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(54) Title: CHIMERIC ANTIGEN RECEPTORS DIRECTED AGAINST TRBC1 AND TRBC2

(57) Abstract: The present invention provides a chimeric antigen receptor (CAR) comprising an anti-TRBC2 antigen-binding domain, a CD28 spacer, a CD28 transmembrane domain, and a CD28- CDSzeta endodomain.

WO 2024/256824 A1

FIELD OF THE INVENTION

The present invention relates to CARs that are specific for TCR beta chain (TRBC) 1 or TRBC2 and their use in the treatment of diseases, for example T cell lymphomas or leukaemias.

5 BACKGROUND TO THE INVENTION

Lymphoid malignancies can largely be divided into those which are derived from either T-cells or B-cells. T-cell malignancies are a clinically and biologically heterogeneous group of disorders, together comprising 10-20% of non-Hodgkin's lymphomas and 20% of acute leukaemias. The most commonly identified histological subtypes are peripheral T-cell
10 lymphoma, not otherwise specified (PTCL-NOS); angio-immunoblastic T-cell lymphoma (AITL) and anaplastic large cell lymphoma (ALCL). Of all acute Lymphoblastic Leukaemias (ALL), some 20% are of a T-cell phenotype.

These conditions typically behave aggressively, compared for instance with B-cell malignancies, with estimated 5-year survival of only 30%. In the case of T-cell lymphoma, they
15 are associated with a high proportion of patients presenting with disseminated disease, unfavourable International Prognostic Indicator (IPI) score and prevalence of extra-nodal disease. Chemotherapy alone is not usually effective and less than 30% of patients are cured with current treatments.

Further, unlike in B-cell malignancies, where immunotherapies such as the anti-CD20
20 monoclonal antibody rituximab have dramatically improved outcomes, there is currently no equivalently effective, minimally toxic immunotherapeutic available for the treatment of T-cell malignancies. An important difficulty in the development of immunotherapy for T-cell disorders is the considerable overlap in marker expression of clonal and normal T-cells, with no single antigen clearly able to identify clonal (malignant) cells.

25 Chimeric antigen receptors (CARs) T-cells have shown promise in the treatment of refractory B-cell malignancies. Targeting T cell malignancies is likely to be equally efficacious, but the application of CARs in diseases such as T-cell lymphoma has been hampered by a paucity of suitable target antigens. Unlike B cell lymphomas, in which ablation of the B cell compartment is a manageable toxicity and can be treated with the administration of intravenous
30 immunoglobulin, destroying the T cell compartment will not be well tolerated and will lead to complications associated with suppression of cell mediated immunity.

A method for treating T cell lymphomas and leukaemias consisting in targeting the constant region of the TCR beta chain (TRBC) has been previously described in WO2015/132598. This approach is based on a unique feature of the T cell receptor, i.e. that each TCR encodes either TRBC1 or TRBC2 in a mutually exclusive fashion. Because T-cell lymphomas and leukaemias are a clonal population of cells, each lymphoma will express one TCR, with either TRBC1 or TRBC2, on the surface.

The monoclonal antibody Jovi-1 specifically binds to TRBC1 and has been used as the binding domain in a CAR for a therapy treating T cell lymphomas (Maciocia et al., 2017, Nat Med 23:1416-23; WO2015/132598). This proposed therapy allows the treatment of a subset of patients that express a TCR with the TRBC1 constant region. A humanized (HuJovi-1) version of the known Jovi-1 (MuJovi-1) antibody has been previously described (WO 2018/224844).

In order to treat the entire patient population, a binder/CAR targeting TRBC2 is necessary. One method for obtaining antibodies which are specific to TRBC2 is by phage selections on a human phage display library. Another method consists in immunising animals with TRBC2-derived peptides and subsequently selecting specific antibodies. Both these approaches have been carried out with success and TRBC2-specific binders generated, as disclosed in WO2015/132598. Alternative binders specific for TRBC2 are described in WO2020/089644.

The present invention provides CARs that are specific for either TRBC1 or TRBC2 and which are potential therapeutic agents for the treatment of either TRBC1+ or TRBC2+ lymphomas or leukaemias.

SUMMARY OF THE INVENTION

The present invention provides CARs that are specific for either TRBC1 or TRBC2 and which are potential therapeutic agents for the treatment of either TRBC1+ or TRBC2+ lymphomas or leukaemias. The CARs provide efficient and specific killing of tumors carrying the respective TCR beta chain target.

