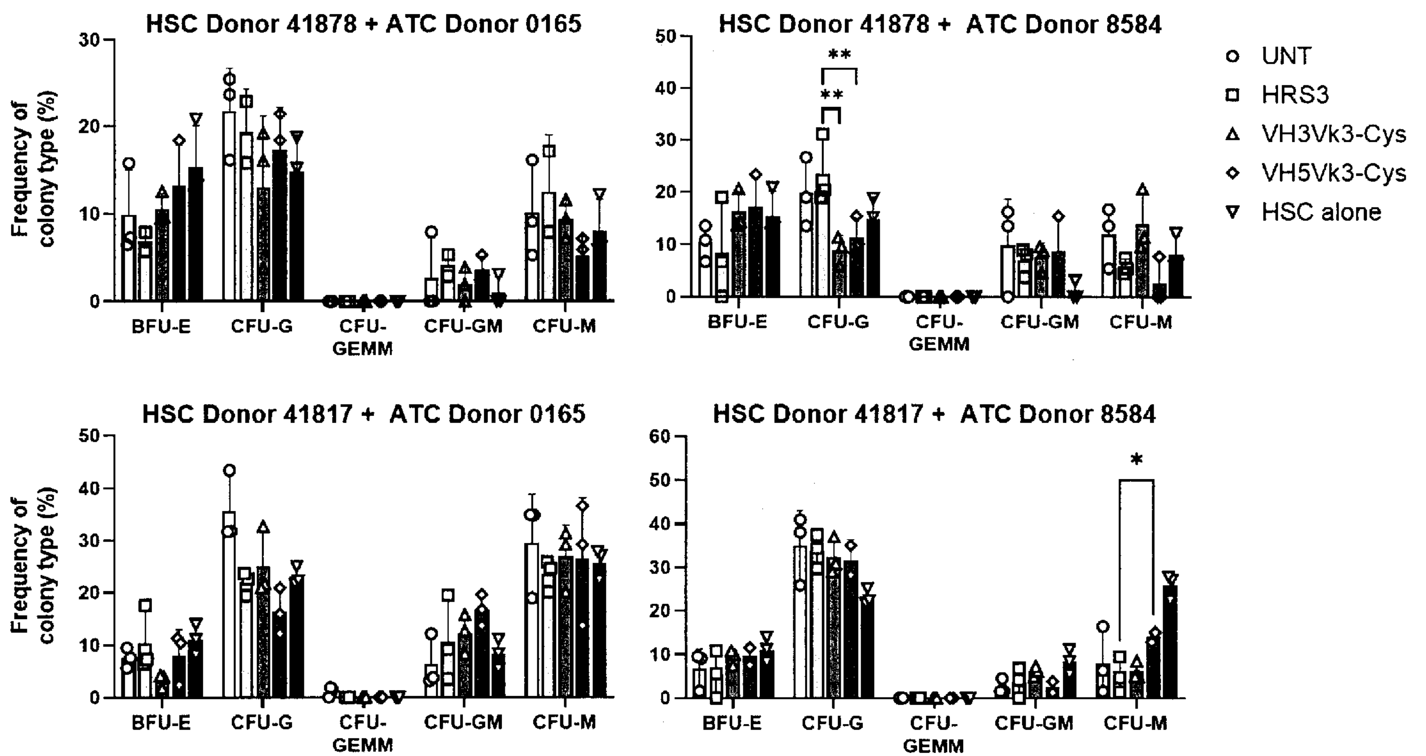
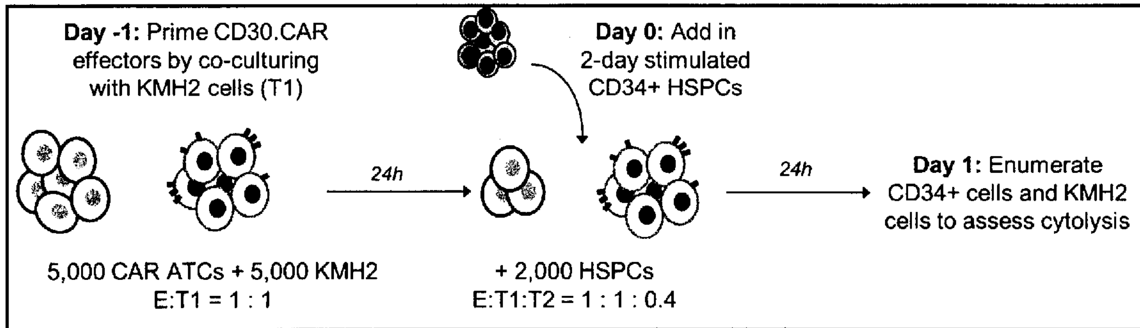


