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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 July 2021 (29.07.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/148788 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *A61P 35/00* (2006.01)
C12N 5/10 (2006.01)

(21) International Application Number:
PCT/GB2021/050128

(22) International Filing Date:
21 January 2021 (21.01.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2000934.6 22 January 2020 (22.01.2020) GB

(71) Applicant: **UCL BUSINESS LTD** [GB/GB]; The Network Building, 97 Tottenham Court Road, London W1T 4TP (GB).

(72) Inventors: **FISHER, Jonathan**; c/o UCL BUSINESS LTD, The Network Building, 97 Tottenham Court Road, London W1T 4TP (GB). **CHESTER, Kerry**; c/o UCL BUSINESS LTD, The Network Building, 97 Tottenham Court Road, London W1T 4TP (GB). **ANDERSON, John**; c/o UCL BUSINESS LTD, The Network Building, 97 Tottenham Court Road, London W1T 4TP (GB). **ROTA, Enrique Miranda**; c/o UCL BUSINESS LTD, The Network Building, 97 Tottenham Court Road, London W1T 4TP (GB).

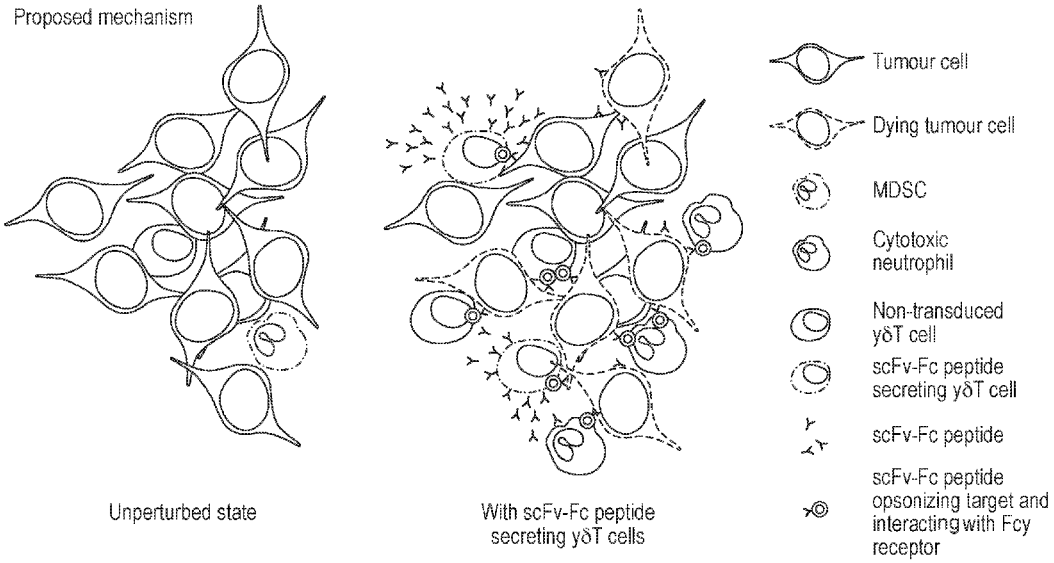
(74) Agent: **J A KEMP LLP**; 14 South Square, Gray's Inn, London Greater London WC1R 5JJ (GB).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,

(54) Title: ENGINEERED IMMUNE CELLS

Fig. 1



(57) Abstract: The invention relates to an immune cell that is capable of antibody-dependent cellular cytotoxicity and which comprises a nucleic acid sequence encoding a secreted antigen binding protein. The invention also concerns a method of producing the immune cell and medical uses for the immune cell.

[Continued on next page]

WO 2021/148788 A1

EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

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

— *with sequence listing part of description (Rule 5.2(a))*

ENGINEERED IMMUNE CELLS

Field of the invention

The invention relates to an immune cell that is capable of antibody-dependent cellular cytotoxicity and which comprises a nucleic acid sequence encoding an antigen binding molecule. The invention also concerns a method of producing the immune cell and medical uses for the immune cell.

Background to the invention

Alpha beta (ab) T cells expressing chimeric antigen receptors (CARs) form an important part of the immunotherapeutic toolkit. By expressing a CAR in an abT cell, the antigen specificity of the abT cell can be changed. In this way, a subject's adaptive immune system can be reprogrammed to target an antigen of particular interest in the subject, such as a tumour antigen.

Recently, strategies to improve the therapeutic potential of CAR abT cells have been developed. In particular, "*armoured*" CAR abT cells have been produced. Armoured CAR abT cells are endowed with the ability to secrete function-enhancing molecules. The CAR abT cell may, for instance, secrete a cytokine that enhance its therapeutic effect. CAR abT cells may secrete a peptide which inhibits protein kinase A (Newick *et al.*, Cancer Immunol Res, June 2016, 4(6): 541-551). CAR abT cells may also secrete molecules that overcome the immune checkpoint. For example, Rafiq *et al.* (Nature Biotechnology, 13 Aug 2018, 36(9):847-856) equipped CAR abT cells with the ability to secrete an anti-PDL1 antibody, and Li *et al.* (Clinical Cancer Research, November 2017, 23(22): 6982-6992) engineered CAR abT cells to secrete a scFv specific for PD-1 on the effector cell surface.

In contrast to abT cells, gamma delta (gd) T cells are a relatively overlooked innate-like immune cell subset. Unlike abT cells, gdT cells (especially Vδ2+ gdT cells) are capable of potent antibody-dependent cellular cytotoxicity (ADCC) against antibody-labelled tumour cells. Accordingly, gdT cell infiltration into tumours correlates with favourable clinical outcome and gdT cells have potential in cancer immunotherapy. Myeloid cells are also capable of ADCC and may be used for cancer immunotherapy. To

optimise their therapeutic potential, it would be desirable to engineer gdT cells and myeloid cells to secrete molecules that enhance their anti-cancer effects.

Summary of the invention

The present inventors have demonstrated that immune cells capable of ADCC may be engineered to secrete an antigen binding molecule (e.g. an antibody or antibody-like protein such as a scFv-Fc) targeting an antigen that is expressed in the tumour microenvironment. Secretion of the antigen binding molecule may enhance the anti-cancer effects of the immune cell. For example, the secreted antigen binding molecule may increase target cell killing, for instance by ADCC. This exemplary mechanism is shown in Figure 1, in which a scFv-Fc secreted by an engineered immune cell (e.g. gdT cell) marks target cells expressing the cognate antigen for ADCC. This allows the engineered immune cells to kill the target cells by ADCC. Bystander, non-engineered immune cells can also exert ADCC against the target cells. In this way, the antigen-specific cytotoxicity of engineered and bystander immune cells is improved. Administration of the engineered immune cells to a subject therefore provides an improved anti-cancer therapy.

Accordingly, the present invention provides:

- an immune cell that is capable of ADCC and which comprises a nucleic acid sequence encoding an antigen binding molecule that comprises an antigen binding region;
- a method of producing an immune cell of the invention, comprising introducing a nucleic acid sequence encoding an antigen binding molecule into an immune cell;
- a method of treating disease in an individual, the method comprising administering to the individual a therapeutically effective number of immune cells of the invention; and
- immune cells of the invention for use in a method of treating disease in an individual, the method comprising administering to the individual a therapeutically effective number of the immune cells.

Description of the Figures

Figure 1 – Example of a proposed mechanism whereby scFv-Fcs secreted by γδT cells engage Fc receptors on ADCC competent cells in the tumour micro-environment.

Figure 2 – Binding of scFv-Fc to cells expressing the target antigen detected using flow cytometry. CEA^+ CAPAN-1 or CEA^- HELA cells were incubated with supernatant from Vδ2 cells or Jurkat cells secreting an anti-CEA scFv-Fc fusion protein. Binding of the scFv-Fc fusion protein was detected using anti-human Fc. Purified scFv-fusion protein was used as a positive control. A similar experiment was performed using $GD2^{+/-}$ SupT1 cells and supernatant from Vδ2 cells secreting anti-GD2 scFv-Fc fusion protein. In this case, clinical grade dinutuximab (anti-GD2) was used as a positive control.

Figure 3 – Experimental setup showing conditions used in cytotoxicity assays to test the direct and indirect cytotoxic benefit of scFv-Fc fusion protein expression.

Figure 4 – cytotoxicity in cell-contact dependent and independent settings.
All cytotoxicity experiments were performed at an effector : target ratio of 1:1, using 18h co-culture. Target cells were labelled with CellTrace Violet™ and death detected by staining with GhostRed fixable viability dye. Target cell death is shown with the background death (in absence of effectors) subtracted from all values.

A) Killing of CEA^+ CAPAN-1 or CEA^- HELA cells by anti-CEA scFv-Fc secreting Vδ2 cells or non-transduced Vδ2 cells.
B) Killing of CEA^+ CAPAN-1 or CEA^- HELA cells by non-transduced Vδ2 cells in the presence or absence of supernatant from anti-CEA-scFv-Fc secreting Vδ2 cells.
C) Killing of CEA^+ CAPAN-1 or CEA^- HELA cells by non-transduced Vδ2 cells. where anti-CEA scFv-Fc secreting Vδ2 cells or non-transduced controls were sequestered behind a semi-permeable membrane.
D) Killing of $GD2^+$ SupT1 or $GD2^-$ wild type SupT1 cells by anti-GD2 scFv-Fc secreting or non-transduced Vδ2 cells.
E) Killing of $GD2^+$ SupT1 or GD2- wild type SupT1 cells by non-transduced Vδ2 cells in the presence or absence of supernatant from anti-GD2-scFv-Fc secreting Vδ2 cells.

F) Killing of $GD2^+$ SupT1 or $GD2^-$ wild type SupT1 cells by non-transduced Vδ2 cells where anti-GD2 scFv-Fc secreting Vδ2 cells or non-transduced controls were sequestered behind a semi-permeable membrane.

Figure 5 – Concentrations of IFNγ in supernatant after 18h co-culture of CEA^+ CAPAN-1 or CEA^- HELA cells with non-transduced Vδ2 cells where anti-CEA scFv-Fc secreting Vδ2 cells or non-transduced controls were sequestered behind a semi-permeable membrane.

Figure 6 – Binding of anti-GD2 antibody (SEQ ID NO: 17) produced by 293T cells to $GD2^{+/-}$ target cells detected by flow cytometry. 293T cells were treated with reducing volumes of lentivirus encoding the whole anti-GD2 antibody 14G2a. Isogenic SupT1_wt ($GD2^-$) or SupT1_GD2 ($GD2^+$) were incubated with supernatant from the transduced 293T cells. Antibody binding was detected using anti-human Fc antibody conjugated to PE. Pure anti-GD2 antibody (dinutuximab, ch14.18, clone 14G2a) was used as a positive control. Antibody was detected in the supernatant of transduced 293T cells, at a level dependent on the viral dose applied.

Figure 7 – Binding of antibody produced by γδT cells to cells expressing target antigen. Isogenic SupT1_wt ($GD2^-$) or SupT1_GD2 ($GD2^+$) were incubated with supernatant from Vδ2 transduced to express whole anti-Gd2 antibody (clone 14G2a). Antibody binding was detected using anti-human IgG secondary antibody, and pure anti-GD2 (dinutuximab, ch14.18, clone 14G2a) was used as a positive control.

Figure 8 – cytotoxicity in cell-contact dependent and independent settings. All cytotoxicity experiments were performed at an effector : target ratio of 1:1, using 18h co-culture. Target cells were labelled with CellTrace Violet™ and death detected by staining with Live/Dead Blue fixable viability dye (detected on the DAPI channel). Live/Dead Blue staining of the target cells is shown and dead cell percentages are marked

A) Experimental setup showing how direct and bystander cytotoxicity was determined.

B) Killing of $GD2^+$ SupT1 or $GD2^-$ wild type SupT1 cells by anti-GD2 antibody secreting or non-transduced Vδ2 cells.

C) Killing of $GD2^+$ SupT1 or $GD2^-$ wild type SupT1 cells by non-transduced Vδ2 cells in the presence or absence of supernatant from anti-GD2-antibody secreting Vδ2 cells.

Description of the sequence listing

SEQ ID NO: 1 provides the sequence of the V_H domain of a CEA-specific scFv used in the examples.

SEQ ID NO: 2 provides the sequence of the V_L domain of a CEA-specific scFv used in the examples.

SEQ ID NO: 3 provides the sequence of a CEA-specific scFv-Fc used in the examples.

SEQ ID NO: 4 provides the sequence of the V_H domain of a GD2-specific scFv used in the examples.

SEQ ID NO: 5 provides the sequence of the V_L domain of a GD2-specific scFv used in the examples.

SEQ ID NO: 6 provides the sequence of a GD2-specific scFv-Fc used in the examples.

SEQ ID NO: 7 provides the sequence of a CEA-specific scFv used in the examples

SEQ ID NO: 8 provides the sequence of a GD2-specific scFv used in the examples

SEQ ID NO: 9 provides the sequence of the V_H domain of a B7H3-specific scFv .

SEQ ID NO: 10 provides the sequence of the V_L domain of a B7H3-specific scFv.

SEQ ID NO: 11 provides the sequence of a B7H3-specific scFv-Fc.

SEQ ID NO: 12 provides the sequence of a B7H3-specific scFv.

SEQ ID NO: 13 provides the sequence of the V_H domain of a CD20-specific scFv.

SEQ ID NO: 14 provides the sequence of the V_L domain of a CD20-specific scFv.

SEQ ID NO: 15 provides the sequence of a CD20-specific scFv-Fc.

SEQ ID NO: 16 provides the sequence of a CD20-specific scFv.

SEQ ID NO: 17 provides the sequence of a GD2 specific IgG1 used in the examples, having a cleavage site between the light and heavy chains.

SEQ ID NO: 18 provides the sequence of the light chain of the GD2 IgG1 of SEQ ID NO: 17.

SEQ ID NO: 19 provides the sequence of the cleavage sequence (Furin-V5-SG-P2A) of the GD2 IgG1 of SEQ ID NO: 17.

SEQ ID NO: 20 provides the sequence of the heavy chain of the GD2 IgG1 of SEQ ID NO: 17.

Detailed description of the invention

It is to be understood that different applications of the disclosed products and methods may be tailored to the specific needs in the art. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

In addition, as used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a nucleic acid" includes "nucleic acids", reference to "an scFv-Fc" includes two or more such scFv-Fcs, reference to "a T cell" includes two or more such T cells, and the like.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety.

Immune cells

The invention provides an immune cell that is capable of ADCC and which comprises a nucleic acid sequence encoding an antigen binding molecule. The immune cell may be any immune cell that is capable of ADCC. Immune cells capable of ADCC are known in the art.

ADCC is a well-known mechanism of adaptive, cell-mediated immunity. During ADCC, an immune effector cell actively lyses a target cell whose surface antigens have been bound by specific antibodies. Specifically, antibodies bind their cognate antigen on the surface of target cells. Fc receptors present on the surface of immune effector cells recognise the Fc region of the bound antibodies. Cross-linking of Fc receptors triggers the formation of a lytic synapse between the immune effector cell and the target cell, into which the immune effector cell degranulates lytic granules. Apoptosis of the target cell is therefore triggered. Immune effector cells capable of ADCC are known to include natural

killer (NK) cells, macrophages, neutrophils and eosinophils. gdT cells are also capable of ADCC.

The immune cell may be from any species, such as a human, dog, cat, mouse, rat, pig, sheep, cow, goat or horse. The immune cell is typically a human immune cell. The immune cell may be a canine, feline, murine, porcine, ovine, caprine, bovine or equine immune cell.

Preferably, the immune cell is not an abT cell. abT cells are T cells that possess a T cell receptor (TCR) that comprises an alpha chain and a beta chain. They are usually activated in an MHC-dependent manner. ADCC has not been reported for abT cells. abT cells are often thought of as "*conventional*" T cells.

The immune cell may be a gdT cell. gdT cells are T cells that have a gd T cell receptor (TCR) on their surface. That is, gdT cells possess a TCR that comprises a gamma chain and a delta chain. Therefore, gdT cells are structurally different from abT cells. gdT cells are also functionally different from abT cells. In particular, gdT cells are capable of ADCC. gdT cells are usually activated in an MHC-independent matter. gdT cells are often thought of as "*unconventional*" T cells.

Several subsets of gdT cell exist. For example, the gdT cell may be a Vδ2+ gdT cell, a Vδ1+ gdT cell, or a Vδ1-/ Vδ2- gdT cell. Preferably, the gdT cell is a Vδ2+ gdT cell. Vδ2+ gdT cells, Vδ1+ gdT cells, and Vδ1-/ Vδ2- T cells all have an excellent capacity for ADCC and exhibit good anti-tumour toxicity.