In a first aspect, the invention provides a chimeric antigen receptor (CAR) comprising an anti-TRBC2 antigen-binding domain, a CD28 spacer, a CD28 transmembrane domain, and a CD28-CD3zeta endodomain.

The present inventors have determined that a CAR comprising a spacer, transmembrane domain, and endodomain derived from CD28 has higher surface CAR density compared to other CAR formats. Without wishing to be bound by theory, it is hypothesised that this is due to the stabilizing effect of the CD28TM domain, potentially further in combination with other domains from CD28. Additionally, the CD28stk region has been shown to reduce the target

antigen density threshold for CAR-T activity, possibly due to heterodimerization with endogenous CD28. Together, this may provide an avidity effect to counteract lower binding affinities.

In some embodiments, the anti-TRBC2 antigen-binding domain has a variable heavy chain (VH) and a variable light chain (VL) which comprise the following complementarity determining regions (CDRs):

- a. VH CDR1: GYKFTGF (SEQ ID No: 1)
- b. VH CDR2: NPYNDD (SEQ ID No: 2)
- c. VH CDR3: GNGYNFDGAYRFFDF (SEQ ID No: 3)
- d. VL CDR1: RSSQRLVHSNGNTYLH (SEQ ID No: 4)
- e. VL CDR2: RVSNRFP (SEQ ID No: 5)
- f. VL CDR3: SQSTHVPYT (SEQ ID No: 6)

In some embodiments, the anti-TRBC2 antigen-binding domain comprises:

- a. A VH domain comprising SEQ ID No: 7, or a sequence with at least 80% identity to SEQ ID No: 7, and
- b. A VL domain comprising SEQ ID NO: 8, or a sequence with at least 80% identity to SEQ ID No: 8.

In some embodiments, the anti-TRBC2 antigen-binding domain comprises SEQ ID No: 9, or a sequence with at least 80% identity to SEQ ID No: 9.

In some embodiments, the CD28 spacer comprises SEQ ID No: 16, or a sequence with at least 80% identity to SEQ ID No: 16.

In some embodiments, the CD28 transmembrane comprises SEQ ID No: 18, or a sequence with at least 80% identity to SEQ ID No: 18.

In some embodiments, the CD28-CD3Zeta endodomain comprises SEQ ID No: 20 or SEQ ID No: 21, or a sequence with at least 80% identity to SEQ ID No: 20 or SEQ ID No: 21.

In some embodiments, the CAR comprises or consists of an amino acid sequence having SEQ ID No: 22, or a sequence with at least 80% identity thereto.

In a second aspect, the invention provides a chimeric antigen receptor (CAR) comprising:

- a. an anti-TRBC1 antigen-binding domain, an IgG1 hinge domain, a TYRP-1 transmembrane domain, and a 41BB-CD3zeta endodomain;

- b. an anti-TRBC1 antigen-binding domain, a CD8 spacer, a TYRP-1 transmembrane domain, and a CD28-CD3zeta endodomain; or
- c. an anti-TRBC1 antigen-binding domain, a CD28 spacer, a CD28 transmembrane domain, and a CD28-CD3zeta endodomain.

5 In some embodiments, the anti-TRBC1 antigen-binding domain has a variable heavy chain (VH) and a variable light chain (VL) which comprise the following complementarity determining regions (CDRs):

- a. VH CDR1: GYTFTGY (SEQ ID No: 10)
- b. VH CDR2: NPYNDD (SEQ ID No: 2)
- 10 c. VH CDR3: GAGYNFDGAYRFFDF (SEQ ID No: 11)
- d. VL CDR1: RSSQRLVHSNGNTYLH (SEQ ID No: 4)
- e. VL CDR2: RVSNRFP (SEQ ID No: 5)
- f. VL CDR3: SQSTHVPYT (SEQ ID No: 6)

In some embodiments, the anti-TRBC1 antigen-binding domain comprises:

- 15 a. A VH domain comprising SEQ ID No: 12, or a sequence with at least 80% identity to SEQ ID No: 12, and
- b. A VL domain comprising SEQ ID No: 8, or a sequence with at least 80% identity to SEQ ID No: 8.

20 In some embodiments, the anti-TRBC1 antigen-binding domain comprises SEQ ID No: 13, or a sequence with at least 80% identity to SEQ ID No: 13.

In some embodiments, the IgG1 hinge domain comprises SEQ ID No: 14, or a sequence with at least 80% identity to SEQ ID No: 14.

In some embodiments, the CD8 spacer comprises SEQ ID No: 15, or a sequence with at least 80% identity to SEQ ID No: 15.