Figure 8E



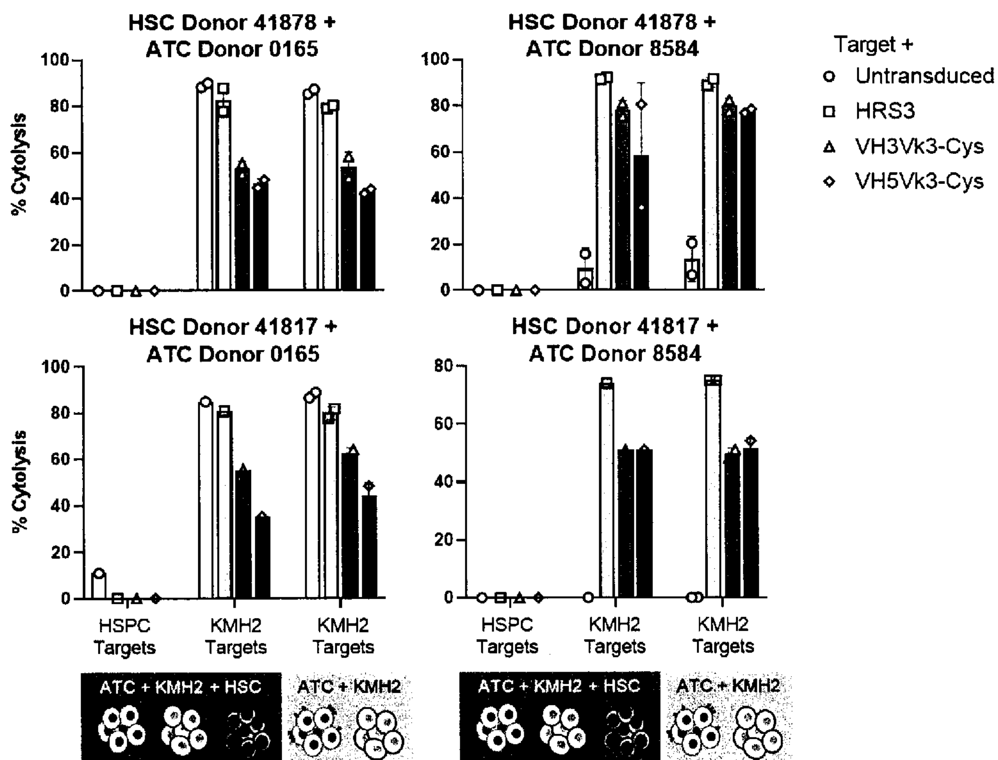


Figure 8F

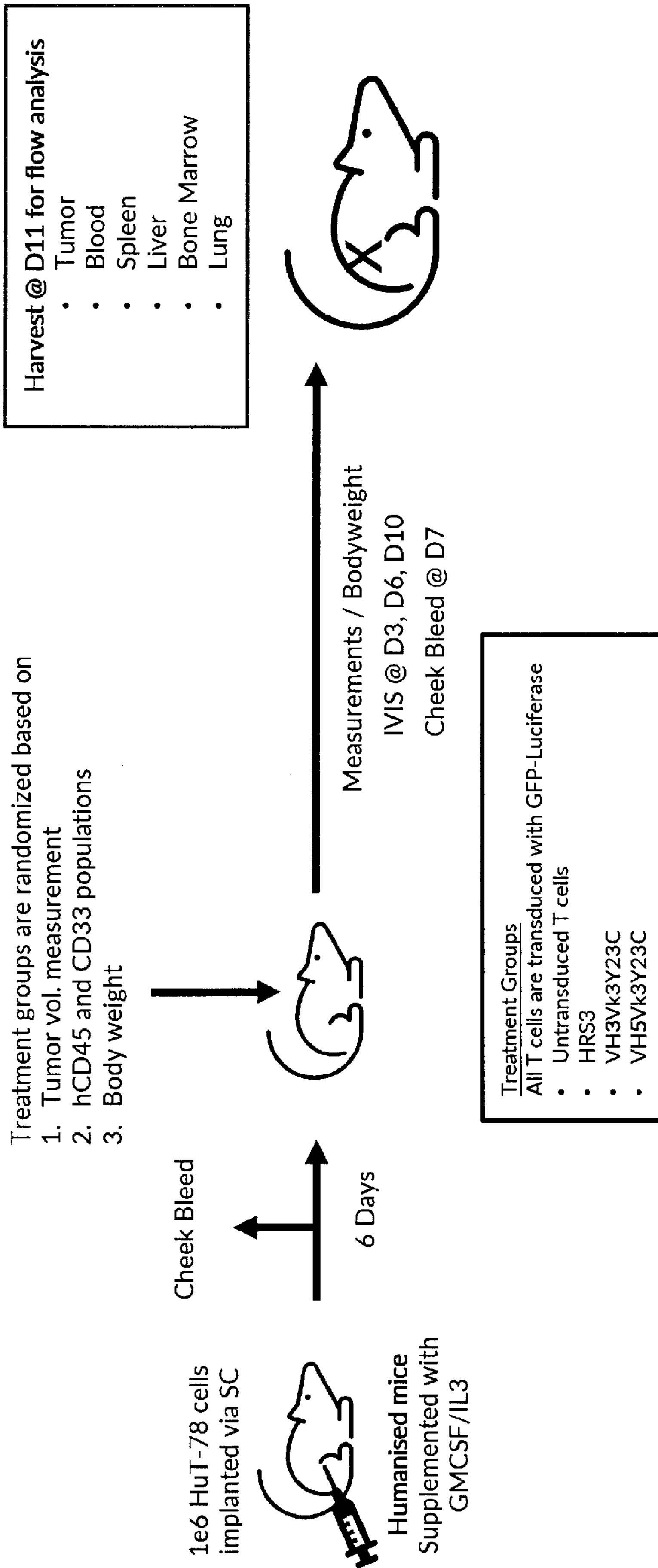


Figure 9A

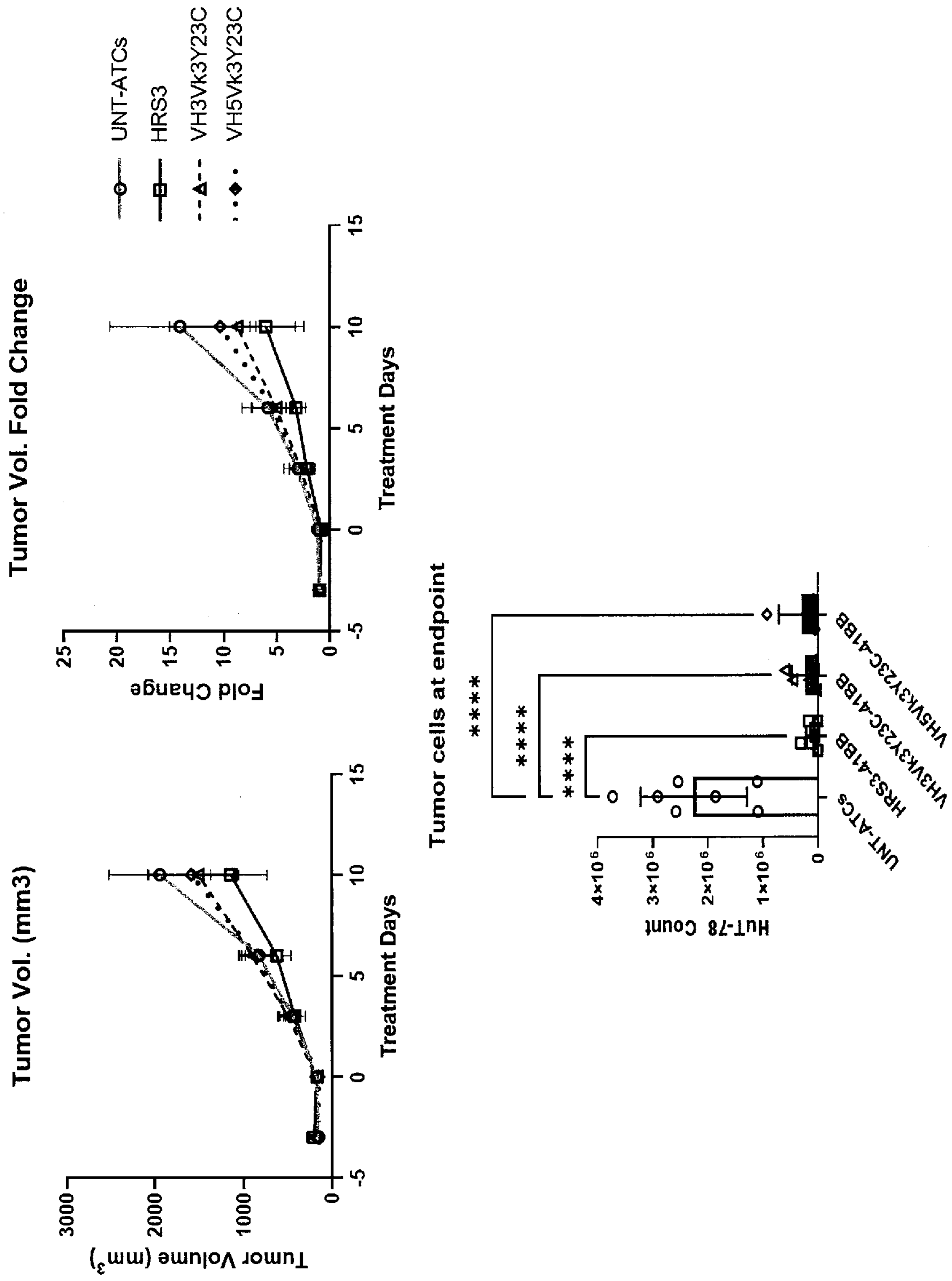