Methods for expanding gdT cells are known in the art. For instance, gdT cells may be expanded by culturing in the presence of IL-2 and zoledronic acid (Fisher J *et al.*. Effective combination treatment using anti-GD2 ch14.18/CHO antibody with Vδ2+ γδT cells in Ewing sarcoma and neuroblastoma. Oncoimmunology 2015 Apr 27;5(1):e1025194.). gdT cells are thus readily available for use in the invention.

The immune cell may be a myeloid cell. Myeloid cells are cells that arise from a common myeloid progenitor cell, such as platelets, erythrocytes, mast cells, macrophages, basophils, neutrophils, and eosinophils. ADCC has been reported for macrophages, basophils, neutrophils, and eosinophils. Preferably, therefore, the myeloid cell is a macrophage, basophil, neutrophil or eosinophil. Methods for isolating and expanding myeloid cells are known in the art.

Natural killer (NK) cells are also capable of ADCC. The immune cell may be a

NK cell. NK cells are a class of innate lymphocytes with roles in immunity against a variety of diseases. For example, NK cells have roles in detecting and controlling cancer, and in killing virally-infected cells. Methods for isolating and expanding NK cells are known in the art.

Preferably, the immune cell does not express a chimeric antigen receptor (CAR). Preferably, therefore, the immune cell is not a CAR T cell.

Nucleic acid sequence

The immune cell of the invention comprises a nucleic acid sequence encoding an antigen binding molecule. The nucleic acid sequence may comprise DNA. The nucleic acid sequence may comprise RNA. The nucleic acid sequence may comprise DNA and RNA.

The immune cell may comprise one or more nucleic acid sequences each encoding an antigen binding molecule. For example, the immune cell may comprise 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 15 or more, or 20 or more nucleic acid sequences each encoding as antigen binding molecule. If the immune cell comprises multiple nucleic acid sequences each encoding an antigen binding molecule, the antigen binding molecule encoded by each nucleic acid sequence may be the same or different. Preferably, each of the antigen binding molecules is different. Preferably, each of the antigen binding molecules is specific for a different antigen. Preferably, the antigen is a tumour antigen. The antigen may be expressed on or by a cancer cell. The antigen may be expressed on or by a non-cancer cell in the tumour microenvironment. The antigen may be secreted into the tumour microenvironment.

Each nucleic acid sequence may encode one or more antigen binding molecules. For example, the nucleic acid sequence may encode 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 15 or more, or 20 or more antigen binding molecules. If the nucleic acid sequence encodes multiple antigen binding molecules, each of the antigen binding molecules may be the same or different. Preferably, each of the antigen binding molecules is different. Preferably, each of the antigen binding molecules is specific for a different antigen. Preferably, the antigen is a tumour antigen. The antigen may be expressed on or by a cancer cell. The antigen may be expressed on or by a non-cancer cell in the tumour microenvironment. The antigen may be

secreted into the tumour microenvironment.

The nucleic acid sequence may comprise an exogenous promoter sequence to which the sequence encoding the antigen binding molecule is operably linked. The exogenous promoter may be an inducible promoter. Alternatively, the nucleic acid sequence may lack an exogenous promoter sequence. In this case, the nucleic acid sequence may integrate to the genome of the immune cell such that expression of the antigen binding molecule is controlled by an endogenous promoter in the genome. Activation of the exogenous or endogenous promoter may be controlled by an inducible signalling pathway. For example, the exogenous or endogenous promoter may be activated following engagement of a synNotch receptor with cognate antigen.

The nucleic acid sequence may be integrated to the genome of the immune cell. Alternatively, the nucleic acid sequence may not be integrated to the genome of the immune cell. If the nucleic acid sequence is not integrated to the genome of the immune cell, it may be comprised in a plasmid, a vector, or an artificial chromosome. The vector may be a viral vector or a non-viral vector. The artificial chromosome may be a yeast artificial chromosome (YAC), a bacterial artificial chromosome (BAC) or a human artificial chromosome (HAC). Preferably, the artificial chromosome is a HAC.

Antigen binding molecule

The immune cell of the invention comprises a nucleic acid sequence encoding an antigen binding molecule.

The antigen binding molecule comprises an antigen binding region. An antigen binding region is a region of an antigen binding molecule that is capable of specifically binding to one or more antigens. For example, an antigen binding region may be capable of specifically binding to 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more or 10 or more different antigens. Exemplary antigen binding regions are known in the art, and include at least a scFv (single chain variable fragment), a Fab, a modified Fab, a Fab', a modified Fab', a F(ab')2, a Fv, a dAb, a Fd, a dsFv, a ds-scFv, a scFv2, a Bi-specific T-cell engager, a nanobody, a DARPin, an antibody mimetic, a diabody, a triabody and a tetrabody. The antibody binding region may therefore comprise a scFv, a Fab, a modified Fab, a Fab', a modified Fab', a F(ab')2, a Fv, a dAb, a Fd, a dsFv, a ds-scFv, a scFv2, a Bi-specific T-cell engager, a nanobody, a DARPin, an antibody

mimetic, a diabody, a triabody or a tetrabody, alone or in any combination. The antigen binding molecule may comprise a scFv, a Fab, a modified Fab, a Fab', a modified Fab', a F(ab')2, a Fv, a dAb, a Fd, a dsFv, a ds-scFv, a scFv2, a Bi-specific T-cell engager, a nanobody, a DARPin, an antibody mimetic, a diabody, a triabody, a tetrabody, or a polypeptide ligand for a receptor expressed on the surface of a cell that is targeted by the immune cell, alone or in any combination.

Preferably, the antigen binding molecule comprises an antigen binding region comprising a scFv. The antigen binding region may comprise 2 or more, such as 3 or more, 4 or more, 5 or more, 6 or more, 7 or more 8, or more, 9 or more or 10 or more scFvs. scFvs are known in the art. An scFv is a fusion protein comprising the variable region of the heavy chain (V_H) tethered to the variable region of the light chain (V_L) of an antibody. Typically, the V_H and the V_L are tethered by a linker peptide. The linker peptide may be from about 5 to about 30 amino acids in length. For example, the linker peptide may be from about 6 to about 29, about 7 to about 28, about 8 to about 27, about 9 to about 26, about 10 to about 25, about 11 to about 24, about 12 to about 23, about 13 to about 22, about 14 to about 21, about 15 to about 20, about 16 to about 19, about 17 or about 18 amino acids in length.

The antigen binding molecule may be capable of binding to a Fc receptor. For example, the antigen binding molecule may comprise a Fc (fragment crystallisable) region. Fc regions are known in the art. The Fc region is the tail region of an antibody that interacts with Fc receptors and some proteins of the complement system. This property allows antibodies to activate the immune system. The Fc region comprises at least two heavy chain constant (CH) domains. Specifically, in Fc domains derived from an IgG, IgA or an IgD antibody, the Fc region comprises the CH2 and CH3 domains of the antibody. In Fc regions derived from an IgM or an IgE antibody, the Fc region comprise the CH2, CH3 and CH4 regions of the antibody. The Fc region may be a modified Fc region. For example, the Fc region may be an Fc region that has been modified to optimise its ability to bind to FcR. Such optimisation is known in the art and is described, for example in (i) Mössner E *et al.* Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood. 2010;115(22):4393–4402; and (ii) Wang *et al.* 2018

IgG Fc engineering to modulate antibody effector functions. Protein Cell. 2018 Jan; 9(1): 63–73. doi: 10.1007/s13238-017-0473-8 (and references therein).

The antigen binding molecule may be capable of binding to a Fc receptor via a region other than a Fc region. That is, the antigen binding molecule need not comprise a Fc region in order to be capable of binding to a Fc receptor. For example, the antigen binding molecule may contain an antigen binding region (such as a scFv, a Fab, a modified Fab, a Fab', a modified Fab', a F(ab')2, a Fv, a dAb, a Fd, a dsFv, a ds-scFv, a scFv2, a Bi-specific T-cell engager, a nanobody, a DARPin, an antibody mimetic, a diabody, a triabody, a tetrabody, or a polypeptide ligand for a receptor expressed on the surface of a cell that is targeted by the immune cell) that is capable of binding to a Fc receptor. The antigen binding molecule may comprise an antigen binding region that is capable of binding to a Fc receptor, and an antigen binding region that is capable of binding to a different antigen, such as an antigen that is expressed in the tumour microenvironment. For instance, the antigen binding molecule may comprise a scFv that is capable of binding to a Fc receptor, and a scFv that is capable of binding to a different antigen, such as an antigen that is expressed in the tumour microenvironment. The antigen binding molecule may comprise a bi-specific T-cell engager the comprises a scFv that is capable of binding to CD3, and a scFv that is capable of binding to a different antigen, such as an antigen that is expressed in the tumour microenvironment.

The antigen binding molecule may comprise an antigen binding region and a Fc region. The antigen binding molecule may be an antibody, a scFv-Fc, a dAb-Fc, or a heavy chain antibody. Exemplary heavy chain antibodies include an IgNAR and a camelid antibody. The antibody may, for example, comprise (a) a light chain encoded by SEQ ID NO: 18, (b) a heavy chain encoded by SEQ ID NO: 20, and/or (c) a cleavage sequence encoded by SEQ ID NO: 19. The antibody molecule may, for example, comprise: (a); (b); (c); (a) and (b); (a) and (c); (b) and (c); or (a), (b) and (c). The antibody may be encoded by SEQ ID NO: 17. The light chain encoded by SEQ ID NO: 18 comprises the V_L encoded by SEQ ID NO: 5. The heavy chain encoded by SEQ ID NO: 20 comprises the V_H encoded by SEQ ID NO:4.

Preferably, the antigen binding molecule is a scFv-Fc. An scFv-Fc is a fusion protein that comprises a scFv fused to a Fc region. The structure of scFvs and the Fc region is known in the art and described above. In accordance with these structures, the

scFv-Fc may comprise a V_H and a V_L (together forming the scFv) and a CH2 domain and a CH3 domain (together forming the Fc region). The scFv-Fc may comprise a V_H and a V_L (together forming the scFv), and a CH2 domain, a CH3 domain and a CH4 domain (together forming the Fc region). In a scFv-Fc, the scFv is linked to the Fc region. Preferably, the V_L or the VH in the scFv is linked to the CH2 in the Fc region in order to link the scFv to the Fc region. The scFv may be linked to the Fc region directly, i.e. in the absence of a linker. The scFv may be linked to the Fc region by a linker. The linker may be $(Ser(Gly)_4)$, $(Ser(Gly)_4)_2$, $(Ser(Gly)_4)_3$, $(Ser(Gly)_4)_4$, or $(Ser(Gly)_4)_5$. The linker may be an amino acid, or a short oligopeptide, consisting of about 2 amino acids. The linker may form a hinge region. The scFv-Fc may be a bivalent scFv-Fc. A bivalent scFv-Fc comprises two different scFvs each linked to a Fc region. In essence, a bivalent scFv-Fc comprises two arms, each comprising a scFv linked to a Fc region. scFvs, Fc regions and linkage are discussed above. The two arms are preferably linked. Linkage between the two arms preferably connects a linker between the scFv and Fc region in one arm with a linker between the scFv and Fc region in the other arm. The two arms are preferably connected at a point that is between the scFv and the Fc region in each arm.

The antigen binding molecule may function to increase target cell cytotoxicity. That is, the antigen binding molecule may increase the killing of cells expressing the cognate antigen of the antigen binding region. For example, the antigen binding molecule may increase the killing of tumour cells, endothelial cells, and/or immune cells. Increased killing may be mediated by engineered immune cells (i.e. by immune cells comprising the nucleic acid sequence encoding an antigen binding molecule). Increased killing may be mediated by non-engineered ("*bystander*") immune cells (i.e. by immune cells that do not comprise the nucleic acid sequence encoding an antigen binding molecule). Increased killing may be mediated by both engineered immune cells and non-engineered immune cells. Increased killing may be by any mechanism known in the art. Preferably, increased killing is mediated by increased ADCC. Increased killing may be mediated by increased engagement of ab T cells.

The antigen binding molecule is preferably an opsonin. An opsonin is a molecule that binds to an antigen to enhance its phagocytosis. Binding of an opsonin to an antigen may favour interactions between the antigen and cell surface receptors on immune cells, thereby boosting the kinetics of phagocytosis. Accordingly, the antigen binding molecule

may enhance phagocytosis. In particular, the antigen binding molecule may enhance phagocytosis of an antigen for which the antigen binding region is specific. In other words, the antigen binding molecule may enhance phagocytosis of an antigen bound by the antigen binding region (and, therefore, the antigen binding molecule).

The antigen binding region (and the antigen binding molecule) may be capable of binding to any antigen. That is, any antigen may be bound by the antigen binding region (and the antigen binding molecule). Preferably, the antigen binding region (and the antigen binding molecule) is capable of binding to an antigen that is expressed on or by cells in the tumour microenvironment. An antigen is expressed in the tumour microenvironment if it is expressed by any type of cell present in the tumour microenvironment. For example, the antigen may be expressed by a tumour cell, an endothelial, or an immune cell in the tumour microenvironment. The antigen binding region (and the antigen binding molecule) may therefore be capable of binding to a tumour antigen, an endothelial antigen, or an immune cell antigen. The immune cell may, for example, be a CD4+ T cell, a CD8+ T cell, a gdT cell, a B cell, a NK cell, a NKT cell, a macrophage, a monocyte, a basophil, an eosinophil or a neutrophil.

The antigen binding region (and the antigen binding molecule) may be capable of binding to an antigen selected from a group consisting of TSHR, CD19, CD123, CD22, CD20, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, CEA, EPCAM, B7-H3, KIT, IL-13Ra2, Mesothelin, IL-llRa, PSCA, PRSS21, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gplOO, bcr-abl, tyrosinase, EphA2, Fucosyl GMl, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17, XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin and telomerase, PCTA-l/Galectin 8, MelanA/MARTl, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OYTES1, LCK, AKAP-4,

SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, CEA, LINGO1, CD70, IL13Rα2, MUC-16, PSCA, ROR1, and IGLL1.

The antigen binding region (and antigen binding molecule) may be capable of binding to carcinoembryonic antigen (CEA). The CEA may be CEA-CAM5. CEA may be expressed in many carcinomas, including those of the colon and/or rectum, stomach, breast, pancreas, lung, thyroid, uterine cervix, or ovary.

The CEA-specific antigen binding region (and antigen binding molecule) may comprise a V_H domain encoded by SEQ ID NO: 1. The CEA-specific antigen binding region (and antigen binding molecule) may comprise a V_L domain encoded by SEQ ID NO: 2. The CEA-specific antigen binding region (and antigen binding molecule) may comprise a V_H domain encoded by SEQ ID NO: 1 and a V_L domain encoded by SEQ ID NO: 2. The CEA-specific antigen binding region (and antigen binding molecule) may comprise complementarity determining regions (CDRs) from a V_H domain encoded by SEQ ID NO: 1 and/or a V_L domain encoded by SEQ ID NO: 2. That is, the CEA-specific antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 1. The CEA-specific antigen binding region (and antigen binding molecule) may comprise light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 2. The CEA-specific antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 1 and light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 2. The CEA-specific antigen binding molecule may be a scFv-Fc encoded by SEQ ID NO: 3. The scFv may have the amino acid sequence of SEQ ID NO: 7.

The antigen binding region (and antigen binding molecule) may be capable of binding to GD2. GD2 may be expressed by cancers of neuroectodermal origin, such as neuroblastoma and melanoma.

The GD2-specific antigen binding region (and antigen binding molecule) may comprise a V_H domain encoded by SEQ ID NO: 4. The GD2-specific antigen binding region (and antigen binding molecule) may comprise a V_L domain encoded by SEQ ID NO: 5. The GD2-specific scFv-Fc antigen binding region (and antigen binding molecule)

may comprise a V_H domain encoded by SEQ ID NO: 4 and a V_L domain encoded by SEQ ID NO: 5. The GD2-specific antigen binding region (and antigen binding molecule) may comprise CDRs from a V_H domain encoded by SEQ ID NO: 4 and/or a V_L domain encoded by SEQ ID NO: 5. That is, the GD2- antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 4. The GD2-specific antigen binding region (and antigen binding molecule)may comprise light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 5. The GD2-specific antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 4 and light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 5. The GD2-specific antigen binding molecule may be a scFv-Fc encoded by SEQ ID NO: 6. The scFv may have the amino acid sequence of SEQ ID NO: 8.