25 In some embodiments, the CD28 spacer comprises SEQ ID No: 16, or a sequence with at least 80% identity to SEQ ID No: 16.

In some embodiments, the TYRP-1 transmembrane domain comprises SEQ ID No: 17, or a sequence with at least 80% identity to SEQ ID No: 17.

30 In some embodiments, the CD28 transmembrane comprises SEQ ID No: 18, or a sequence with at least 80% identity to SEQ ID No: 18.

In some embodiments, the 41BB-CD3zeta endodomain comprises SEQ ID No: 19, or a sequence with at least 80% identity to SEQ ID No: 19.

In some embodiments, the CD28-CD3Zeta endodomain comprises SEQ ID No: 20 or SEQ ID No: 21, or a sequence with at least 80% identity to SEQ ID No: 20 or SEQ ID No: 21.

- 5 In some embodiments, the CAR comprises or consists of an amino acid sequence having SEQ ID No: 25, or a sequence with at least 80% identity thereto.

In some embodiments, the CAR comprises or consists of an amino acid sequence having SEQ ID No: 26, or a sequence with at least 80% identity thereto.

- 10 In some embodiments, the CAR comprises or consists of an amino acid sequence having SEQ ID No: 27, or a sequence with at least 80% identity thereto.

The invention further provides a nucleic acid which encodes a CAR according to the invention.

The invention further provides a vector which comprises a nucleic acid according to the invention.

The invention further provides a cell which comprises a CAR according to the invention.

- 15 The invention further provides a composition comprising a plurality of cells according to the invention.

In some embodiments, the cell(s) is a TRBC1+ cell comprising an anti-TRBC2 CAR according to the first aspect of the invention.

- 20 In some embodiments, the cell(s) is a TRBC2+ cell comprising an anti-TRBC1 CAR according to the second aspect of the invention.

The invention further provides a method of making a cell, or a composition of cells, according to the invention, which comprises the step of transducing or transfecting a cell, or a sample of cells, with a nucleic acid or a vector according to the invention.

- 25 In some embodiments, a TRBC1+ cell(s) is transduced or transfected with a nucleic acid or vector encoding an anti-TRBC2 CAR according to the invention.

In some embodiments, a TRBC2+ cell(s) is transduced or transfected with a nucleic acid or vector encoding an anti-TRBC1 CAR according to the invention.

The invention further provides a pharmaceutical composition comprising a nucleic acid, vector, cell, or cell composition according to the invention, together with a pharmaceutically acceptable carrier, diluent or excipient.

5 The invention further provides a method of treating T-cell lymphoma or leukaemia, comprising administering a nucleic acid according to the invention to a subject.

The invention further provides a method of treating T-cell lymphoma or leukaemia, comprising administering a vector according to the invention to a subject.

The invention further provides a method of treating T-cell lymphoma or leukaemia, comprising administering a cell or cell composition according to the invention to a subject.

10 The invention further provides a method of treating T-cell lymphoma or leukaemia, comprising administering a pharmaceutical composition according to the invention to a subject.

The invention further provides a nucleic acid according to the invention for use in a method of treating T-cell lymphoma or leukaemia.

15 The invention further provides a vector according to the invention for use in a method of treating T-cell lymphoma or leukaemia.

The invention further provides a cell or cell composition according to the invention for use in a method of treating T-cell lymphoma or leukaemia.

The invention further provides a pharmaceutical composition according to the invention for use in a method of treating T-cell lymphoma or leukaemia.

20 The invention further provides for use of a nucleic acid according to the invention for the manufacture of a medicament for the treatment of T-cell lymphoma or leukaemia.

The invention further provides for use of a vector according to the invention for the manufacture of a medicament for the treatment of T-cell lymphoma or leukaemia.

25 The invention further provides for use of a cell or cell composition according to the invention for the manufacture of a medicament for the treatment of T-cell lymphoma or leukaemia.

The invention further provides for use of a pharmaceutical composition according to the invention for the manufacture of a medicament for the treatment of T-cell lymphoma or leukaemia.

30 The T-cell lymphoma or leukaemia may be selected from: peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS); angio-immunoblastic T-cell lymphoma (AITL), anaplastic

large cell lymphoma (ALCL), enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL), extranodal NK/T-cell lymphoma nasal type, cutaneous T-cell lymphoma, primary cutaneous ALCL, T cell prolymphocytic leukaemia and T-cell acute lymphoblastic leukaemia.