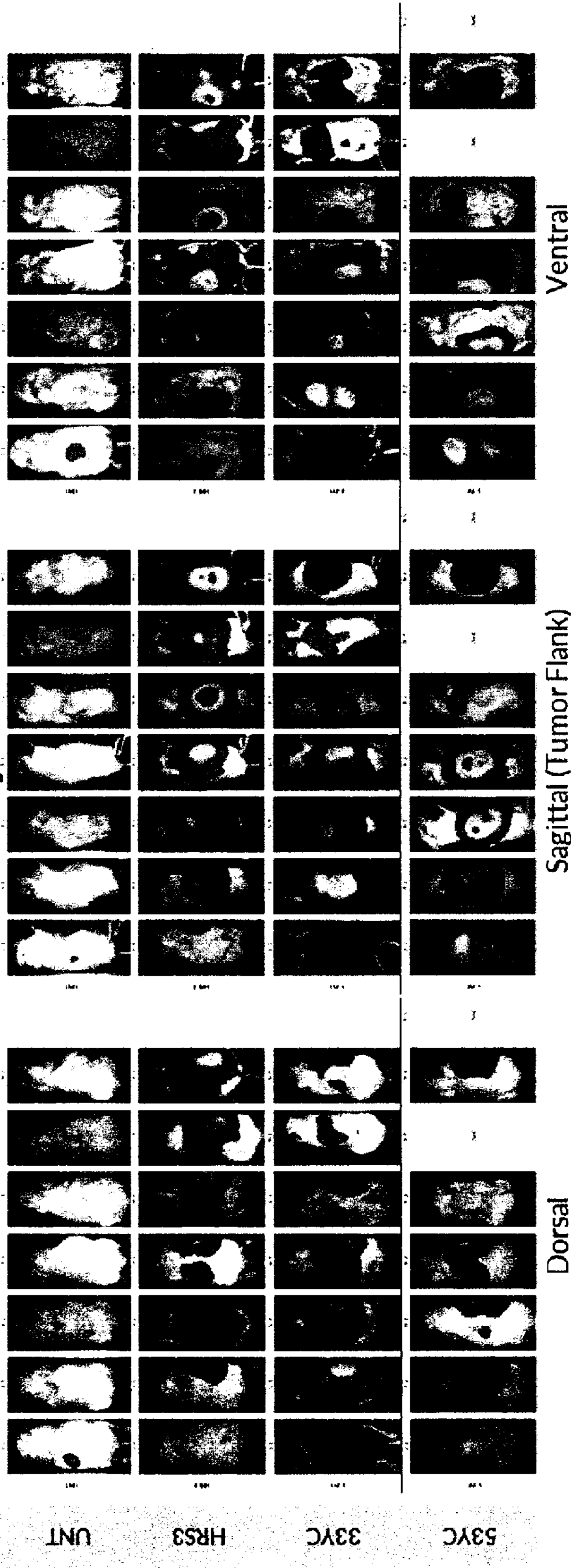
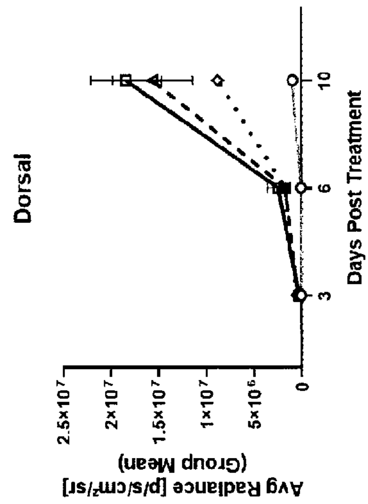
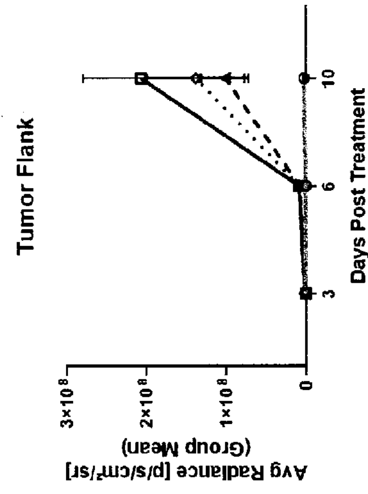