The GD2-specific antigen binding molecule may comprise (a) a light chain encoded by SEQ ID NO: 18, (b) a heavy chain encoded by SEQ ID NO: 20, and/or (c) a cleavage sequence. The GD2-specific antigen binding molecule may, for example, comprise: (a); (b); (c); (a) and (b); (a) and (c); (b) and (c); or (a), (b) and (c). Preferably, the GD2-specific antigen binding molecule comprises (a), (b) and (c) in that order. In any of the aspects described herein, the cleavage sequence (c) may, for example, be Furin-V5-SG-P2A. The cleavage sequence may, for example, be encoded by SED ID NO: 19. Other cleavage sequences are also known in the art and may be used as the cleavage sequence (c). For example, the cleavage sequence (c) may comprise or consist of P2A, E2A, F2A or T2A. An IRES or IRES 2 sequence may be used in place of the cleavage sequence (c), in any aspect described herein. The GD2-specific antigen binding molecule may be an antibody encoded by SEQ ID NO: 17.

The antigen binding region (and antigen binding molecule) may be capable of binding to B7-H3. B7-H3 may be expressed by cancers of neuroectodermal origin, such as neuroblastoma and melanoma.

The B7-H3-specific antigen binding region (and antigen binding molecule) may comprise a V_H domain encoded by SEQ ID NO: 9. The B7-H3-specific antigen binding region (and antigen binding molecule) may comprise a V_L domain encoded by SEQ ID NO: 10. The B7-H3-specific antigen binding region (and antigen binding molecule) may comprise a V_H domain encoded by SEQ ID NO: 9 and a V_L domain encoded by SEQ ID

NO: 10. The B7-H3-specific antigen binding region (and antigen binding molecule) may comprise CDRs from a V_H domain encoded by SEQ ID NO: 9 and/or a V_L domain encoded by SEQ ID NO: 10. That is, the B7-H3-specific antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 9. The B7-H3-specific antigen binding region (and antigen binding molecule) may comprise light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 10. The B7-H3-specific antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 9 and light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 10. The B7-H3-specific antigen binding molecule may be a scFv-Fc encoded by SEQ ID NO: 11. The scFv may have the amino acid sequence of SEQ ID NO: 12.

The antigen binding region (and antigen binding molecule) may be capable of binding to CD20. CD20 is expressed during B cell development, the late pro-B cell stage through memory cells (though not on early pro-B cells or plasma blasts and plasma cells). CD20 is also expressed in B-cell lymphomas, hairy cell leukemia, B-cell chronic lymphocytic leukemia, and melanoma cancer stem cells.

The CD20-specific antigen binding region (and antigen binding molecule) may comprise a V_H domain encoded by SEQ ID NO: 13. The CD20-specific antigen binding region (and antigen binding molecule) may comprise a V_L domain encoded by SEQ ID NO: 14. The CD20-specific antigen binding region (and antigen binding molecule) may comprise a V_H domain encoded by SEQ ID NO: 13 and a V_L domain encoded by SEQ ID NO: 14. The CD20-specific antigen binding region (and antigen binding molecule) may comprise CDRs from a V_H domain encoded by SEQ ID NO: 13 and/or a V_L domain encoded by SEQ ID NO: 14. That is, the CD20-specific antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 13. The CD20-specific antigen binding region (and antigen binding molecule) may comprise light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 14. The CD20-specific antigen binding region (and antigen binding molecule) may comprise heavy chain CDR1, CDR2 and/or CDR3 from a V_H domain encoded by SEQ ID NO: 13 and light chain CDR1, CDR2 and/or CDR3 from a V_L domain encoded by SEQ ID NO: 14. The CD20-specific antigen binding molecule

may be a scFv-Fc encoded by SEQ ID NO: 15. The scFv may have the amino acid sequence of SEQ ID NO: 16.

The antigen binding molecule is preferably expressed by the immune cell that comprises the nucleic acid sequence encoding the antigen binding molecule. Expression of the antigen binding molecule may be determined based on the presence of mRNA encoding the antigen binding molecule in the immune cell. Preferably, expression of the antigen binding molecule is determined based on the presence of the antigen binding molecule itself in the immune cell. Methods for determining the presence of a mRNA or a protein in a cell are well-known in the art. For example, reverse transcriptase PCR or Northern blotting may be used to determine the presence of an mRNA in a cell. Flow cytometry, immunofluorescent imaging or western blotting may be used to determine the presence of a protein in a cell.

Method of producing an immune cell

The invention provides a method of producing an immune cell of the invention. The method comprises introducing a nucleic acid sequence encoding an antigen binding molecule into an immune cell. Antigen binding molecules are described in detail above. The nucleic acid sequence may, for example, be introduced to the immune cell by transduction or transfection. For example, T-cell transduction methods known in the art may be used to transduce gdT cells may be with the nucleic acid sequence. gdT cells may be expanded prior to such transduction, for example by culturing in the presence of IL-2 and zoledronic acid for a period of about 5 days (such as about 3 days, about 4 days, about 6 days, or about 7 days).

The term "transduction" may be used to describe virus-mediated nucleic acid transfer. A viral vector may be used to transduce the cell with the nucleic acid sequence. Thus, the nucleic acid sequence may be comprised in a viral vector. The viral vector may be a retroviral, lentiviral, adenoviral, adeno-associated (AAV) or herpes simplex virus (HSV) vector. Preferably, the viral vector is a retroviral vector. Methods for producing and purifying such vectors are known in the art. The immune cell may be transduced using any method known in the art. Transduction may be *in vitro* or *ex vivo*.

The term "transfection" may be used to describe non-virus-mediated nucleic acid transfer. The immune cell may be transfected using any method known in the art.

Transfection may be *in vitro* or *ex vivo*. Any vector capable of transfecting the immune cell may be used, such as conventional plasmid DNA or RNA transfection. A human artificial chromosome and/or naked RNA and/or siRNA may be used to transfect the cell with the nucleic acid sequence. Human artificial chromosomes are described in e.g. Kazuki et al., Mol. Ther. 19(9): 1591-1601 (2011), and Kouprina et al., Expert Opinion on Drug Delivery 11(4): 517-535 (2014). Alternative non-viral delivery systems include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle, such as a liposome. Methods of non-viral delivery of nucleic acids include lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA. Lipofection is described in e.g., U.S. Pat. Nos. 5,049,386, 4,946,787; and 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™). Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Felgner, WO 91/17424; WO 91/16024. The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (see, e.g., Crystal, Science 270:404-410 (1995); Blaese et al., Cancer Gene Ther. 2:291-297 (1995); Behr et al., Bioconjugate Chem. 5:382-389 (1994); Remy et al., Bioconjugate Chem. 5:647-654 (1994); Gao et al., Gene Therapy 2:710-722 (1995); Ahmad et al., Cancer Res. 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

Nanoparticle delivery systems may be used to transfect the cell with the nucleic acid sequence or nucleic acid construct. Such delivery systems include, but are not limited to, lipid-based systems, liposomes, micelles, microvesicles and exosomes. With regard to nanoparticles that can deliver RNA, see, e.g., Alabi et al., Proc Natl Acad Sci U S A. 2013 Aug 6;110(32):12881-6; Zhang et al., Adv Mater. 2013 Sep 6;25(33):4641-5; Jiang et al., Nano Lett. 2013 Mar 13;13(3):1059-64; Karagiannis et al., ACS Nano. 2012 Oct 23;6(10):8484-7; Whitehead et al., ACS Nano. 2012 Aug 28;6(8):6922-9 and Lee et al., Nat Nanotechnol. 2012 Jun 3;7(6):389-93. Lipid Nanoparticles, Spherical Nucleic Acid (SNA™) constructs, nanoplexes and other nanoparticles (particularly gold nanoparticles) are also contemplated as a means for delivery of a construct or vector in accordance with the invention.

Uptake of nucleic acid constructs may be enhanced by several known transfection techniques, for example those including the use of transfection agents. Examples of these agents includes cationic agents, for example, calcium phosphate and DEAE-Dextran and lipofectants, for example, lipofectAmine, fugene and transfectam.

The immune cell may be transfected under suitable conditions. The immune cell and agent or vector may, for example, be contacted for between five minutes and ten days, preferably from an hour to five days, more preferably from five hours to two days and even more preferably from twelve hours to one day.

The nucleic acid sequence transduced or transfected into the immune cell gives rise to expression of the antigen binding molecule in the immune cell. The nucleic acid sequence preferably comprises a promoter which is operably linked to the sequence encoding the antigen binding molecule. The promoter may be constitutively active in the immune cell. The promoter may be inducible in the immune cell.

Medicaments, methods and therapeutic use

The immune cells of the invention may be used in a method of therapy of the human or animal body. Thus, the invention provides a method of treating disease in an individual, the method comprising administering to the individual a therapeutic number of immune cells of the invention. The invention further provides immune cells of the invention for use in a method of treating disease in an individual, the method comprising administering to the individual a therapeutic amount of the immune cells

The individual may be of species. For example, the individual may be a human, dog, cat, mouse, rat, pig, sheep, cow, goat or horse. Preferably, the individual is human. Preferably, the immune cells are of the same species as the individual. The immune cells may be autologous with respect to the individual. The immune cells may be allogeneic with respect to the individual. The individual may be an infant, a juvenile or an adult. The individual may have, be susceptible to, or be at risk from, the disease.

The invention concerns administering to the individual a therapeutically effective number of immune cells of the invention. A therapeutically effective number is a number which ameliorates one or more symptoms of the disease. A therapeutically effective number is preferably a number which treats the disease. Any suitable number of immune cells may be administered to the individual. As a guide, the number of immune cells to be administered is

typically from 10^5 to 10^9, preferably from 10^6 to 10^8. For example, at least, or about, 0.2 x 10^6, 0.25 x 10^6, 0.5 x 10^6, 1.5 x 10^6, 4.0 x 10^6 or 5.0 x 10^6 immune cells per kg of individual may administered. For example, at least, or about, 10^5, 10^6, 10^7, 10^8, or 10^9 immune cells may be administered. At least about 1 x 10^6, at least about 2 x 10^6, at least about 2.5 2 x 10^6, at least about 5 x 10^6, at least about 1 x 10^7, at least about 2 x 10^7, at least about 5 x 10^7, at least about 1 x 10^8 or at least about 2 x 10^8 immune cells may be administered.

The immune cells may be used in combination with other means of, and substances for, treating the disease. For example, the immune cells may be used in combination with one or more cancer therapies. For example,. the immune cells may be used in combination with one or more chemotherapeutic agent. The immune cells may be used in combination with one or more CAR-expressing ab T cells. The immune cells may be used in combination with radiotherapy. The immune cells may be used in combination with surgery, for example surgery to resect or remove a tumour.

The immune cells may be used in combination with one or more therapies for treating an infectious disease, such as a viral infection or a bacterial infection. For example, the immune cells may be used in combination with one or more anti-viral drug. The immune cells may be used in combination with one or more antibiotics.

The immune cells may be used in combination with substances that support immune cell function. For example, the immune cells may be used in combination with an aminobisphosphonate, such as zoledronic acid. The immune cells may be used in combination with an aminobisphosphonate when, for example, the immune cells are Vδ2+ gdT cells. The immune cells may be used in combination with one or more immune stimulating cytokines, such as IL-2, GM-CSF or G-CSF. IL-2, GM-CSF or G-CSF may each enhance other populations of ADCC-competent cells.

When immune cells are used in combination with one or more other substances, immune cells may be administered simultaneously with, sequentially with or separately from the other substance(s). The immune cells may be used in combination with existing treatments for treating the disease and may, for example, be simply mixed with such treatments. Thus, the immune cells may be used to increase the efficacy of existing treatments for the disease.

The immune cells may be formulated for administration using any suitable method. Formulation of cells with standard pharmaceutically acceptable carriers and/or excipients may be carried out using routine methods in the pharmaceutical art. The exact nature of a formulation will depend upon several factors including the cells to be administered and the

desired route of administration. Suitable types of formulation are fully described in Remington's Pharmaceutical Sciences, 19th Edition, Mack Publishing Company, Eastern Pennsylvania, USA.

The immune cells may be formulated with a physiologically acceptable carrier or diluent. Typically, such formulations are prepared as liquid suspensions of cells. The cells may be mixed with an excipient which is pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, of the like and combinations thereof. In addition, if desired, the pharmaceutical compositions of the invention may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance effectiveness.

The immune cells may be administered by any route. Suitable routes include, but are not limited to, intravenous, intramuscular, intraperitoneal or other appropriate administration routes. The immune cells are preferably administered intravenously.

The disease may be any disease in which the patients may benefit from targeted ADCC. The disease may be any disease in which the patients may benefit from a targeted T-cell response. For instance, the disease may be a disease in which the subject may benefit from the killing of unwanted cells. The unwanted cells may, for example, be cancer cells. The unwanted cells may be or cells infected with bacteria, a virus, a fungus, protozoa, or a parasite. The unwanted cells may be aberrant immune cells, i.e. immune cells that give rise to a detrimental immune response. For example, the aberrant immune cells may be autoimmune cells. Accordingly, the disease may be an infection (such as a bacterial, viral, fungal, protozoal or other parasitic infection) or an autoimmune disease. Preferably, the disease is cancer. The cancer may be a cancer of the haematopoietic tissue and/or lymphoid tissue. Preferably, the cancer is a solid tumour.

The cancer may be primary cancer or secondary cancer. The cancer may be anal cancer, bile duct cancer (cholangiocarcinoma), bladder cancer, blood cancer, bone cancer, bowel cancer, brain tumours, breast cancer, colorectal cancer, cervical cancer, endocrine tumours, eye cancer (such as ocular melanoma), fallopian tube cancer, gall bladder cancer, head and/or neck cancer, Kaposi's sarcoma, kidney cancer, larynx cancer, leukaemia, liver cancer, lung cancer, lymph node cancer, lymphoma, melanoma, mesothelioma, myeloma, neuroendocrine tumours, ovarian cancer, oesophageal cancer, pancreatic cancer, penis cancer, primary peritoneal cancer, prostate cancer, skin cancer, small bowel cancer, soft tissue sarcoma, spinal cord tumours, stomach cancer, testicular cancer, thymus cancer, thyroid

cancer, trachea cancer, unknown primary cancer, vagina cancer, vulva cancer or endometrial cancer. The leukaemia may be acute lymphoblastic leukaemia, acute myeloid leukaemia (AML), chronic lymphocytic leukaemia or chronic myeloid leukaemia. The lymphoma may be Hodgkin lymphoma or non-Hodgkin lymphoma. The cancer may be neuroblastoma or melanoma. The cancer may be colon cancer, rectal cancer, stomach cancer, breast cancer, lung cancer, thyroid cancer or ovary cancer. The cancer may be a carcinoma. Preferably, the cancer is a colorectal cancer or a neuro-endocrine tumour.

EXAMPLES

V$\delta2^+$ $\gamma\delta$T cells can be transduced to secrete proteins which specifically bind to antigen on target cells.

V$\delta2^+$ $\gamma\delta$T cells were transduced to secrete an scFv fusion peptide targeting the tumour associated antigens CEA and GD2. Supernatant from transduced cells was harvested and applied to CEA^+ CAPAN-1 cells and CEA- HELA cells, or to $GD2^{+/-}$ SupT1 cells. Binding of scFv-Fc fusion protein was detected using a phycoerythrin conjugated anti-human Fc antibody. Purified anti-CEA scFv-Fc fusion protein or purified anti-GD2 antibody, produced in cell lines were used as a positive controls.

As shown in Figure 2, a constituent of the supernatant from transduced cell lines and transduced V$\delta2$ $\gamma\delta$T cells was able to specifically bind antigen positive cells and led to detection of human Fc on their surface.

Cytotoxicity experiments demonstrating the effects of direct and indirect contact with scFv-Fc protein secreting V$\delta2$

Because the engineered T-cells secrete a protein which may have effects on the cytotoxicity of bystander, non-engineered cells, a series of experimental conditions were developed to demonstrate this. The experimental setup is illustrated in Figure 3. Briefly, in addition to comparting cytotoxicity of transduced and non-transduced V$\delta2$ upon direct co-culture with target cells, two conditions were added to demonstrate the cell-contact autonomous effects. Firstly, supernatant from transduced V$\delta2$ was added to a co-culture of

non-transduced Vδ2 and target cells. Secondly, non-transduced cells were co-cultured with targets in the presence of transduced cells which were sequestered behind a semi-permeable membrane which blocks passage of cells but not of secreted molecules. All co-cultures were 18h, at an effector:target ratio of 1:1. For demonstration of anti-CEA scFv-Fc fusion protein action, CEA+ CAPAN-1 and CEA- HELA cells were used. For demonstration of anti-GD2 scFv-Fc fusion protein action, SupT1 engineered to express GD2 or isogenic $GD2^-$ wild type SupT1 were used.