5 In some embodiments, the invention provides a method of treating T-cell lymphoma or leukaemia associated with the clonal expansion of a cell expressing a T-cell receptor (TCR) comprising TRBC2, comprising administering the nucleic acid or vector encoding the anti-TRBC2 CAR, or the cell or composition expressing the anti-TRBC2 CAR according to the invention to a subject.

10 In some embodiments, the invention provides a nucleic acid or vector encoding the anti-TRBC2 CAR, or the cell or composition expressing the anti-TRBC2 CAR according to the invention for use in a method of treating a T-cell lymphoma or leukaemia associated with the clonal expansion of a cell expressing a TCR comprising TRBC2.

15 In some embodiments, the invention provides for use of a nucleic acid or vector encoding the anti-TRBC2 CAR, or the cell or composition expressing the anti-TRBC2 CAR according to the invention for the manufacture of a medicament for the treatment of T-cell lymphoma or leukaemia associated with the clonal expansion of a cell expressing a TCR comprising TRBC2.

20 In some embodiments, the invention provides a method of treating T-cell lymphoma or leukaemia associated with the clonal expansion of a cell expressing a TCR comprising TRBC1, comprising administering the nucleic acid or vector encoding the anti-TRBC1 CAR, or the cell or composition expressing the anti-TRBC1 CAR according to the invention to a subject.

25 In some embodiments, the invention provides a nucleic acid or vector encoding the anti-TRBC1 CAR, or the cell or composition expressing the anti-TRBC1 CAR according to the invention for use in a method of treating T-cell lymphoma or leukaemia associated with the clonal expansion of a cell expressing a TCR comprising TRBC1.

30 In some embodiments, the invention provides for use of a nucleic acid or vector encoding the anti-TRBC1 CAR, or the cell or composition expressing the anti-TRBC1 CAR according to the invention for the manufacture of a medicament for the treatment of a T-cell lymphoma or leukaemia may be associated with the clonal expansion of a cell expressing a TCR comprising TRBC1.

DESCRIPTION OF THE FIGURES

Figure 1 – A diagram of the $\alpha\beta$ T-cell Receptor/CD3 Complex. The T-cell receptor is formed from 6 different protein chains which must assemble in the endoplasmic reticulum to be expressed on the cell surface. The four proteins of the CD3 complex (CD3 ζ , CD3 γ , CD3 ϵ and CD3 δ) sheath the T-cell Receptor (TCR). This TCR imbues the complex with specificity of a particular antigen and is composed of two chains: TCR α and TCR β . Each TCR chain has a variable component distal to the membrane and a constant component proximal to the membrane. Nearly all T-cell lymphomas and many T-cell leukaemias express the TCR/CD3 complex.

Figure 2 – The segregation of T-cell Receptor β -constant region (TRBC)-1 and TRBC2 during T-cell receptor rearrangement. Each TCR beta chain is formed from genomic recombination of a particular beta variable (V), diversity (D), joining (J) and constant (TRBC) regions. The human genome contains two very similar and functionally equivalent TRBC loci known as TRBC1 and TRBC2. During TCR gene re-arrangement, a J-region recombines with either TRBC1 or TRBC2. This rearrangement is permanent. T-cells express many copies of a single TCR on their surface, hence each T-cell will express a TCR whose β -chain constant region is coded for by either TRBC1 or TRBC2.

Figure 3 – Alignment of human TRBC1 and TRBC2 at the amino acid level. The TCR β constant chain coded for by TRBC1 and TRBC2 differ by only 4 amino acid differences: K / N at position 3 of the TRBC; N / K at position 4 of the TRBC; F / Y at position 36 of the TRBC; V / E at position 135 of the TRBC.

Figure 4 – Diagram of the structure of TRBC1 and TRBC2 specific chimeric antigen receptors (CARs).

Figure 5 – Functional characterization of HuJovi-1 and KFN CAR. Schematic of anti-TRBC1 (a) and anti-TRBC2 (b) CAR architectures. Flow cytometry-based killing of Jurkat TRBC1, Jurkat TRBC2, or Jurkat TCR KO cells by HuJovi-1 (c) and KFN (d) CAR-T cells at 1:8 E:T ratio, 72h; donor n = 9. *p < 0.05, *** p < 0.001, **** p < 0.0001 by two-way ANOVA and Dunnett's test for multiple comparisons versus aCD19 CAR. IFN- γ and IL-2 secretion by HuJovi-1 (e) and KFN (f) –based CARs against Jurkat TRBC1, TRBC2 and TCR KO. Donor n = 6, *p < 0.05, ** p < 0.01, **** p < 0.0001 by two-way ANOVA and Dunnett's test for multiple comparisons versus aCD19 CAR. Sorted healthy donor T cells (TRBC1⁺ and TRBC2⁺) forward (left) and reverse (right) killing for HuJovi-1 (g) and KFN (h) –based CARs at 4:1, 1:1 and 1:4 E:T ratios, 72h. Donor n = 4, *p < 0.05, ** p < 0.01, two-way ANOVA and Sidak's post-test for multiple comparisons TRBC1 versus TRBC2. (i) FACS-based killing of primary T-PLL tumor