Figure 9B







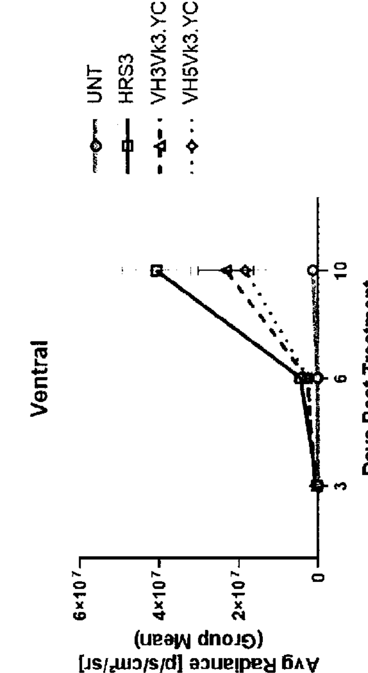


Figure 9C

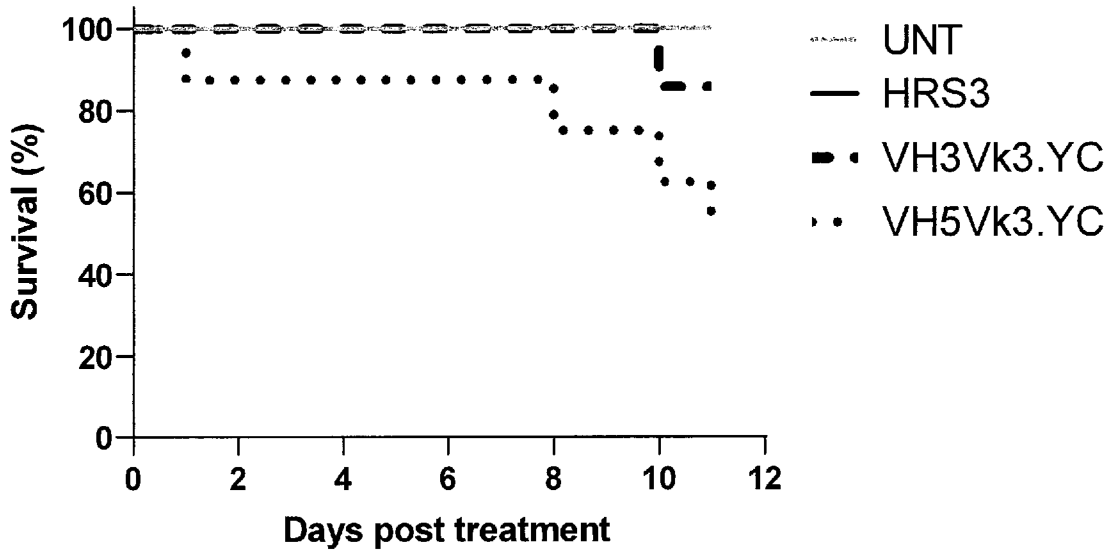


Figure 9D

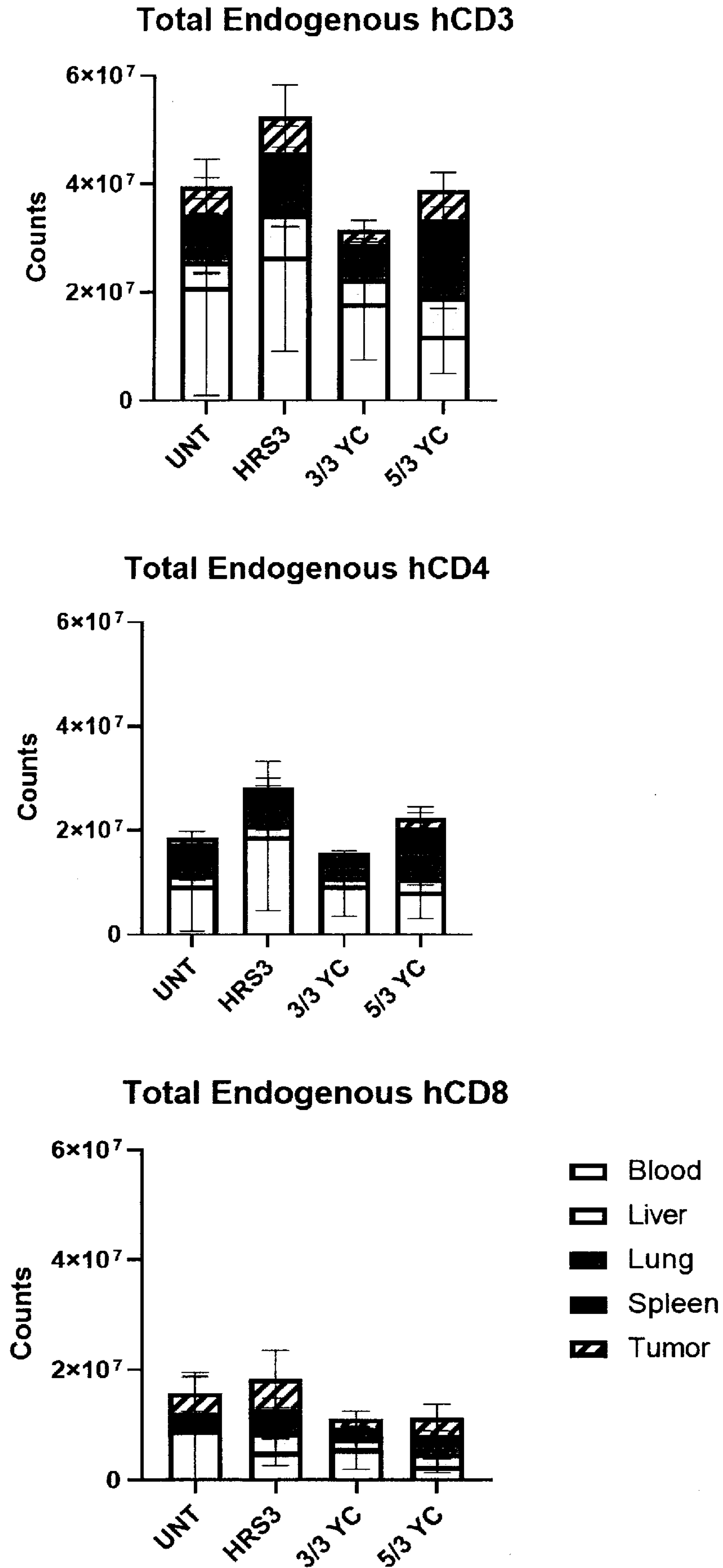


Figure 9E

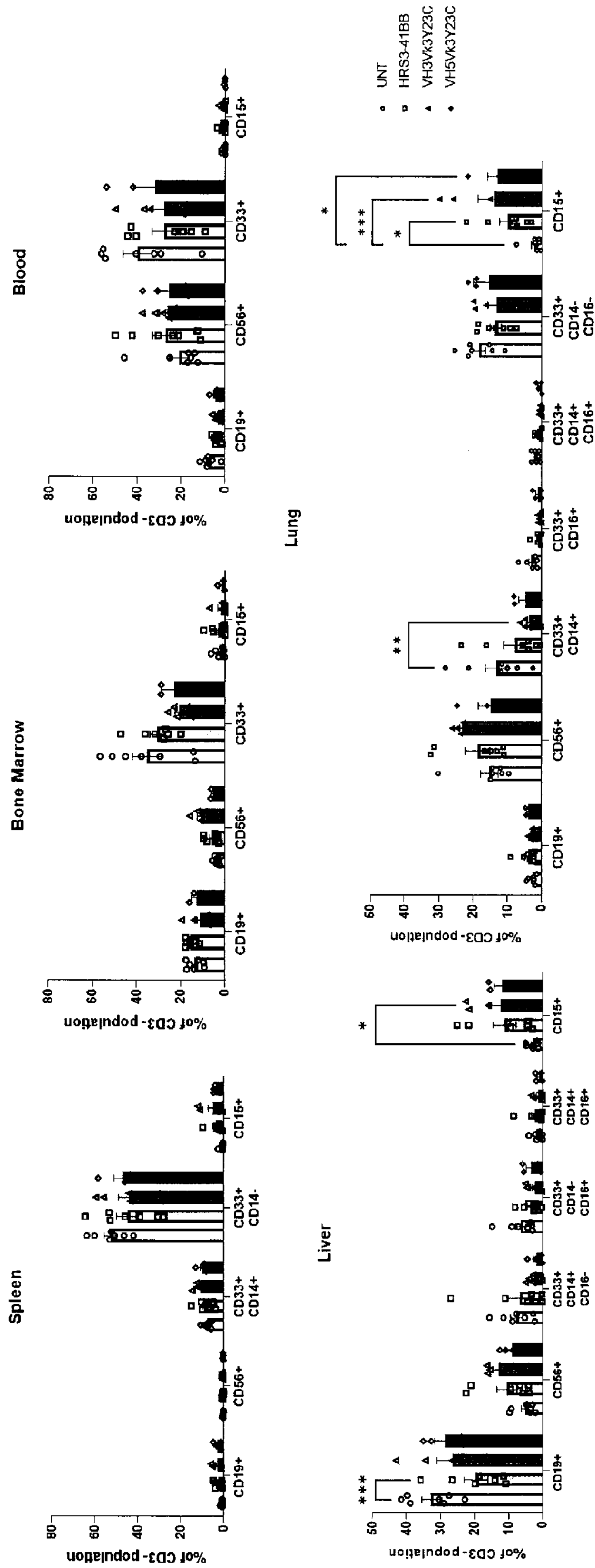