Vδ2 cells transduced to secrete anti-CEA scFv-Fc fusion proteins led to significantly higher death of CEA^+ targets compared to NT Vδ2 ($p<0.0001$, Figure 4A). Supernatant from $V\delta2^+$ cells transduced to express anti-CEA scFv-Fc fusion proteins enhanced killing of CEA^+ targets by non-transduced Vδ2 ($p<0.0001$) and also led to a small increase in killing of CEA^- targets ($p = 0.031$, Figure 4B). When anti-CEA scFv-Fc fusion protein secreting Vδ2 were sequestered behind a semi-permeable membrane, they were also able to enhance the ability of non-transduced Vδ2 to kill CEA^+ targets when compared to sequestration of non-transduced cells ($p = 0.012$, Figure 4C).

Similar data were observed for Vδ2 transduced to secrete an anti-GD2 scFv-Fc fusion protein. Transduced Vδ2 killed $GD2^+$ targets better than non-transduced ($p = 0.03$) and exhibited greater cytotoxicity against $GD2^+$ targets than isogenic $GD2^-$ controls ($p = 0.0049$, Figure 4D). Supernatant from anti-GD2-scFv-Fc fusion protein expressing Vδ2 significantly enhanced the ability of non-transduced Vδ2 to kill $GD2^+$ targets when compared to non-transduced controls ($p = 0.0025$) or isogenic $GD2^-$ targets ($p = 0.0002$, Figure 4E). When anti-GD2 scFv-Fc fusion protein secreting Vδ2 were sequestered behind a semi-permeable membrane, they were also able to enhance the ability of non-transduced Vδ2 to kill $GD2^+$ targets when compared to sequestration of non-transduced cells ($p = 0.0158$) or killing of $GD2^-$ targets ($p = 0.0003$, Figure 4F).

When non-transduced Vδ2 were co-cultured with $CEA^\pm$ targets in the presence of anti-CEA scFv-Fc fusion protein secreting Vδ2 or non-transduced controls sequestered behind a semi-permeable membrane, significant increases in IFNγ production were only observed

when anti-CEA scFv-Fc fusion protein secreting cells were sequestered ($p = 0.0014$ comparted to sequestered NT Vδ2) and CEA^+ targets were used ($p = 0.012$ compared to CEA^- targets, Figure 5).

$V\delta2^+$ γδT cells can be transduced to secrete whole antibody which specifically binds to antigen on target cells.

HEK293T and $V\delta2^+$ γδT cells were transduced to secrete an IgG1 antibody targeting the tumour associated antigen GD2 (SEQ ID NO: 17). Supernatant from HEK293T cells transduced with reducing volumes of virus was harvested and applied to $GD2^{+/-}$ SupT1 cells. Binding of scFv-Fc fusion protein was detected using a phycoerythrin conjugated anti-human Fc antibody (Figure 6). Supernatant from transduced $V\delta2^+$ γδT cells was also harvested and applied to $GD2^{+/-}$ SupT1 cells, and antibody binding detected using AlexaFluor 647 conjugated anti-human IgG antibody (Figure 7). Purified anti-GD2 antibody, produced in cell lines was used as a positive control.

As shown in Figures 6 and 7, a constituent of the supernatant from transduced cell lines and transduced $V\delta2^+$ γδT cells was able to specifically bind antigen positive cells and led to detection of human Fc or IgG on their surface.

Cytotoxicity experiments demonstrating the effects of direct and indirect contact with antibody secreting Vδ2

In order to demonstrate the cell-contact dependent and cell-contact autonomous effects, two experimental systems were used (Figure 8A). Briefly, non-transduced $V\delta2^+$ γδT cells or Vδ2+ γδT cells transduced to express anti-GD2 IgG1 were co-cultured at a 1:1 effector:target ratio with $GD2^{+/-}$ SupT1. In the second experiment, supernatant from non-transduced $V\delta2^+$ γδT cells or supernatant from Vδ2+ γδT cells transduced to express anti-GD2 IgG1 was added to a 1:1 co-culture of non-transduced Vδ2+ γδT cells and $GD2^{+/-}$ SupT1. Target cell death was determined using flow cytometry, and Figures 8B and 8C show representative data from three replicates. $V\delta2^+$ γδT cells transduced to express anti-GD2 IgG1 had enhanced cytotoxicity against SupT1-GD2 but not against SupT1-wt (Figure 8B). Supernatant from $V\delta2^+$ γδT cells transduced to express anti-GD2 IgG1

enhanced the cytotoxicity of non-transduced Vδ2$^+$ γδT cells against SupT1-GD2 but not against SupT1-wt (Figure 8C).

Materials and methods

Cell lines

CAPAN-1, HELA and SupT1 cell lines were obtained from ATCC. SupT1-GD2 were generated by transducing wild type SupT1 with vector encoding GD2/GD3 synthase and isolating clones of successfully transduced cells.

Donor selection for PBMC

PBMC were isolated from the blood of healthy donors aged 20-36y. Donors were screened for cross-reactivity between SFP constructs and expanding Vδ2 cells prior to inclusion. Donors where there was evidence of cross-reactivity were not used for cytotoxicity experiments.

Isolation and pre-stimulation handling of fresh PBMC

20 ml of whole blood were diluted with 10 ml PBS + 500 µl 100 mM EDTA and layered on 20 ml Percoll. Interface PBMCs (20 min, 300xg, RT) were washed in PBS and re-suspended in 25 ml T-cell medium (X-VIVO 15 (Lonza BioWhittaker, Maryland, USA) supplemented with penicillin/streptomycin (100 IU/ml penicillin, 100 µg/ml streptomycin (Sigma-Aldrich, Missouri, USA)) and cultured overnight before use.

Vδ2$^+$ T-cell expansion

For specific Vδ2$^+$ γδ T-cell expansion, PBMC were isolated as described above. They were cultured in RPMI-1640 medium supplemented with L-glutamine (2 mM, Sigma-Aldrich), penicillin/streptomycin (100 IU/ml penicillin, 100 µg/ml streptomycin (Sigma-Aldrich)) and 10% FCS (v/v, (Gibco, Massachusetts, USA)). Vδ2$^+$ γδT cell expansion was stimulated using 5 µM zoledronic acid (Actavis, New Jersey, USA) and 100 IU/ml IL-2 (Aldesleukin, Novartis, Frimley, UK), which was added to PBMC suspension after PBMC isolation (day 1). IL-2 was replenished every 2-3 days by removing half of the media from the well and replacing with fresh media containing 200 IU/ml IL-2.

Construction of retroviral constructs

The gammaretroviral vector used in all constructs was SFG (Riviere et al 1995), pseudotyped with an RD114 envelope. DNA fragments were amplified using the Phusion HT II polymerase according to the manufacturer's instructions (Thermo Scientific, Massachusetts, USA). PCR was carried out in a PTC-200 DNA Engine (MJ Research, Massachusetts, USA). PCR products were extracted from 1% agarose gels using the Wizard SV Gel & PCR Clean-Up kit (Promega, Wisconsin, USA). Sample concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Massachusetts, USA). The construct comprised one of a range of scFvs against a panel of targets including human GD2 (clone 14G2A) or human CEA (clone SM3EL) linked to the Fc portion of human IgG1.

In addition to the scFv-Fc fusion protein construct, RQR8 which is a marker bearing a CD34 epitope (Philip et al, 2014) was included, separated from the scFv-Fc fusion protein by a cleavable 2A peptide. This allows scFv-Fc fusion protein expressing cells to be detected by flow cytometry by staining using the anti-CD34 antibody clone QBend10.

Construction of lentiviral constructs

The lentiviral vector used for whole antibody transduction was pCCL (Dull et al 1998), pseudotyped with an RDPro envelope (Cosset et al 1995). DNA fragments were amplified using the Phusion HT II polymerase according to the manufacturer's instructions (Thermo Scientific, Massachusetts, USA). PCR was carried out in a PTC-200 DNA Engine (MJ Research, Massachusetts, USA). PCR products were extracted from 1% agarose gels using the Wizard SV Gel & PCR Clean-Up kit (Promega, Wisconsin, USA). Sample concentrations were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Massachusetts, USA). The construct comprised an anti-GD2 IgG1 (clone 14G2A).

In addition to the IgG1 construct, eGFP was included, separated from the IgG1 protein by a cleavable 2A peptide. This allows IgG1 expressing cells to be detected by flow cytometry.

Production of viral particles by transfection

$1.5x10^6$ 293T cells/100 mm^2 dish (Nucleon Delta Surface, Thermo Fisher) were plated at day 1 in 293T medium (D-MEM, 10 % FCS (v/v)). γ-retroviral or lentiviral particles were produced by co-transfection of 293T cells at day 2 using Gene Juice Transfection Reagent (Novagen/Millipore, Massachusetts, USA) in accordance with manufacturer's directions. Viral particle-containing supernatants were harvested at day 4; medium was replenished, and harvested at day 5. γ-retroviral supernatants were pooled, filtered (0.45-μm filter, Millipore) and directly used for transductions or stored overnight at 4°C before use. Lentiviral supernatants were concentrated by ultracentrifugation and frozen for later use.

γ-retroviral transduction of T cells

Transduction of T cells was carried out in Retronectin (Takara Bio, Tokyo, Japan) coated 24-well plates, which were pre-loaded with viral supernatant. $0.5x10^6$ T cells suspended in 0.5 ml T-cell medium + 400 IU IL-2 were combined with 1.5 ml viral supernatant and centrifuged for 40 min, 1000xg at RT. Typically, $12x10^6$ T cells per donor were plated for transduction.

γδT-cell expansion was stimulated with 5 μM zoledronic acid (Actavis, New Jersey, USA) and 100 IU/ml IL-2 (Aldesleukin,) and transduction performed at day 5. At day 8 of culture (day 3 after transduction) cells were pooled, washed and plated at $2x10^6$ cells/ml in T-cell medium + 100 IU IL-2/ml (24-well plates, Nucleon Delta Surface, Thermo Scientific, Massachusetts, USA). Transduction efficiency was determined by flow cytometry at day 10 (day 5 after transduction).

Lentiviral transduction of T-cells

Transduction of T cells was carried out in 96-well plates, each well containing $0.3x10^6$ T cells suspended in 0.3 ml T-cell medium. Concentrated lentivirus was added and the plates centrifuged for 40 min, 1000xg at RT.

γδT-cell expansion was stimulated with 5 μM zoledronic acid (Actavis, New Jersey, USA) and 100 IU/ml IL-2 (Aldesleukin,) and transduction performed at day 2. At day 5 of culture (day 3 after transduction) cells were pooled, washed and plated at $1x10^6$ cells/cm^2 in T-cell

medium + 100 IU IL-2/ml. Transduction efficiency was determined by flow cytometry at day 7 (day 5 after transduction).

Cytotoxicity assays

Target cell lines were labelled with CellTrace Violet (Thermo Fisher) before co-culture with effector cells at a 1:1 effector:target ratio. The media was either RPMI 1640 + 10% FCS + 1% Pen/Step + 1% L-glutamine supplemented with 100u/ml IL-2 or supernatant from scFv-Fc fusion protein secreting Vδ2 which had been cultured in the same media.

For assays involving effector cells sequestered behind semi-permeable membranes, Trans-Wells with 0.4μm pore size (Thermo Fisher) used to separate the cells. An equal number of effectors were placed in the trans-well and the main well. Target cells were placed in the main well, with an effector:target ratio of 1:1 calculated on the cells in the main well.

After 18h co-culture, cells were harvested and analysed by flow cytometry. Cell death was identified by staining using Ghost Red fixable viability dye (Tonbo Biosciences, San Diego, CA) on violet labelled cells.

References for materials and methods

I. Rivière, K. Brose, R. C. Mulligan, Effects of retroviral vector design on expression of human adenosine deaminase in murine bone marrow transplant recipients engrafted with genetically modified cells, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6733–6737 (1995).

B. Philip, E. Kokalaki, L. Mekkaoui, S. Thomas, K. Straathof, B. Flutter, V. Marin, T. Marafioti, R. Chakraverty, D. Linch, S. A. Quezada, K. S. Peggs, M. Pule, A highly compact epitope-based marker/suicide gene for easier and safer T-cell therapy, *Blood* **124**, 1277–1287 (2014).

T. Dull, R. Zufferey. M. Kelly, R. J. Mandel, M. Nguyen, D. Trono, L. Naldini. A Third generation lentivirus vector with a conditional packaging system. Journal of Virology, Nov 1998; 72(11):8463-71

F. L. Cosset, Y. Takeuchi, J. L. Battini, R. A. Weiss, M. K. Collins. High-titer packaging

cells producing recombinant retroviruses resistant to human serum. Journal of Virology, Dec 1995; 69(12):7430-6

5

Claims

1. An immune cell that is capable of antibody-dependent cellular cytotoxicity (ADCC) and which comprises a nucleic acid sequence encoding an antigen binding molecule that comprises an antigen binding region.

2. The immune cell of claim 1, wherein the antigen binding region comprises a scFv, a Fab, a modified Fab, a Fab', a modified Fab', a F(ab')2, a Fv, a dAb, a Fd, a dsFv, a ds-scFv, a scFv2, a bi-specific T-cell engager, a nanobody, a DARPin, an antibody mimetic, a diabody, a triabody, a tetrabody, or a polypeptide ligand for a receptor expressed on the surface of a cell that is targeted by the immune cell.

3. The immune cell of claim 1 or 2, wherein the antigen binding molecule is capable of binding to a Fc receptor.

4. The immune cell of any one of the preceding claims, wherein the antigen binding molecule comprises a Fc region or a modified Fc region.

5. The immune cell any one of the preceding claims, wherein the antigen binding molecule is an antibody, a scFv-Fc, a dAb-Fc, a heavy chain antibody, an IgNAR or a camelid antibody.

6. The immune cell of any one of the preceding claims, wherein the immune cell is not an alpha beta T cell.

7. The immune cell of any one of the preceding claims, wherein the immune cell is a gamma delta T cell or a NK cell.

8. The immune cell of claim 7, wherein the gamma delta T cell is a Vδ1+ gamma delta T cell, a Vδ2+ gamma delta T cell, or a Vδ1-/Vδ2- gamma delta T cell.

9. The immune cell of claim 8, wherein the gamma delta T cell is a Vδ2+ gamma delta T cell.

10. The immune cell of any one of claims 1 to 6, wherein the immune cell is a myeloid cell.

11. The immune cell of claim 10, wherein the myeloid cell is a macrophage, a basophil, an eosinophil, or a neutrophil.

12. The immune cell of any one of the preceding claims, wherein the immune cell does not express a chimeric antigen receptor (CAR).

13. The immune cell of any one of the preceding claims, wherein the antigen binding region is capable of binding to an antigen that is expressed in the tumour microenvironment.

14. The immune cell of any one of the preceding claims, wherein the antigen binding region is capable of binding to a tumour antigen, an endothelial antigen, or an immune cell antigen.

15. The immune cell of any one of the preceding claims , wherein the antigen binding region is capable of binding to an antigen selected from a group consisting of CEA, B7-H3, TSHR, CD3, CD16, CD32, CD64, CD19, CD123, CD22, CD20, CD30, CD171, CS-1, CLL-1, CD33, EGFRvIII , GD2, GD3, BCMA, Tn Ag, PSMA, ROR1, FLT3, FAP, TAG72, CD38, CD44v6, EPCAM, KIT, IL-13Ra2, Mesothelin, IL-llRa, PSCA, PRSS21, VEGFR2, LewisY, CD24, PDGFR-beta, SSEA-4, CD20, Folate receptor alpha, ERBB2 (Her2/neu), MUC1, EGFR, NCAM, Prostase, PAP, ELF2M, Ephrin B2, IGF-I receptor, CAIX, LMP2, gplOO, bcr-abl, tyrosinase, EphA2, Fucosyl GMl, sLe, GM3, TGS5, HMWMAA, o-acetyl-GD2, Folate receptor beta, TEM1/CD248, TEM7R, CLDN6, GPRC5D, CXORF61, CD97, CD179a, ALK, Polysialic acid, PLAC1, GloboH, NY-BR-1, UPK2, HAVCR1, ADRB3, PANX3, GPR20, LY6K, OR51E2, TARP, WT1, NY-ESO-1, LAGE-la, MAGE-A1, legumain, HPV E6,E7, MAGE Al, ETV6-AML, sperm protein 17,

XAGE1, Tie 2, MAD-CT-1, MAD-CT-2, Fos-related antigen 1, p53, p53 mutant, prostein, survivin and telomerase, PCTA-l/Galectin 8, MelanA/MARTl, Ras mutant, hTERT, sarcoma translocation breakpoints, ML-IAP, ERG (TMPRSS2 ETS fusion gene), NA17, PAX3, Androgen receptor, Cyclin Bl, MYCN, RhoC, TRP-2, CYP1B1, BORIS, SART3, PAX5, OYTES1, LCK, AKAP-4, SSX2, RAGE-1, human telomerase reverse transcriptase, RU1, RU2, intestinal carboxyl esterase, mut hsp70-2, CD79a, CD79b, CD72, LAIR1, FCAR, LILRA2, CD300LF, CLEC12A, BST2, EMR2, LY75, GPC3, FCRL5, LINGO1, CD70, IL13Rα2, MUC-16, PSCA, ROR1, and IGLL1

16. The immune cell of claim 15, wherein the tumour antigen is CEA, B7-H3, CD20 or GD2.

17. The immune cell of any one of the preceding claims, wherein the antigen binding molecule is an opsonin.

18. The immune cell of any one of the preceding claims, wherein the immune cell expresses the antigen binding molecule.

19. The immune cell of any one of the preceding claims, wherein the antigen binding molecule comprises a V_H domain encoded by SEQ ID NO: 1 and/or a V_L domain encoded by SEQ ID NO: 2.

20. The immune cell of claim 19, wherein the antigen binding molecule is encoded by SEQ ID NO: 3.

21. The immune cell of any one of claims 1 to 18, wherein the antigen binding molecule comprises a V_H domain encoded by SEQ ID NO: 4 and/or a V_L domain encoded by SEQ ID NO: 5.

22. The immune cell of claim 21, wherein the antigen binding molecule is encoded by SEQ ID NO: 6.

23. The immune cell of any one of claims 1 to 18 and 21, wherein the antigen binding molecule comprises a heavy chain encoded by SEQ ID NO: 20 and/or a light chain encoded by SEQ ID NO: 18.

24. The immune cell of claim 23, wherein the antigen binding molecule is encoded by SEQ ID NO: 17.

25. The immune cell of any one of the preceding claims, wherein the nucleic acid sequence encodes two or more different antigen binding molecules.

26. The immune cell of any one of the preceding claims, wherein the immune cell comprises two or more nucleic acid sequences each encoding a different antigen binding molecule.

27. A method of producing an immune cell according to any one of the preceding claims, comprising introducing a nucleic acid sequence encoding an antigen binding molecule into an immune cell.

28. The method of claim 27, wherein the nucleic acid sequence is comprised in a vector, optionally a viral vector.

29. A method of treating disease in an individual, the method comprising administering to the individual a therapeutically effective number of immune cells according to any one of claims 1 to 26.

30. Immune cells according to any one of claims 1 to 26 for use in a method of treating disease in an individual, the method comprising administering to the individual a therapeutically effective number of the immune cells.

31. The method of claim 29 or immune cells for use of claim 30, wherein the disease is cancer.

32. The method or immune cells for use of claim 31, wherein the cancer is a solid tumour.

Fig. 1

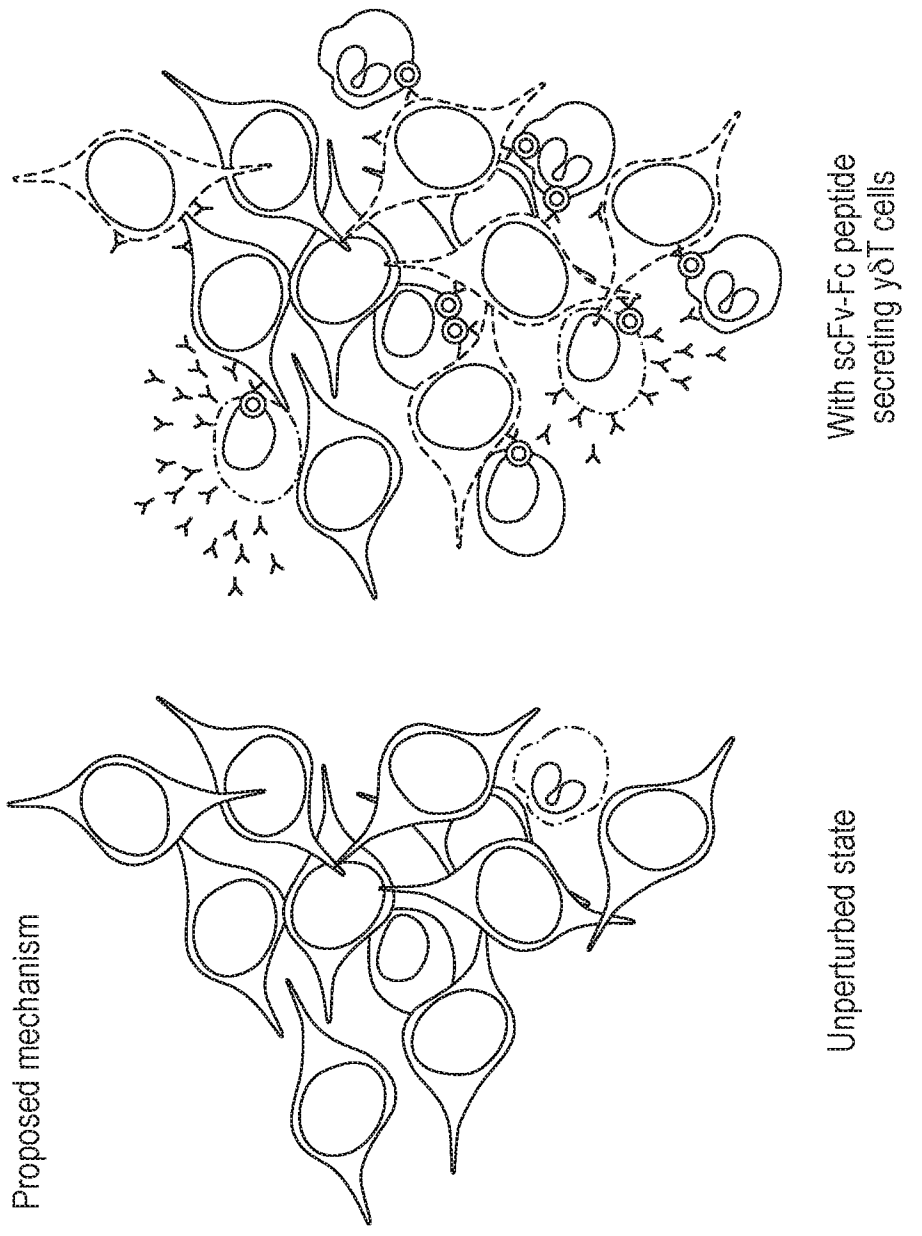

Tumour cell
Dying tumour cell
MDSC
Cytotoxic neutrophil
Non-transduced γδT cell
scFv-Fc peptide secreting γδT cell
scFv-Fc peptide
scFv-Fc peptide opsonizing target and interacting with Fcγ receptor
Proposed mechanism
Unperturbed state
With scFv-Fc peptide secreting γδT cells

Fig. 2

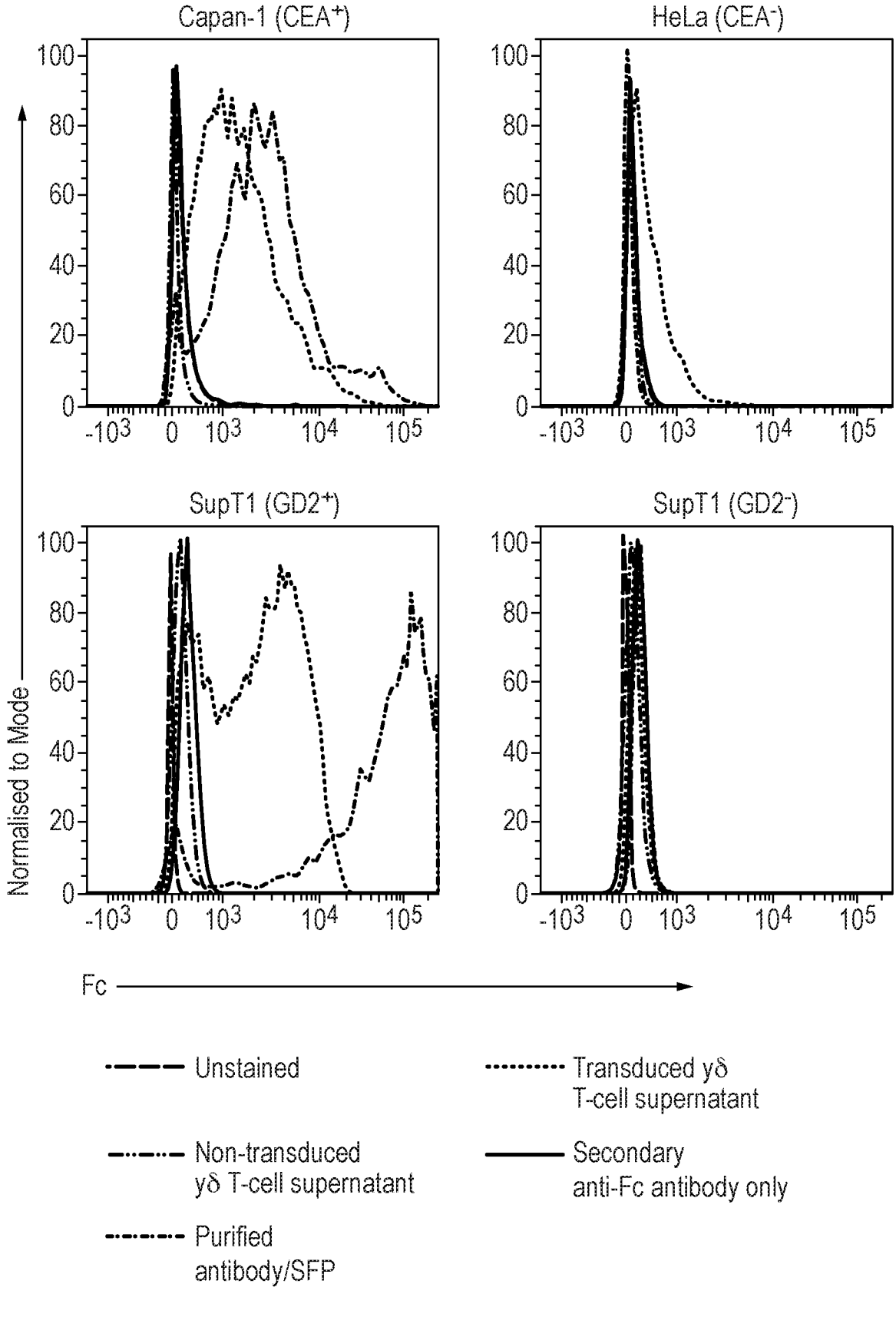

Capan-1 (CEA+)
HeLa (CEA-)
SupT1 (GD2+)
SupT1 (GD2-)
Normalised to Mode
Fc
Unstained
Transduced yδ T-cell supernatant
Non-transduced yδ T-cell supernatant
Secondary anti-Fc antibody only
Purified antibody/SFP

Fig. 3

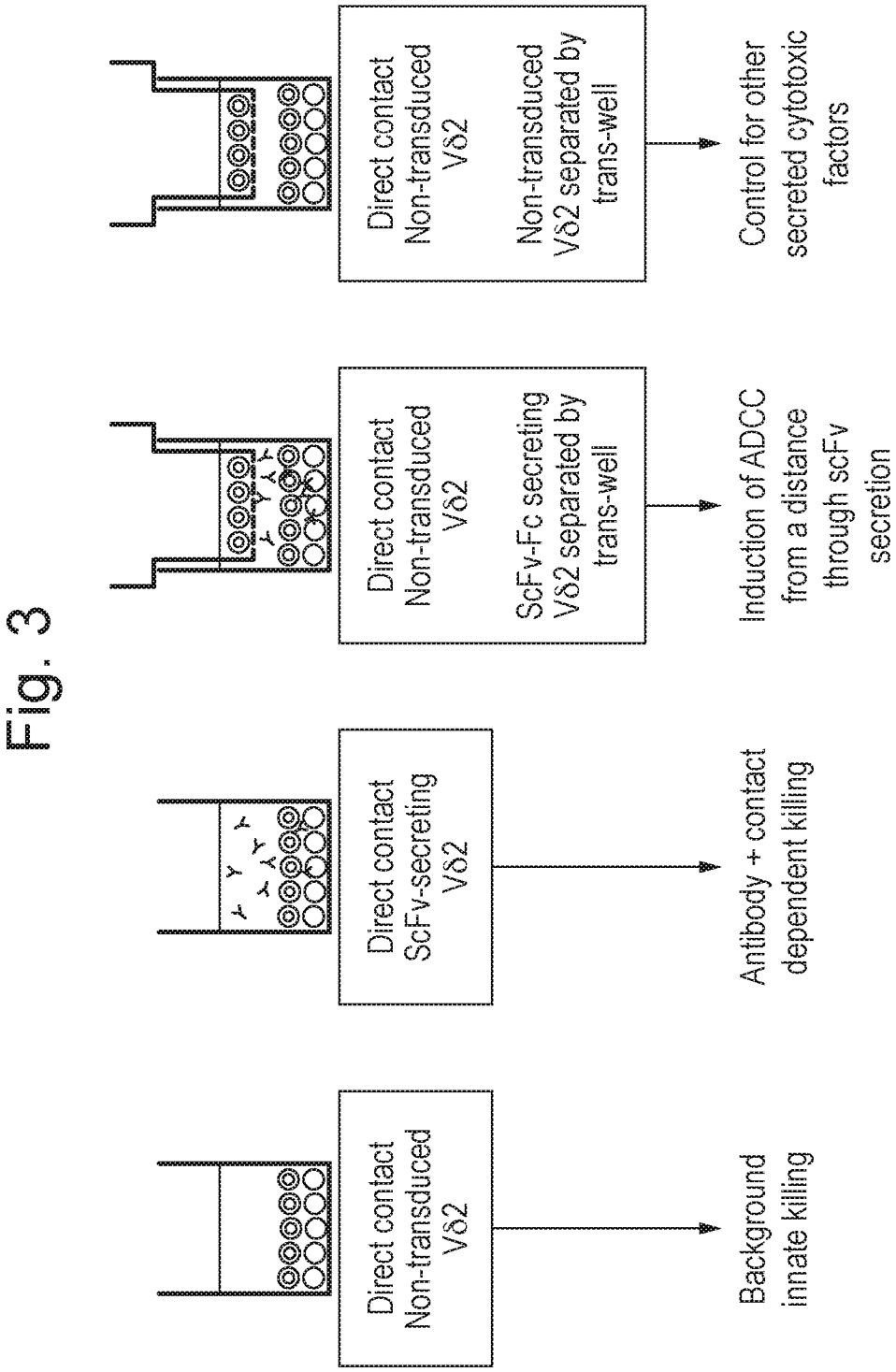

Direct contact
Non-transduced
Vδ2
Background
innate killing
Direct contact
ScFv-secreting
Vδ2
Antibody + contact
dependent killing
Direct contact
Non-transduced
Vδ2
ScFv-Fc secreting
Vδ2 separated by
trans-well
Induction of ADCC
from a distance
through scFv
secretion
Direct contact
Non-transduced
Vδ2
Non-transduced
Vδ2 separated by
trans-well
Control for other
secreted cytotoxic
factors