Figure 9F

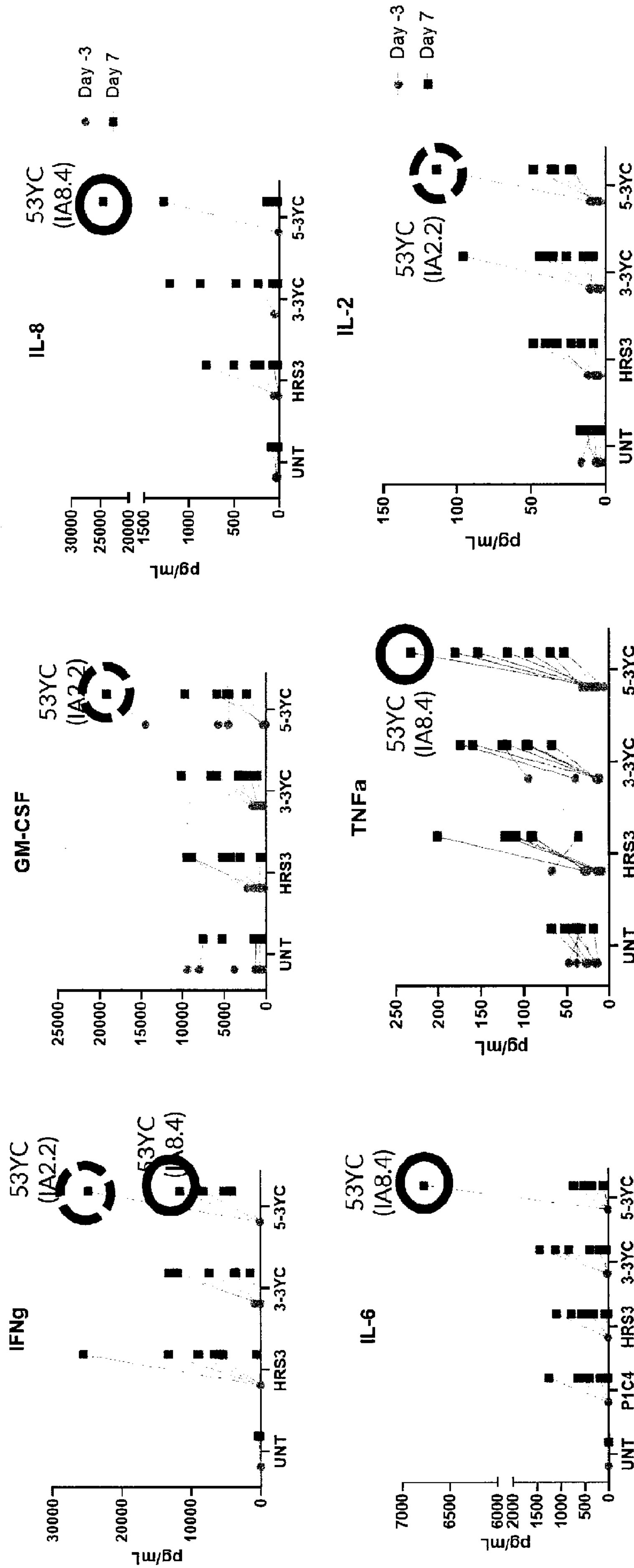


Figure 9G

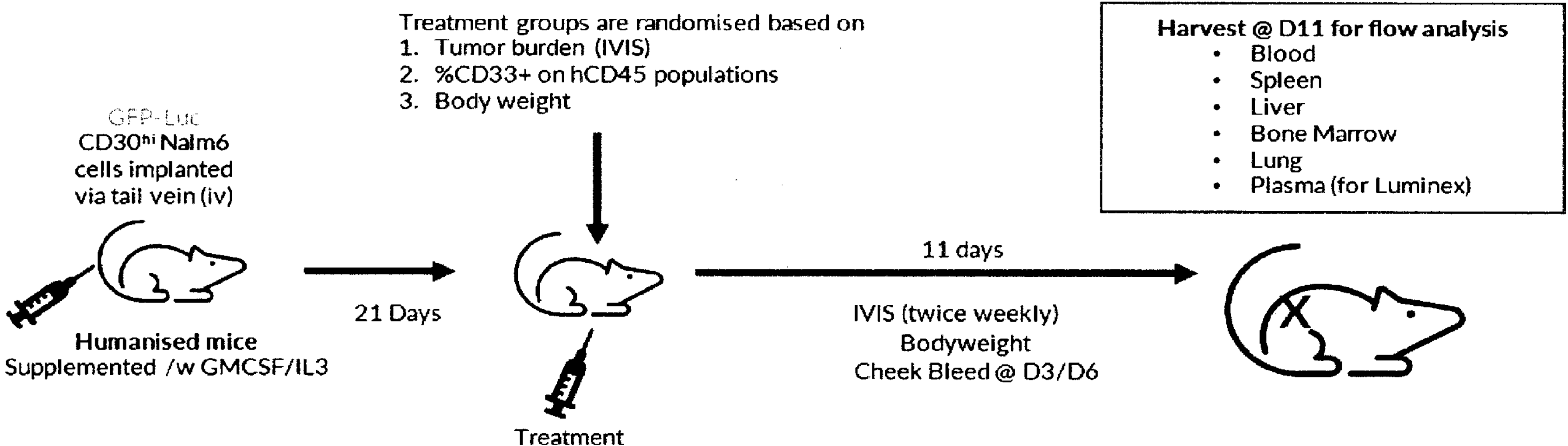


Figure 10A

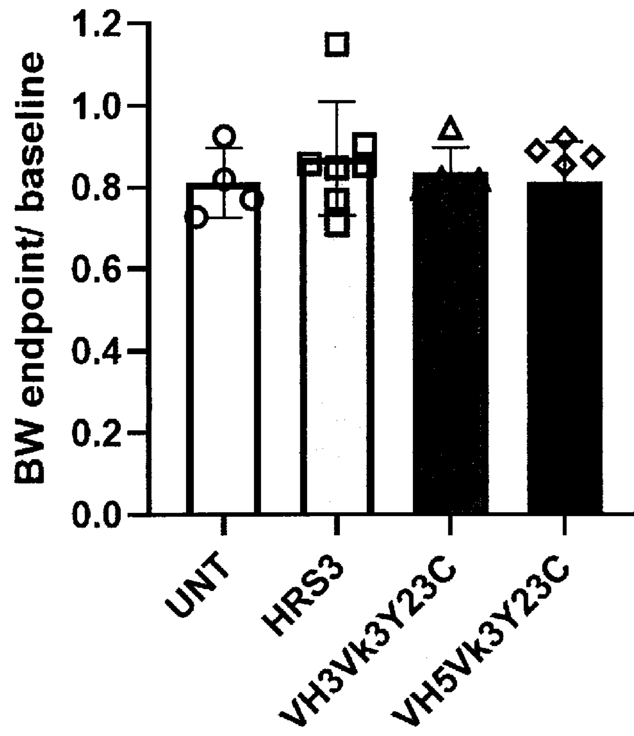
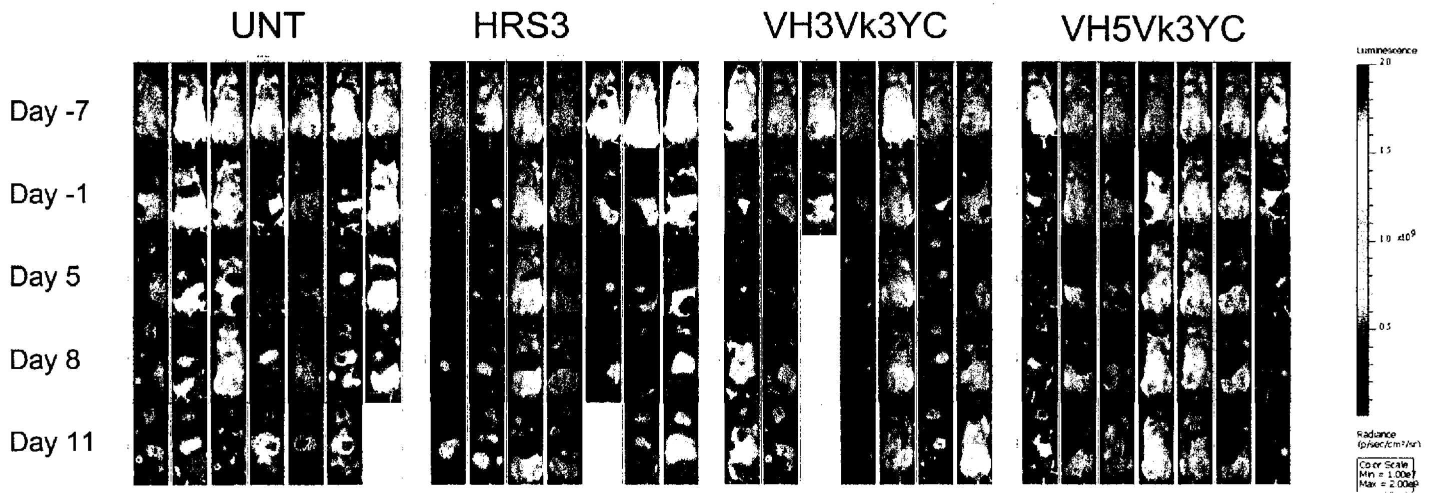