Fig. 4A

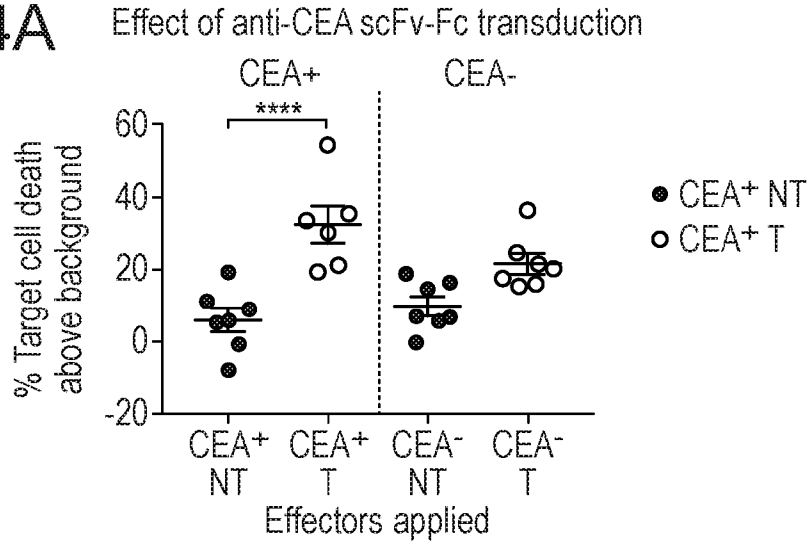


Fig. 4B

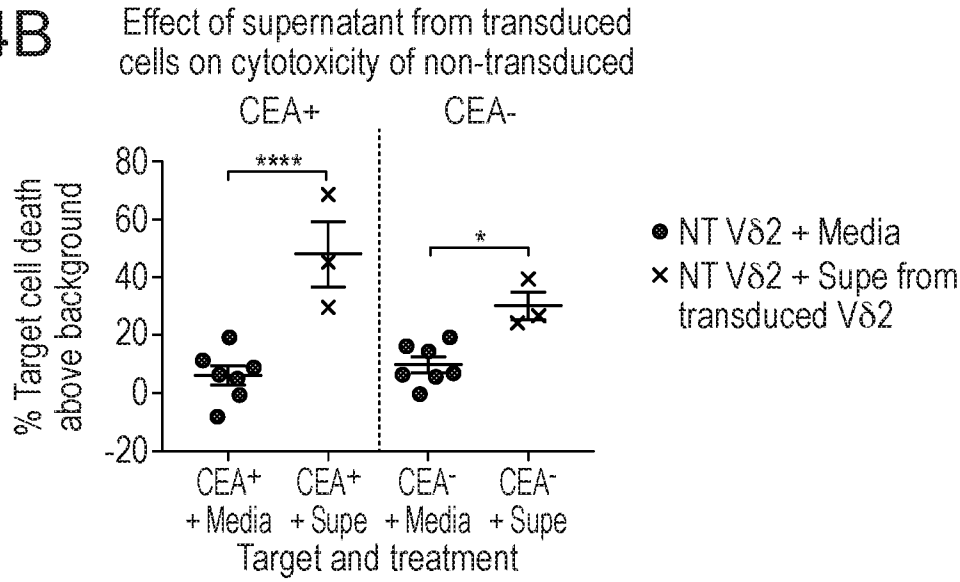


Fig. 4C

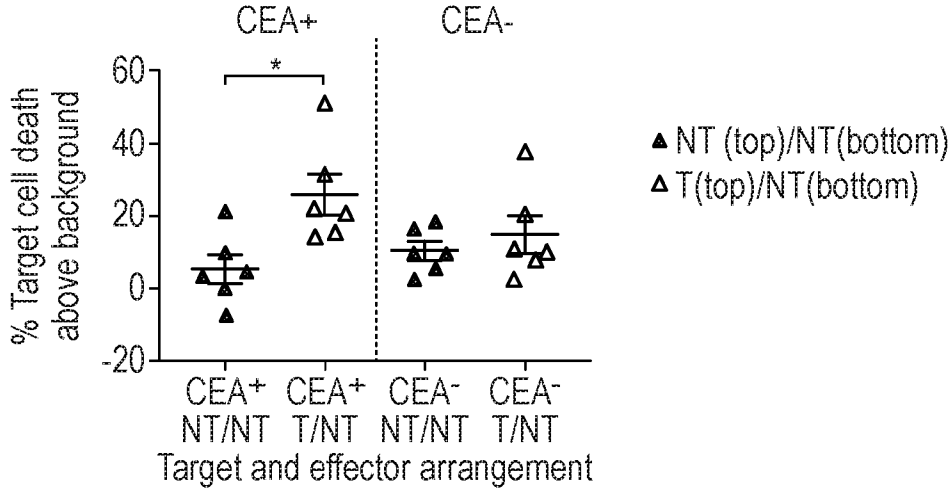


Fig. 4D

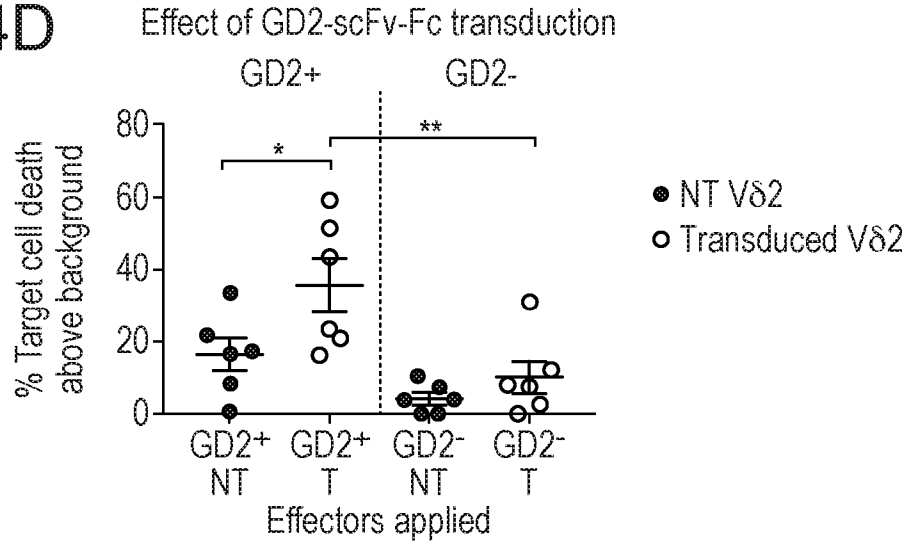


Fig. 4E

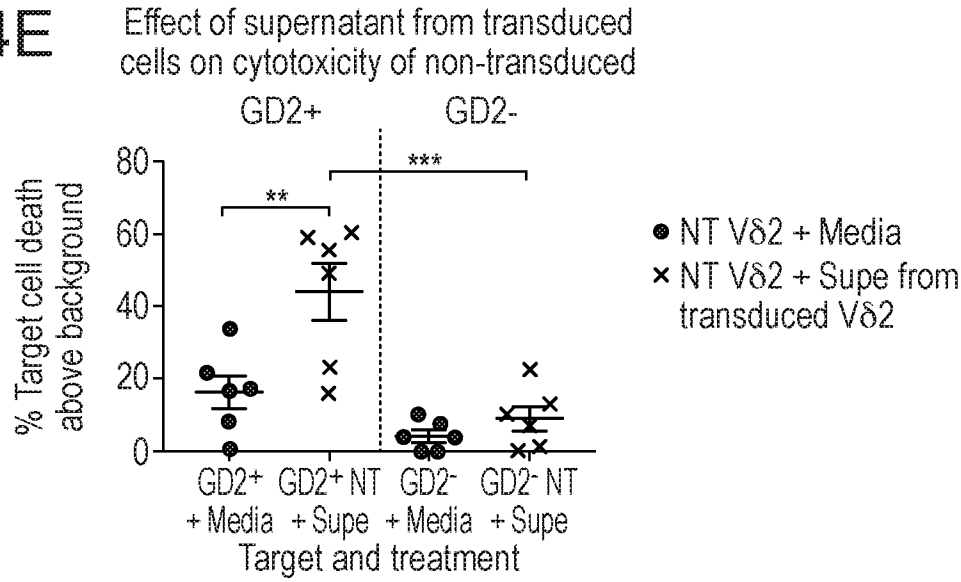


Fig. 4F

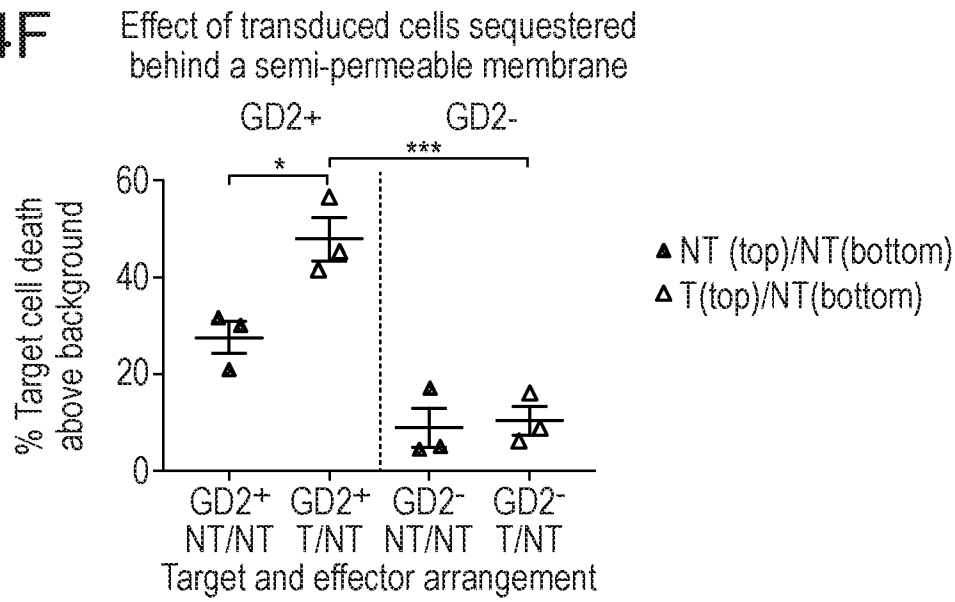


Fig. 5

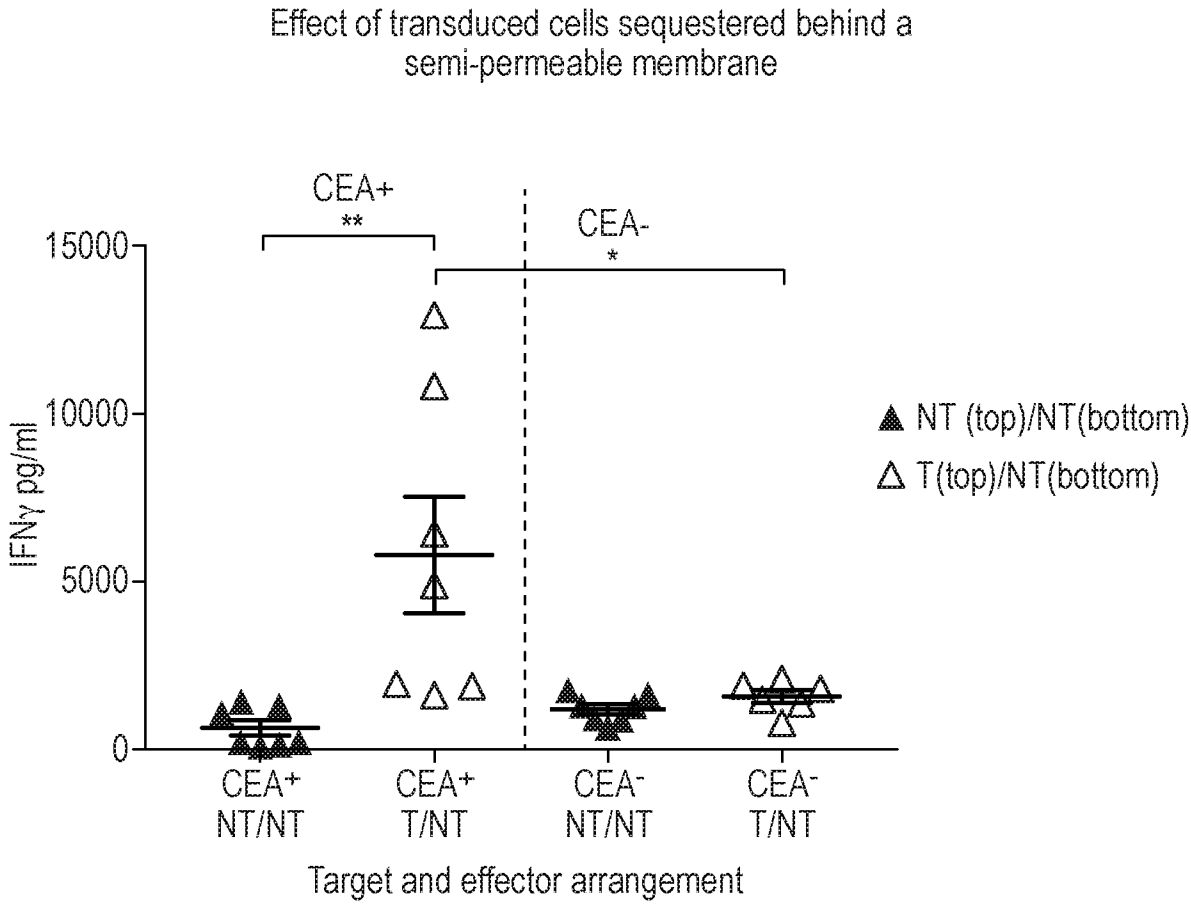

Effect of transduced cells sequestered behind a semi-permeable membrane
CEA+
**
CEA-
*
15000
10000
5000
0
IFNγ pg/ml
NT (top)/NT(bottom)
T(top)/NT(bottom)
CEA+ NT/NT
CEA+ T/NT
CEA- NT/NT
CEA- T/NT
Target and effector arrangement

Fig. 6

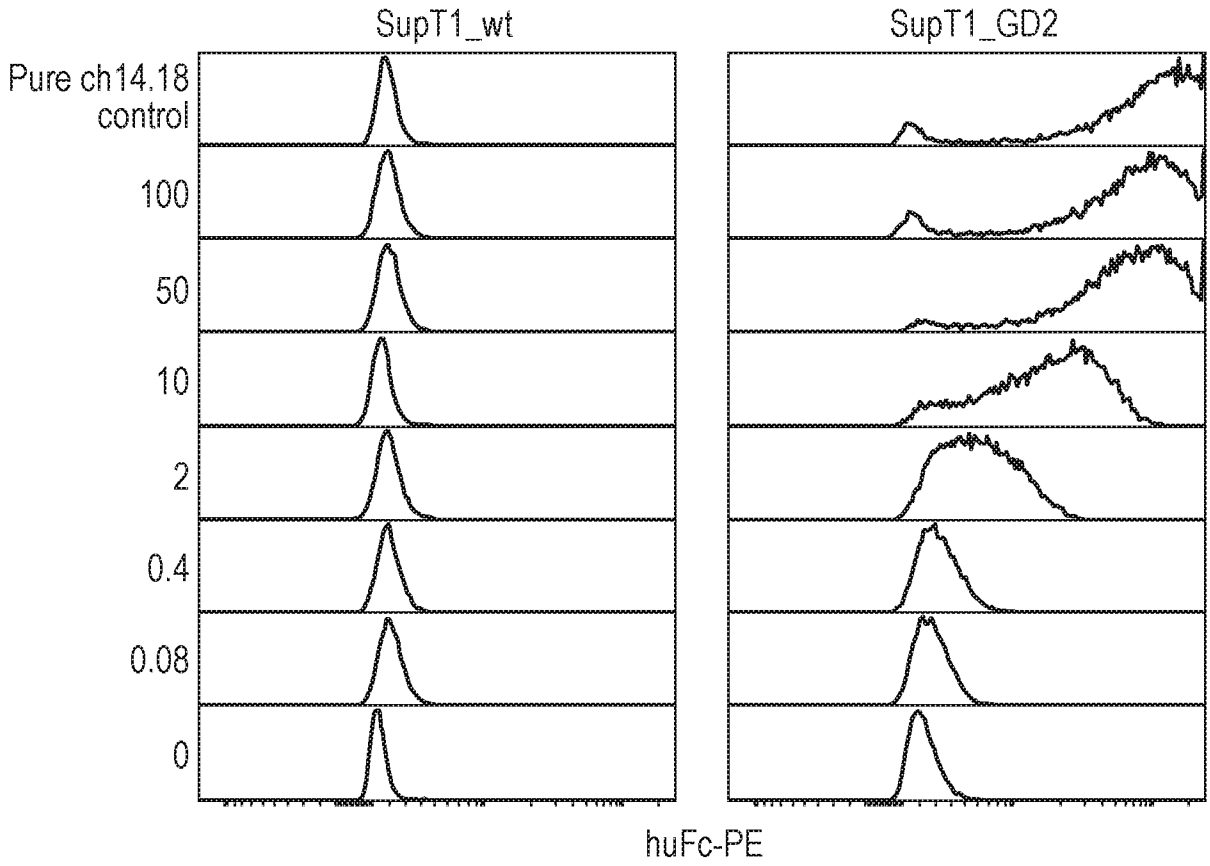

SupT1_wt
SupT1_GD2
Pure ch14.18 control
100
50
10
2
0.4
0.08
0
huFc-PE

Fig. 7

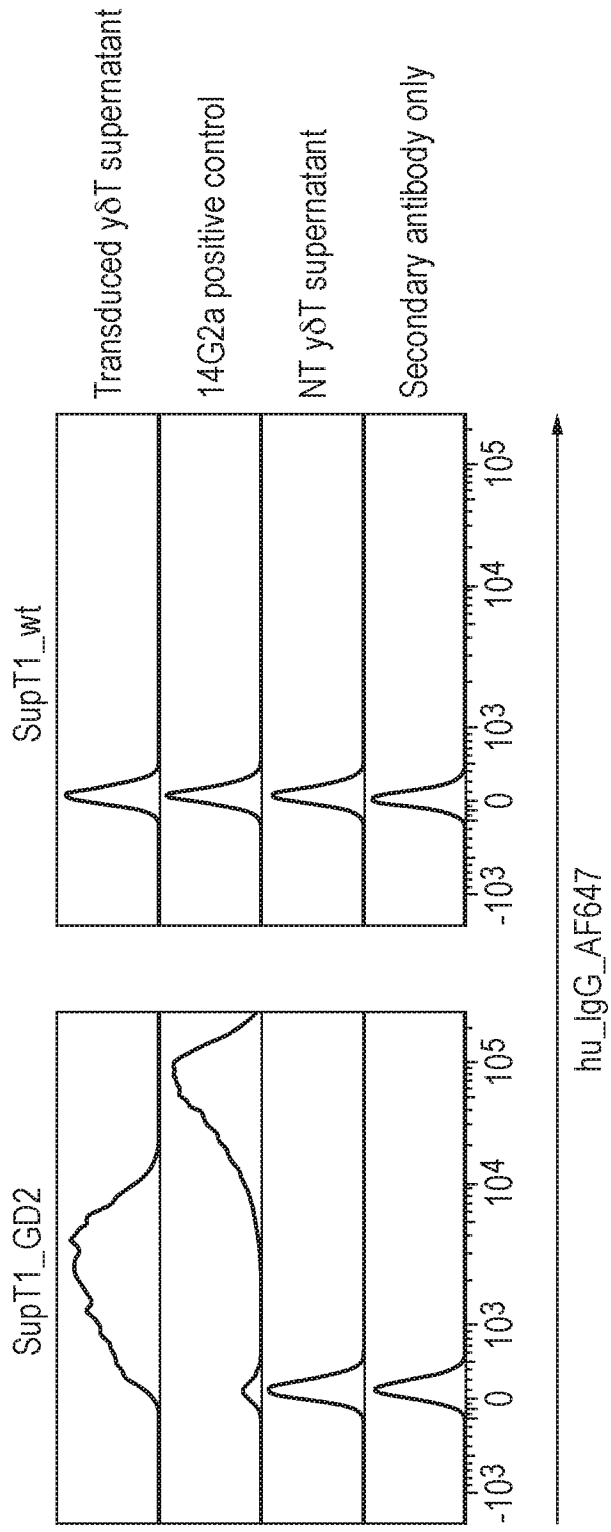

SupT1_GD2
SupT1_wt
Transduced γδT supernatant
14G2a positive control
NT γδT supernatant
Secondary antibody only
-10^3
0
10^3
10^4
10^5
hu_IgG_AF647

Fig. 8A

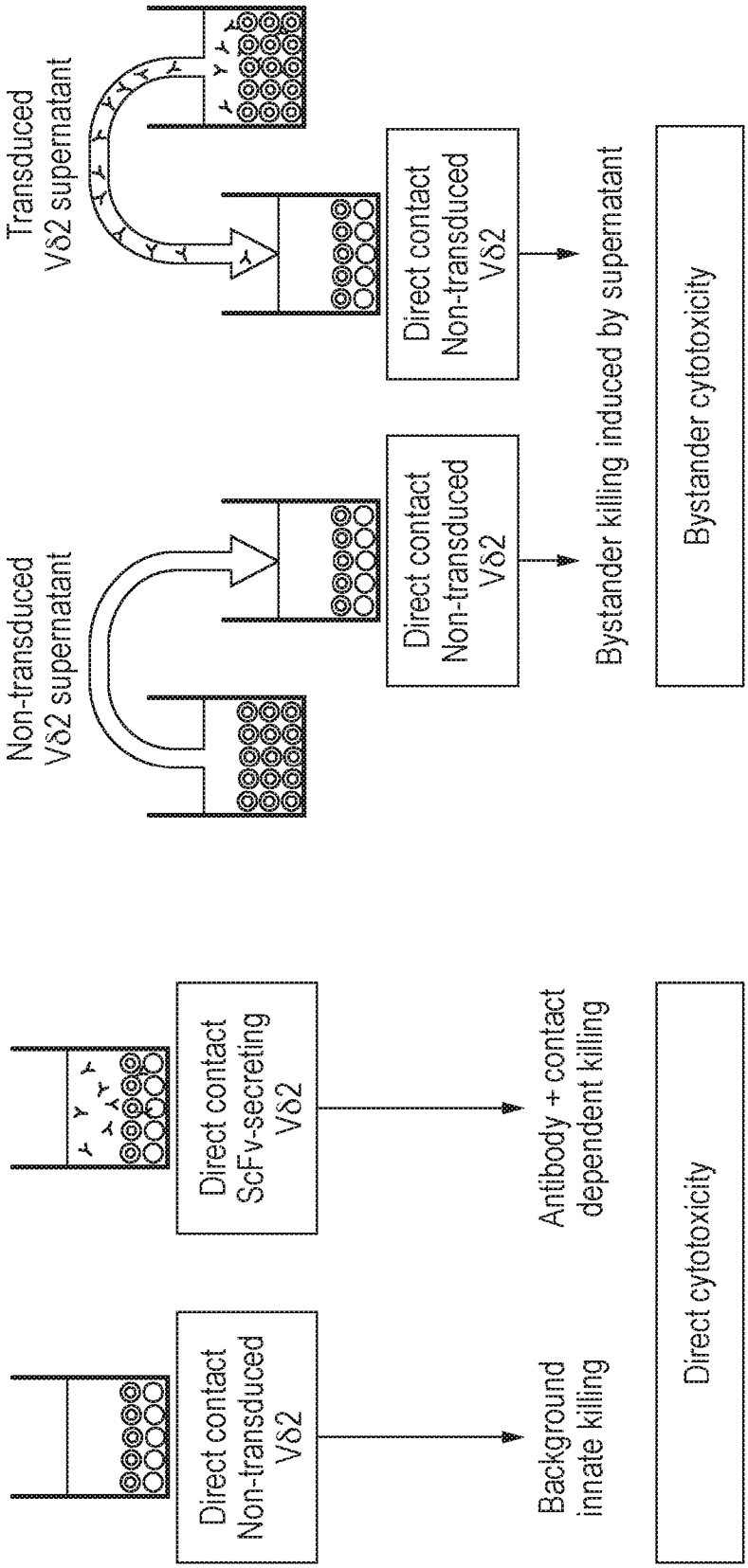

Transduced Vδ2 supernatant
Non-transduced Vδ2 supernatant
Direct contact Non-transduced Vδ2
Direct contact Non-transduced Vδ2
Bystander killing induced by supernatant
Bystander cytotoxicity
Direct contact ScFv-secreting Vδ2
Direct contact Non-transduced Vδ2
Antibody + contact dependent killing
Background innate killing
Direct cytotoxicity

Fig. 8B

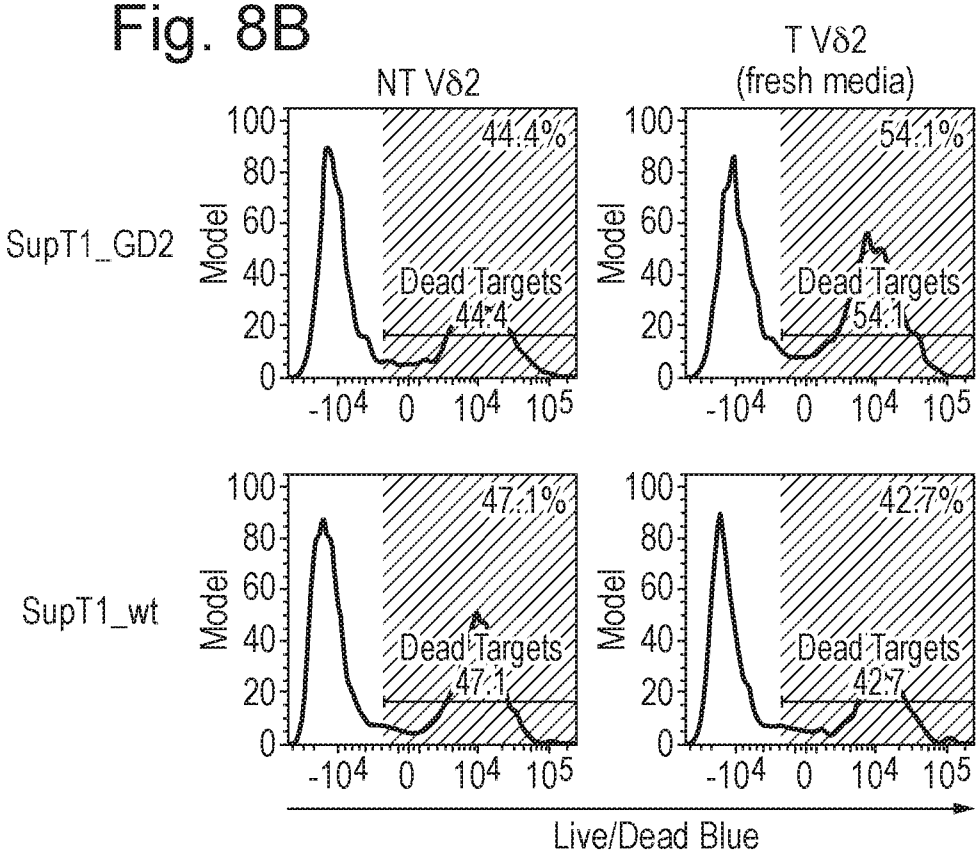
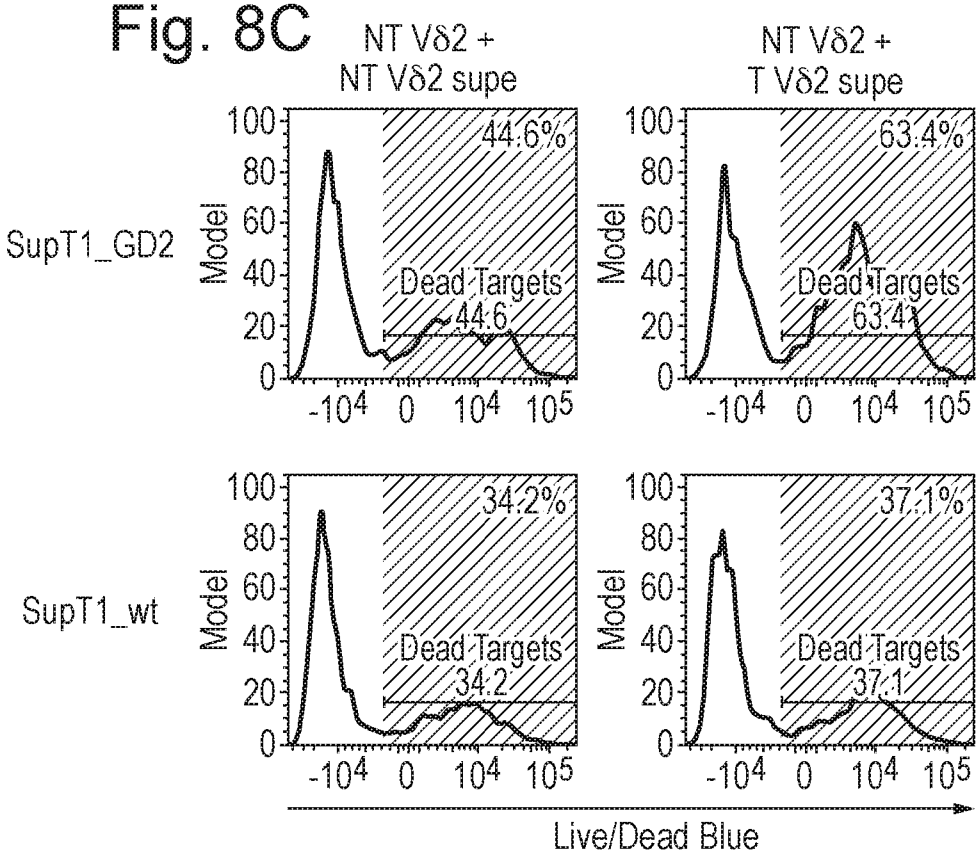

NT Vδ2
T Vδ2
(fresh media)
SupT1_GD2
SupT1_wt
Model
Dead Targets
44.4%
54.1%
47.1%
42.7%
Live/Dead Blue

Fig. 8C


NT Vδ2 +
NT Vδ2 supe
NT Vδ2 +
T Vδ2 supe
SupT1_GD2
SupT1_wt
Model
Dead Targets
44.6%
63.4%
34.2%
37.1%
Live/Dead Blue

SEQUENCE LISTING

<110> UCL Business Ltd

<120> Engineered Immune Cells

<130> N416630WO

<150> GB 2000934.6
<151> 2020-01-22

<160> 20

<170> PatentIn version 3.5

<210> 1
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> VH domain of a CEA-specific scFv used in the examples

<400> 1

Gln Val Lys Leu Glu Gln Ser Gly Ala Glu Val Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Ser
20 25 30

Tyr Met His Trp Leu Arg Gln Gly Pro Gly Gln Cys Leu Glu Trp Ile
35 40 45

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Lys Phe
50 55 60

Gln Gly Lys Ala Thr Phe Thr Thr Asp Thr Ser Ala Asn Thr Ala Tyr
65 70 75 80

Leu Gly Leu Ser Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Asn Glu Gly Thr Pro Thr Gly Pro Tyr Tyr Phe Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser

115 120

<210> 2
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> VL domain of a CEA-specific scFv used in the examples

<400> 2

Glu Asn Val Leu Thr Gln Ser Pro Ser Ser Met Ser Val Ser Val Gly
1 5 10 15

Asp Arg Val Thr Ile Ala Cys Ser Ala Ser Ser Ser Val Pro Tyr Met
20 25 30

His Trp Leu Gln Gln Lys Pro Gly Lys Ser Pro Lys Leu Leu Ile Tyr
35 40 45

Leu Thr Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Ser Val Gln Pro Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Leu Thr
85 90 95

Phe Gly Cys Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 3
<211> 483
<212> PRT
<213> Artificial Sequence

<220>
<223> CEA-specific scFv-Fc used in the examples

<400> 3

Met Ala Gln Val Lys Leu Glu Gln Ser Gly Ala Glu Val Val Lys Pro
1 5 10 15

```
Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys
            20                  25                  30

Asp Ser Tyr Met His Trp Leu Arg Gln Gly Pro Gly Gln Cys Leu Glu
        35                  40                  45

Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro
    50                  55                  60

Lys Phe Gln Gly Lys Ala Thr Phe Thr Thr Asp Thr Ser Ala Asn Thr
65                  70                  75                  80

Ala Tyr Leu Gly Leu Ser Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr
                85                  90                  95

Tyr Cys Asn Glu Gly Thr Pro Thr Gly Pro Tyr Tyr Phe Asp Tyr Trp
            100                 105                 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn
    130                 135                 140

Val Leu Thr Gln Ser Pro Ser Ser Met Ser Val Ser Val Gly Asp Arg
145                 150                 155                 160

Val Thr Ile Ala Cys Ser Ala Ser Ser Ser Val Pro Tyr Met His Trp
                165                 170                 175

Leu Gln Gln Lys Pro Gly Lys Ser Pro Lys Leu Leu Ile Tyr Leu Thr
            180                 185                 190

Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
        195                 200                 205

Gly Thr Asp Tyr Ser Leu Thr Ile Ser Ser Val Gln Pro Glu Asp Ala
    210                 215                 220

Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Leu Thr Phe Gly
```

```
225                 230                 235                 240

Cys Gly Thr Lys Leu Glu Ile Lys Arg Ser Ser Arg Ala Ala Ala Ser
                245                 250                 255

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
            260                 265                 270

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
        275                 280                 285

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
    290                 295                 300

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
305                 310                 315                 320

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
                325                 330                 335

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
            340                 345                 350

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
        355                 360                 365

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
    370                 375                 380

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
385                 390                 395                 400

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
                405                 410                 415

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
            420                 425                 430

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
        435                 440                 445
```

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
450 455 460

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
465 470 475 480

Pro Gly Lys

<210> 4
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> VH domain of a GD2-specific scFv (clone 14G2A) used in the examples