Figure 10B



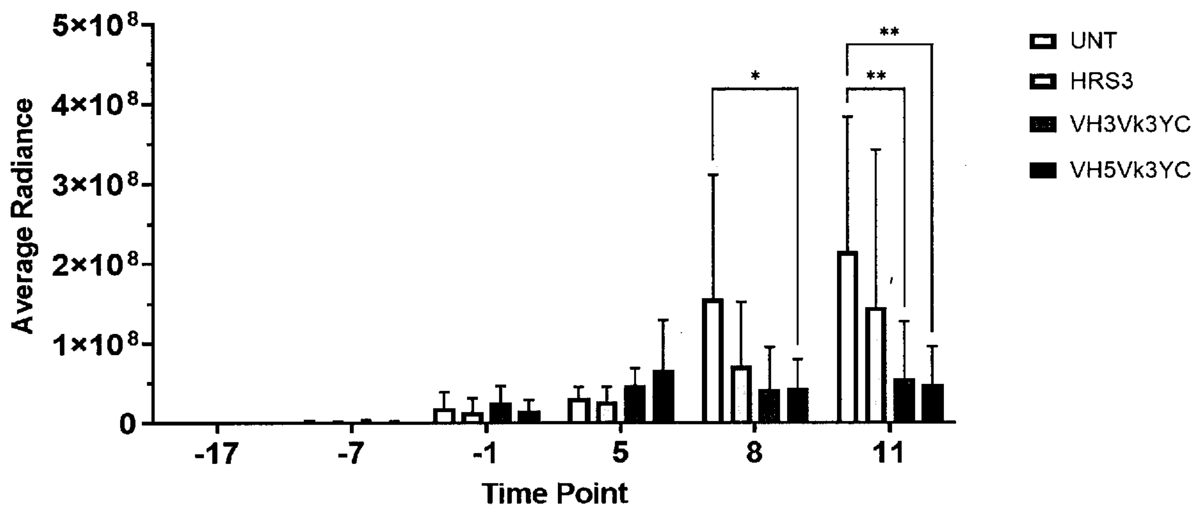


Figure 10C

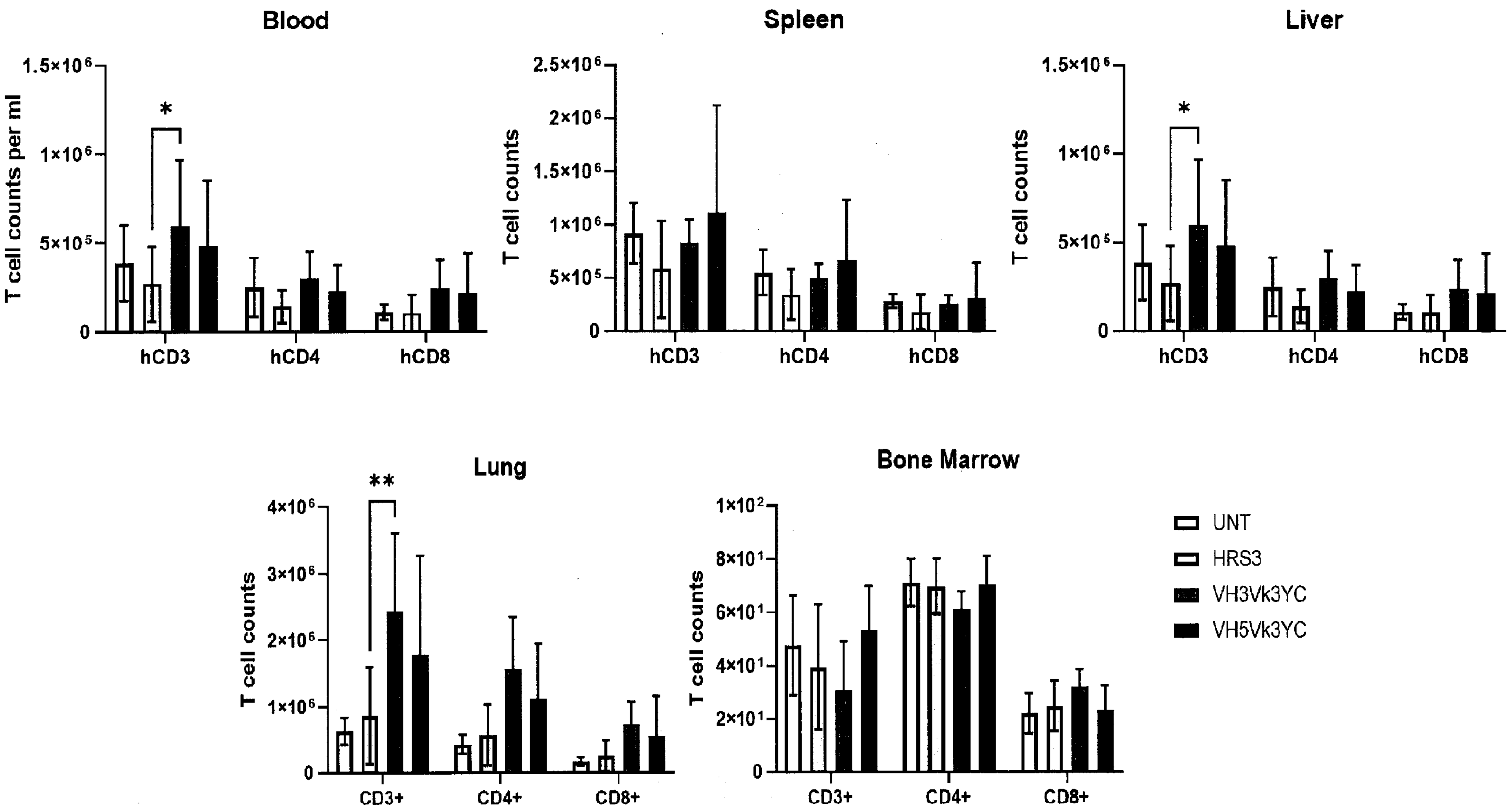


Figure 10D

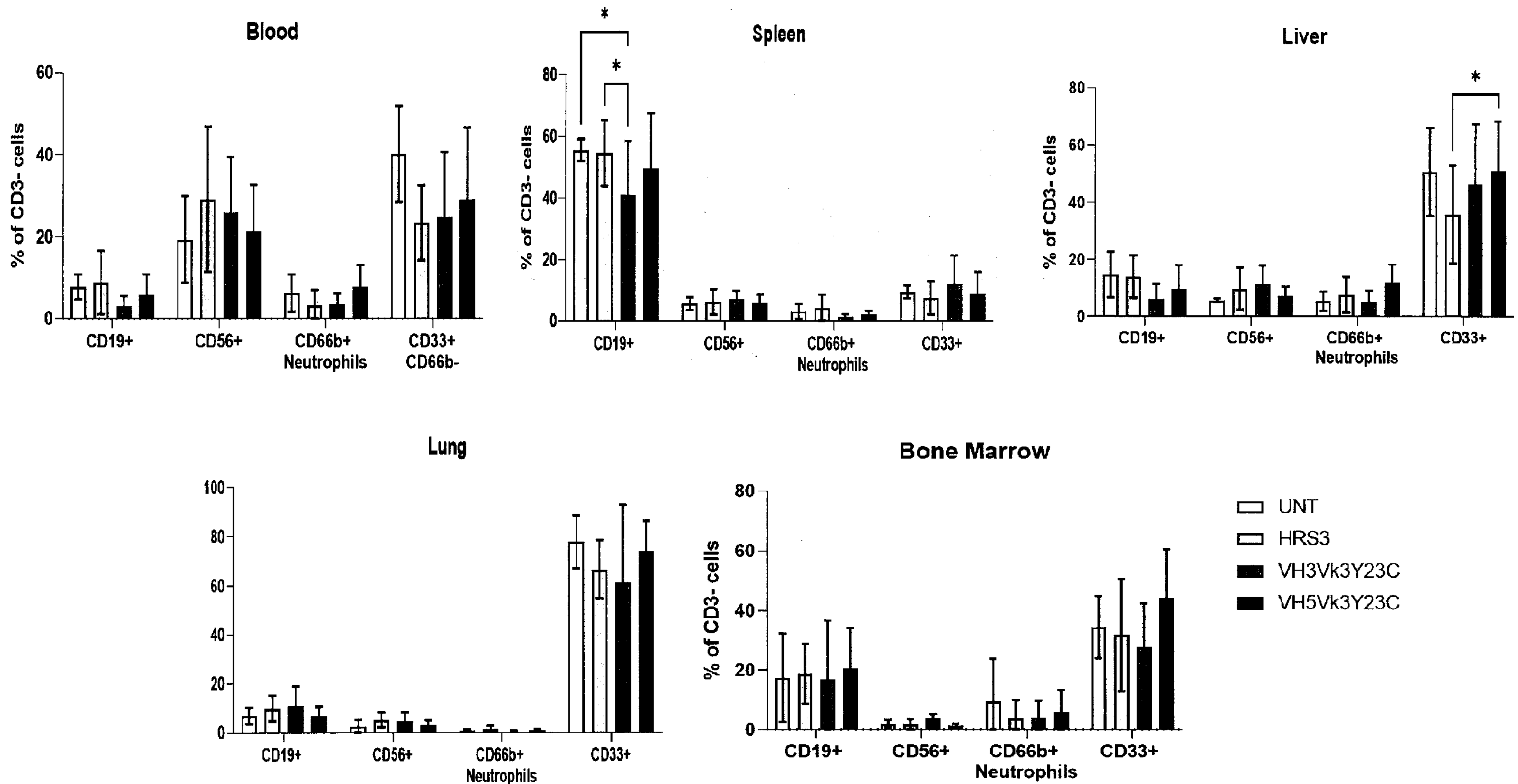


Figure 10E

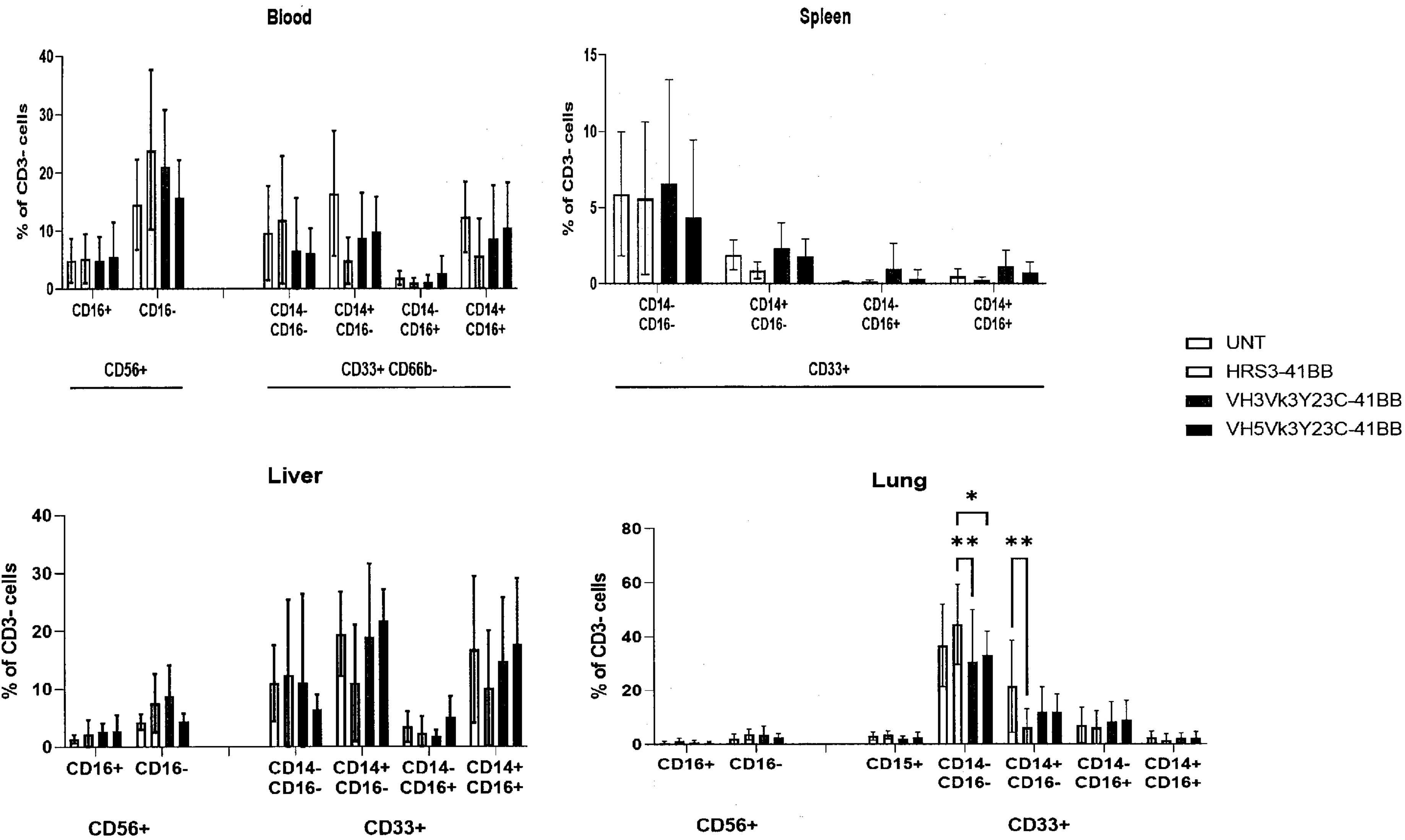


Figure 10E (Cont.)