<400> 4

Glu Val Lys Leu Gln Gln Ser Gly Pro Ser Leu Val Glu Pro Gly Ala
1 5 10 15

Ser Val Met Ile Ser Cys Lys Ala Ser Gly Ser Ser Phe Thr Gly Tyr
20 25 30

Asn Met Asn Trp Val Arg Gln Asn Ile Gly Lys Ser Leu Glu Trp Ile
35 40 45

Gly Ala Ile Asp Pro Tyr Tyr Gly Gly Thr Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met His Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Val Ser Gly Met Glu Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
100 105 110

Ser

<210> 5
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> VL domain of a GD2-specific scFv (clone 14G2A) used in the examples

<400> 5

Asp Ile Leu Leu Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
1 5 10 15

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Arg
20 25 30

Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Lys Leu Leu Ile His Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
85 90 95

Thr His Val Pro Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> 6
<211> 474
<212> PRT
<213> Artificial Sequence

<220>
<223> GD2-specific scFv-Fc (clone 14G2A) used in the examples

<400> 6

```
Glu Val Lys Leu Gln Gln Ser Gly Pro Ser Leu Val Glu Pro Gly Ala
1               5                  10                  15

Ser Val Met Ile Ser Cys Lys Ala Ser Gly Ser Ser Phe Thr Gly Tyr
            20                  25                  30

Asn Met Asn Trp Val Arg Gln Asn Ile Gly Lys Ser Leu Glu Trp Ile
        35                  40                  45

Gly Ala Ile Asp Pro Tyr Tyr Gly Gly Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Arg Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met His Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Val Ser Gly Met Glu Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
            100                 105                 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125

Asp Ile Leu Leu Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
    130                 135                 140

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Arg
145                 150                 155                 160

Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
                165                 170                 175

Pro Lys Leu Leu Ile His Lys Val Ser Asn Arg Phe Ser Gly Val Pro
            180                 185                 190

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
        195                 200                 205

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
```

```
    210                 215                 220

Thr His Val Pro Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
225                 230                 235                 240

Lys Arg Ser Ala Ala Ala Ser Asp Lys Thr His Thr Cys Pro Pro Cys
                245                 250                 255

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
            260                 265                 270

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
        275                 280                 285

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
    290                 295                 300

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
305                 310                 315                 320

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
                325                 330                 335

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
            340                 345                 350

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
        355                 360                 365

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
    370                 375                 380

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
385                 390                 395                 400

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
                405                 410                 415

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
            420                 425                 430
```

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
435 440 445

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
450 455 460

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470

<210> 7
<211> 248
<212> PRT
<213> Artificial Sequence

<220>
<223> CEA-specific scFv used in the examples

<400> 7

Met Ala Gln Val Lys Leu Glu Gln Ser Gly Ala Glu Val Val Lys Pro
1 5 10 15

Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Ser Tyr Met His Trp Leu Arg Gln Gly Pro Gly Gln Cys Leu Glu
35 40 45

Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro
50 55 60

Lys Phe Gln Gly Lys Ala Thr Phe Thr Thr Asp Thr Ser Ala Asn Thr
65 70 75 80

Ala Tyr Leu Gly Leu Ser Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Asn Glu Gly Thr Pro Thr Gly Pro Tyr Tyr Phe Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
115 120 125

```
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu Asn
    130                 135                 140

Val Leu Thr Gln Ser Pro Ser Ser Met Ser Val Ser Val Gly Asp Arg
145                 150                 155                 160

Val Thr Ile Ala Cys Ser Ala Ser Ser Ser Val Pro Tyr Met His Trp
                165                 170                 175

Leu Gln Gln Lys Pro Gly Lys Ser Pro Lys Leu Leu Ile Tyr Leu Thr
            180                 185                 190

Ser Asn Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser
        195                 200                 205

Gly Thr Asp Tyr Ser Leu Thr Ile Ser Ser Val Gln Pro Glu Asp Ala
    210                 215                 220

Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Leu Thr Phe Gly
225                 230                 235                 240

Cys Gly Thr Lys Leu Glu Ile Lys
                245
```

<210> 8
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> GD2-specific scFv (clone 14G2A) used in the examples

<400> 8

```
Glu Val Lys Leu Gln Gln Ser Gly Pro Ser Leu Val Glu Pro Gly Ala
1               5                   10                  15

Ser Val Met Ile Ser Cys Lys Ala Ser Gly Ser Ser Phe Thr Gly Tyr
            20                  25                  30

Asn Met Asn Trp Val Arg Gln Asn Ile Gly Lys Ser Leu Glu Trp Ile
        35                  40                  45
```

```
Gly Ala Ile Asp Pro Tyr Tyr Gly Gly Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Arg Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met His Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Val Ser Gly Met Glu Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser
            100                 105                 110

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125

Asp Ile Leu Leu Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
    130                 135                 140

Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Arg
145                 150                 155                 160

Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
                165                 170                 175

Pro Lys Leu Leu Ile His Lys Val Ser Asn Arg Phe Ser Gly Val Pro
            180                 185                 190

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
        195                 200                 205

Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys Ser Gln Ser
    210                 215                 220

Thr His Val Pro Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
225                 230                 235                 240

Lys
```

<210> 9

<211> 122
<212> PRT
<213> Artificial Sequence

<220>
<223> VH domain of a B7H3-specific scFv

<400> 9

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
20 25 30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ala Tyr Ile Ser Ser Asp Ser Ser Ala Ile Tyr Tyr Ala Asp Thr Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Gly Arg Gly Arg Glu Asn Ile Tyr Tyr Gly Ser Arg Leu Asp Tyr Trp
100 105 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser
115 120

<210> 10
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> VL domain of a B7H3-specific scFv

<400> 10

Asp Ile Gln Leu Thr Gln Ser Pro Ser Phe Leu Ser Ala Ser Val Gly
1 5 10 15

```
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asn Val Asp Thr Asn
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Ala Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Tyr Arg Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Asn Tyr Pro Phe
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
            100                 105
```

<210> 11
<211> 477
<212> PRT
<213> Artificial Sequence

<220>
<223> B7H3-specific scFv-Fc

<400> 11

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                   10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Ser Ser Asp Ser Ser Ala Ile Tyr Tyr Ala Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
```

```
Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Gly Arg Gly Arg Glu Asn Ile Tyr Tyr Gly Ser Arg Leu Asp Tyr Trp
            100                 105                 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser
    130                 135                 140

Pro Ser Phe Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
145                 150                 155                 160

Lys Ala Ser Gln Asn Val Asp Thr Asn Val Ala Trp Tyr Gln Gln Lys
                165                 170                 175

Pro Gly Lys Ala Pro Lys Ala Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr
            180                 185                 190

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
        195                 200                 205

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
    210                 215                 220

Cys Gln Gln Tyr Asn Asn Tyr Pro Phe Thr Phe Gly Gln Gly Thr Lys
225                 230                 235                 240

Leu Glu Ile Lys Arg Ser Ala Ala Ala Ser Asp Lys Thr His Thr Cys
                245                 250                 255

Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu
            260                 265                 270

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
        275                 280                 285

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys
```

290 295 300

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
305 310 315 320

Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu
325 330 335

Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
340 345 350

Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
355 360 365

Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
370 375 380

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
385 390 395 400

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
405 410 415

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly
420 425 430

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
435 440 445

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
450 455 460

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 12
<211> 244
<212> PRT
<213> Artificial Sequence

<220>
<223> B7H3-specific scFv

<400> 12

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1               5                  10                  15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Phe
            20                  25                  30

Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Ala Tyr Ile Ser Ser Asp Ser Ser Ala Ile Tyr Tyr Ala Asp Thr Val
    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80

Leu Gln Met Asn Ser Leu Arg Asp Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Gly Arg Gly Arg Glu Asn Ile Tyr Tyr Gly Ser Arg Leu Asp Tyr Trp
            100                 105                 110

Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly
        115                 120                 125

Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Leu Thr Gln Ser
    130                 135                 140

Pro Ser Phe Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
145                 150                 155                 160

Lys Ala Ser Gln Asn Val Asp Thr Asn Val Ala Trp Tyr Gln Gln Lys
                165                 170                 175

Pro Gly Lys Ala Pro Lys Ala Leu Ile Tyr Ser Ala Ser Tyr Arg Tyr
            180                 185                 190

Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
        195                 200                 205
```

Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr
210 215 220

Cys Gln Gln Tyr Asn Asn Tyr Pro Phe Thr Phe Gly Gln Gly Thr Lys
225 230 235 240

Leu Glu Ile Lys

<210> 13
<211> 121
<212> PRT
<213> Artificial Sequence

<220>
<223> VH domain of a CD20-specific scFv

<400> 13

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110

Ala Gly Thr Thr Val Thr Val Ser Ala
115 120

<210> 14
<211> 106
<212> PRT
<213> Artificial Sequence

<220>
<223> VL domain of a CD20-specific scFv

<400> 14

Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Ile
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 15
<211> 475
<212> PRT
<213> Artificial Sequence

<220>
<223> CD20-specific scFv-Fc

<400> 15

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr

```
            20                  25                  30

Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
        35                  40                  45

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
            100                 105                 110

Ala Gly Thr Thr Val Thr Val Ser Ala Gly Gly Gly Gly Ser Gly Gly
        115                 120                 125

Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile Val Leu Ser Gln Ser Pro
    130                 135                 140

Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg
145                 150                 155                 160

Ala Ser Ser Ser Val Ser Tyr Ile His Trp Phe Gln Gln Lys Pro Gly
                165                 170                 175

Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly
            180                 185                 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
        195                 200                 205

Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
    210                 215                 220

Gln Trp Thr Ser Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
225                 230                 235                 240
```

```
Ile Lys Arg Ser Ala Ala Ala Ser Asp Lys Thr His Thr Cys Pro Pro
                245                 250                 255

Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro
            260                 265                 270

Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr
        275                 280                 285

Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn
    290                 295                 300

Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg
305                 310                 315                 320

Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val
                325                 330                 335

Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser
            340                 345                 350

Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys
        355                 360                 365

Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu
    370                 375                 380

Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
385                 390                 395                 400

Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
                405                 410                 415

Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
            420                 425                 430

Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly
        435                 440                 445

Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr
```

450 455 460

Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
465 470 475

<210> 16
<211> 242
<212> PRT
<213> Artificial Sequence

<220>
<223> CD20-specific scFv

<400> 16

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
35 40 45

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
100 105 110

Ala Gly Thr Thr Val Thr Val Ser Ala Gly Gly Gly Gly Ser Gly Gly
115 120 125

Gly Gly Ser Gly Gly Gly Gly Ser Gln Ile Val Leu Ser Gln Ser Pro
130 135 140

Ala Ile Leu Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Arg

145 150 155 160

Ala Ser Ser Ser Val Ser Tyr Ile His Trp Phe Gln Gln Lys Pro Gly
165 170 175

Ser Ser Pro Lys Pro Trp Ile Tyr Ala Thr Ser Asn Leu Ala Ser Gly
180 185 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
195 200 205

Thr Ile Ser Arg Val Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
210 215 220

Gln Trp Thr Ser Asn Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu
225 230 235 240

Ile Lys

<210> 17
<211> 744
<212> PRT
<213> Artificial Sequence

<220>
<223> GD2 specific IgG1 (clone 14G2A) used in the examples, having a cleavage site between the light and heavy chains

<400> 17

Met Leu Pro Ser Gln Leu Ile Gly Phe Leu Leu Leu Trp Val Pro Ala
1 5 10 15

Ser Arg Gly Asp Ile Leu Leu Thr Gln Thr Pro Leu Ser Leu Pro Val
20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
35 40 45

Val His Arg Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
50 55 60

```
Gly Gln Ser Pro Lys Leu Leu Ile His Lys Val Ser Asn Arg Phe Ser
65                  70                  75                  80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
                85                  90                  95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
            100                 105                 110

Ser Gln Ser Thr His Val Pro Pro Leu Thr Phe Gly Ala Gly Thr Lys
        115                 120                 125

Leu Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
    130                 135                 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                 150                 155                 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
                165                 170                 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
            180                 185                 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
        195                 200                 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
    210                 215                 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys Arg
225                 230                 235                 240

Ala Lys Arg Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp Ser
                245                 250                 255

Thr Ser Gly Ser Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys Gln
            260                 265                 270

Ala Gly Asp Val Glu Glu Asn Pro Gly Pro Met Asp Trp Thr Trp Arg
        275                 280                 285
```

```
Ile Leu Phe Leu Val Ala Ala Ala Thr Gly Ala His Ser Glu Val Lys
    290                 295                 300

Leu Gln Gln Ser Gly Pro Ser Leu Val Glu Pro Gly Ala Ser Val Met
305                 310                 315                 320

Ile Ser Cys Lys Ala Ser Gly Ser Ser Phe Thr Gly Tyr Asn Met Asn
                325                 330                 335

Trp Val Arg Gln Asn Ile Gly Lys Ser Leu Glu Trp Ile Gly Ala Ile
            340                 345                 350

Asp Pro Tyr Tyr Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys Gly Arg
        355                 360                 365

Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr Met His Leu
    370                 375                 380

Lys Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Val Ser Gly
385                 390                 395                 400

Met Glu Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser
                405                 410                 415

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
            420                 425                 430

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
        435                 440                 445

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
    450                 455                 460

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
465                 470                 475                 480

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
                485                 490                 495
```

```
Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
            500                 505                 510

Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        515                 520                 525

Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    530                 535                 540

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
545                 550                 555                 560

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                565                 570                 575

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            580                 585                 590

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        595                 600                 605

Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    610                 615                 620

Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
625                 630                 635                 640

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
                645                 650                 655

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            660                 665                 670

Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        675                 680                 685

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    690                 695                 700

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
705                 710                 715                 720
```

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
725 730 735

Ser Leu Ser Leu Ser Pro Gly Lys
740

<210> 18
<211> 239
<212> PRT
<213> Artificial Sequence

<220>
<223> light chain of the GD2 IgG1 of SEQ ID NO: 17

<400> 18

Met Leu Pro Ser Gln Leu Ile Gly Phe Leu Leu Leu Trp Val Pro Ala
1 5 10 15

Ser Arg Gly Asp Ile Leu Leu Thr Gln Thr Pro Leu Ser Leu Pro Val
20 25 30

Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu
35 40 45

Val His Arg Asn Gly Asn Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Lys Leu Leu Ile His Lys Val Ser Asn Arg Phe Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Phe Cys
100 105 110

Ser Gln Ser Thr His Val Pro Pro Leu Thr Phe Gly Ala Gly Thr Lys
115 120 125

Leu Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

```
Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145                 150                 155                 160


Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
                165                 170                 175


Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
            180                 185                 190


Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
        195                 200                 205


Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
    210                 215                 220


Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235


<210>  19
<211>  43
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  cleavage sequence (Furin-V5-SG-P2A) of the GD2 IgG1 of SEQ ID NO:
       17

<400>  19

Arg Ala Lys Arg Gly Lys Pro Ile Pro Asn Pro Leu Leu Gly Leu Asp
1               5                   10                  15


Ser Thr Ser Gly Ser Gly Ser Gly Ala Thr Asn Phe Ser Leu Leu Lys
            20                  25                  30


Gln Ala Gly Asp Val Glu Glu Asn Pro Gly Pro
        35                  40


<210>  20
<211>  462
<212>  PRT
<213>  Artificial Sequence
```

<220>
<223> heavy chain of the GD2 IgG1 of SEQ ID NO: 17

<400> 20

Met Asp Trp Thr Trp Arg Ile Leu Phe Leu Val Ala Ala Ala Thr Gly
1   5   10   15

Ala His Ser Glu Val Lys Leu Gln Gln Ser Gly Pro Ser Leu Val Glu
            20   25   30

Pro Gly Ala Ser Val Met Ile Ser Cys Lys Ala Ser Gly Ser Ser Phe
        35   40   45

Thr Gly Tyr Asn Met Asn Trp Val Arg Gln Asn Ile Gly Lys Ser Leu
    50   55   60

Glu Trp Ile Gly Ala Ile Asp Pro Tyr Tyr Gly Gly Thr Ser Tyr Asn
65   70   75   80

Gln Lys Phe Lys Gly Arg Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
                85   90   95

Thr Ala Tyr Met His Leu Lys Ser Leu Thr Ser Glu Asp Ser Ala Val
            100   105   110

Tyr Tyr Cys Val Ser Gly Met Glu Tyr Trp Gly Gln Gly Thr Ser Val
        115   120   125

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
    130   135   140

Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
145   150   155   160

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
                165   170   175

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
            180   185   190

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu

```
        195                 200                 205

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
    210                 215                 220

Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
225                 230                 235                 240

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
                245                 250                 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
            260                 265                 270

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
        275                 280                 285

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
    290                 295                 300

Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
305                 310                 315                 320

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
                325                 330                 335

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
            340                 345                 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
        355                 360                 365

Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
    370                 375                 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
385                 390                 395                 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                405                 410                 415
```

```
Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
            420                 425                 430


Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
        435                 440                 445


Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
    450                 455                 460
```