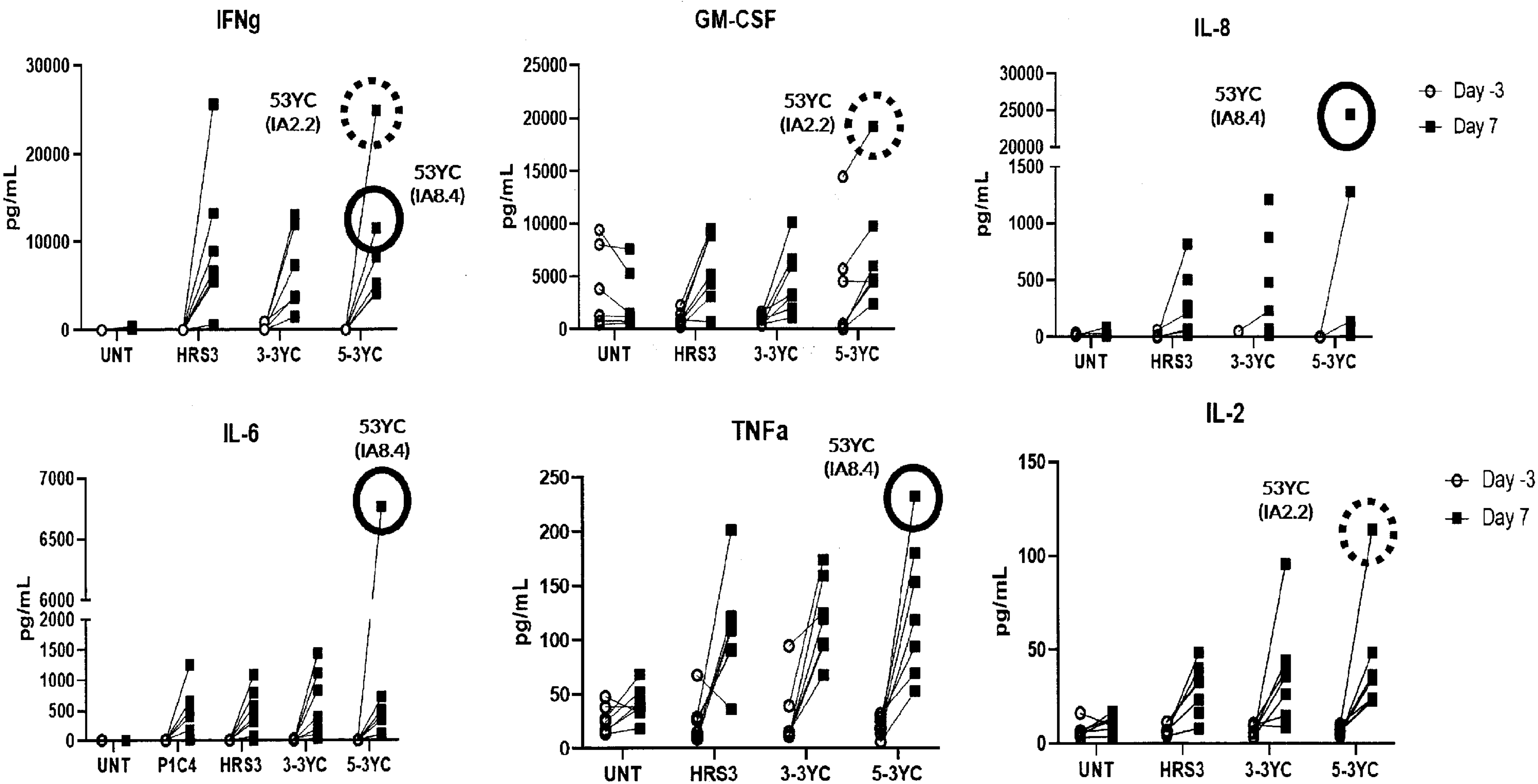


Figure 10F

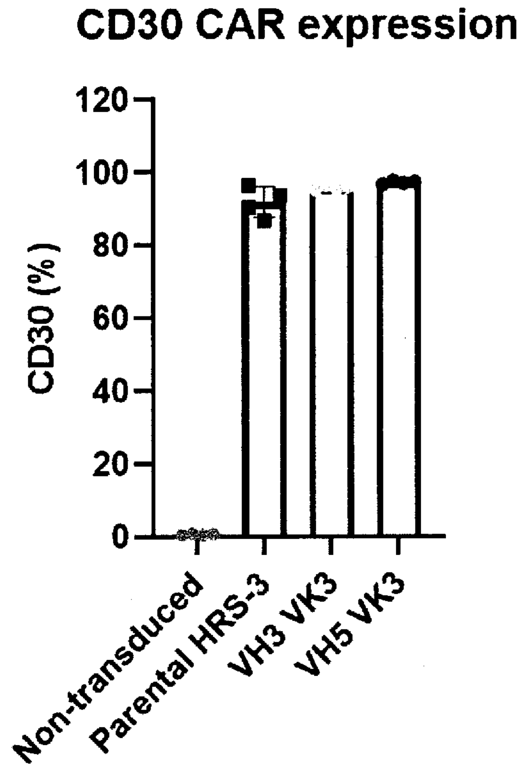


Figure 11A

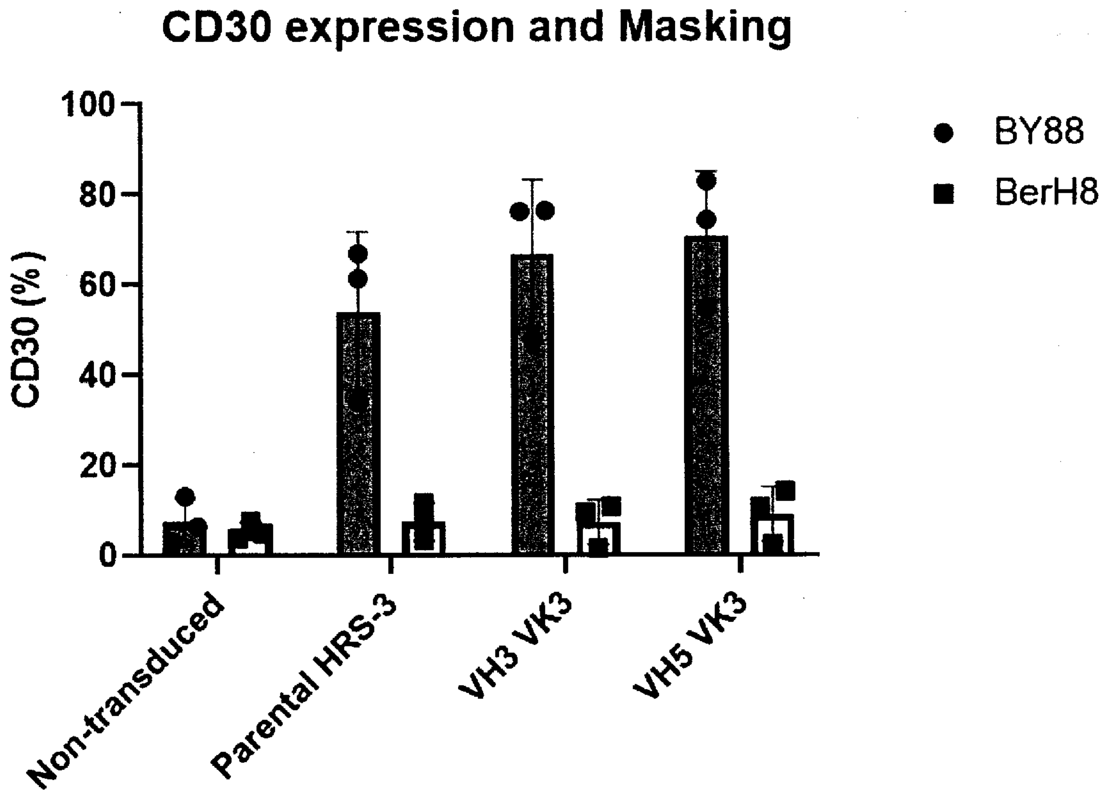
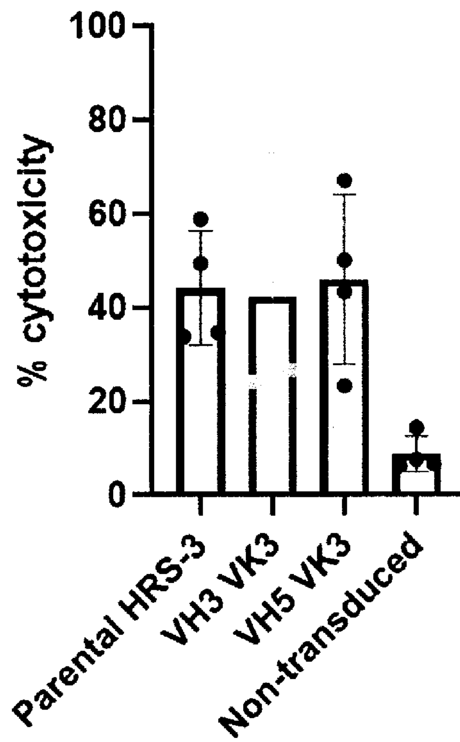


Figure 11B



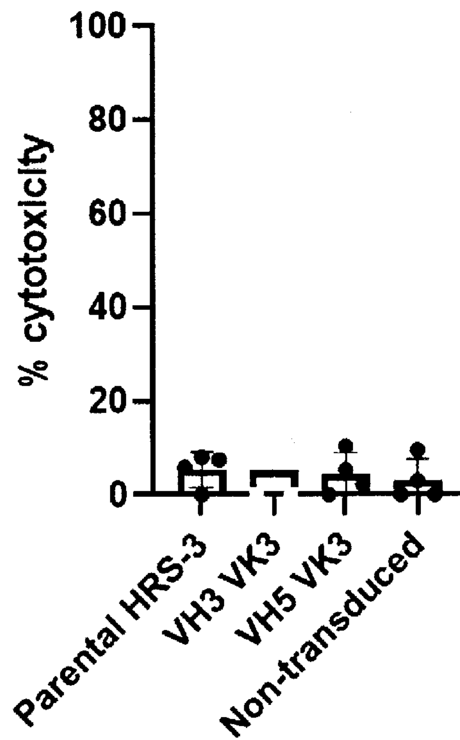


Figure 11C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050544

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28 (2006.01) A61K 39/395 (2006.01) A61P 35/00 (2006.01) A61P 37/06 (2006.01)

According to International Patent Classification (IPC)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DATABASES: FAMPAT, MEDLINE, EMBASE, BIOSIS
SEARCH TERMS: CD30, TNFRSF8, Ki1, antibodies, immunoglobulin, Fab, scFv, Y23C and related terms. SEQ ID NOs: 1-3, 9-12, 17 and 30.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CN 106589139 A (WUHAN BIO-RAID BIOLOGICAL TECH CO LTD) 26 April 2017 (Paragraphs 0008-0015, 0023, 0034; Figures 1-3; SEQ ID NO: 1) of the machine translation	1-3 and 8-28 4
X Y	SCHLAPSCHY, M. ET AL., Functional humanization of an anti-CD30 Fab fragment for the immunotherapy of Hodgkin's lymphoma using an in vitro evolution approach. *Protein Engineering Design and Selection*, 16 February 2005, Vol. 17, No. 12, pages 847-860 [Retrieved on 2024-01-02] <DOI: 10.1093/PROTEIN/GZH098> (Whole document especially Pages 850-851)	5-28 4
X Y	US 2014/0018521 A1 (BUCHHOLZ C., ET AL.) 16 January 2014 (Paragraph 0037, 0235, 0278; Figure 1)	5-28 4

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

*Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02/01/2024 *(day/month/year)*	04/01/2024 *(day/month/year)*

Name and mailing address of the ISA/SG

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2023/050544

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	GUO, J. ET AL., Humanized CD30-Targeted Chimeric Antigen Receptor T Cells Exhibit Potent Preclinical Activity Against Hodgkin's Lymphoma Cells. *Frontiers in Cell and Developmental Biology*, 12 January 2022, Vol. 9, Article 775599, pages 1-10 [Retrieved on 2024-01-02] <DOI: 10.3389/FCELL.2021.775599> (Whole document especially Figure 4)	1-3 and 8-28 4
X Y	WO 2021/245249 A1 (TESSA THERAPEUTICS LTD ET AL.) 9 December 2021 (Claims; Page 14, Lines 20-22; Page 22, Line 30 to Page 23, Line 20; Page 35, Lines 6-9; Example 1; SEQ ID NOs: 8-15, 18 and 35)	1-3 and 8-28 4
A	WO 2020/135426 A1 (INNOVENT BIOLOGICS (SUZHOU) CO. LTD.) 2 July 2020 (Paragraphs 0005, 0007, 0019-0020, 0279-0285, 0302-0311, 0316; Example 1) of the machine translation	25-28
A	QUACH, D.H. ET AL., A Bank of CD30.CAR-Modified, Epstein-Barr Virus-Specific T Cells That Lacks Host Reactivity and Resists Graft Rejection for Patients with CD30-Positive Lymphoma. *Blood*, 5 November 2020, Vol. 136, Supplement 1, page 16 [Retrieved on 2024-01-02] <DOI: 10.1182/BLOOD-2020-141491> (Whole document)	16 and 22-23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SG2023/050544

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

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

   a. ☐ forming part of the international application as filed.

   b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/SG2023/050544

Note: This Annex lists known patent family members relating to the patent documents cited in this International Search Report. This Authority is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
CN 106589139 A	26/04/2017	NONE	
US 2014/0018521 A1	16/01/2014	WO 2012/095535 A1	19/07/2012
		EP 2476754 A1	18/07/2012
		EP 2663643 A1	20/11/2013
WO 2021/245249 A1	09/12/2021	CN 116209461 A	02/06/2023
		EP 4161563 A1	12/04/2023
		CA 3180613 A1	09/12/2021
		US 2023/0263890 A1	24/08/2023
		JP 2023528632 A	05/07/2023
		TW 202210084 A	16/03/2022
		AU 2021283714 A1	19/01/2023
WO 2020/135426 A1	02/07/2020	CN 113227147 A	06/08/2021
		TW 202024135 A	01/07/2020