samples (TRBC1 n=3, TRBC2 n=3) at 4:1, 1:1 and 1:4 E:T ratios, 72h. PBMC donor n = 8 for HuJovi-1 CAR and n = 12 for KFN CAR. ** p > 0.01, ***p < 0.001, **** P < 0.0001, two-way ANOVA and Sidak's post-test for multiple comparisons versus aCD19 CAR. T-PLL = T cell prolymphocytic leukemia.

5 **Figure 6 – Functional characterization of HuJovi-1 CAR.** (a) Representative dot plot for HuJovi-1 CAR T cells stained with anti-CD34 (for RQR8 marker protein) and anti-idiotypic (for CAR). (b) % transduction efficiency based on RQR8⁺ CAR T cell population by flow cytometry (left) and surface CAR expression as CAR (anti-idiotypic)/RQR8 (anti-CD34) ratio. One-way ANOVA with Tukey's post test, * p < 0.05, ** p < 0.01, **** p < 0.0001. (c) FACS-based killing
10 of HPB-ALL TRBC1 (n=9), HPB-ALL TRBC2 (n=9), HPB-ALL TCR KO (n=6), H9 TRBC1 (n=6), T-ALL1 TRBC2 (n=9) and HD-MAR2 TRBC2 (n=4) cell lines by HuJovi-1 CAR T cells at 1:8 E:T ratio, 72h. *p < 0.05, ** p < 0.01, *** p > 0.001 by two-way ANOVA and Dunnett's test for multiple comparisons versus aCD19 CAR. (d) Cytometric bead array assay measurement for cytokine and cytolytic mediators by HuJovi-1 CAR-T cells against target cell lines. H9
15 values for Granzyme A, Granzyme B and Perforin were plotted separately due to high constitutive expression. Data presented as geometric mean.

Figure 7 – Functional characterization of KFN CAR. (a) Representative dot plot for KFN CAR-T cells stained with anti-CD34 (for RQR8 marker protein) and anti-idiotypic (for CAR). (b) % transduction efficiency based on RQR8⁺ CAR-T cell population by flow cytometry (left) and
20 surface CAR expression as CAR (anti-idiotypic)/RQR8 (anti-CD34) ratio. One-way ANOVA with Tukey's post test, *** p < 0.001, **** p < 0.0001. (c) Surface plasmon resonance (SPR) affinity kinetics of anti-idiotypic antibody for KFN antibody. (d) Flow cytometry-based killing of HPB-ALL TRBC1 (n=9), HPB-ALL TRBC2 (n=9), HPB-ALL TCR KO (n=6), H9 TRBC1 (n=6), T-ALL1 TRBC2 (n=9) and HD-MAR2 TRBC2 (n=4) cell lines by HuJovi-1 CAR-T cells at 1:8
25 E:T ratio, 72h. *p < 0.05, ** p < 0.01, *** p > 0.001 by two-way ANOVA and Dunnett's test for multiple comparisons versus aCD19 CAR. (e) Cytometric bead array assay measurement for cytokine and cytolytic mediators by HuJovi-1 CAR-T cells against target cell lines. H9 values for Granzyme A, Granzyme B and Perforin were plotted separately due to high constitutive expression. Data presented as geometric mean.

30 **Figure 8 – Baseline CAR-T differentiation and exhaustion profile.** (a) Differentiation profile of CAR-T cells at baseline, for sorted TRBC1⁺ PBMC (KFN CAR and aCD19 CAR) and TRBC2⁺ PBMC (HuJovi-1 CAR and aCD19 CAR). Tcm = T central memory, Tem = T effector memory, Temra = terminally differentiated effector memory cells, Tn = naïve T cell. (b) Exhaustion profile of CAR T cells at baseline, for sorted TRBC1⁺ PBMC (KFN CAR and aCD19

CAR) and TRBC2⁺ PBMC (HuJovi-1 CAR and aCD19 CAR) based on the expression of 0, 1, 2 or 3 antigens between PD-1, LAG3 and TIGIT.

Figure 9 – Gating strategy for flow cytometry-based killing assay. Representative gating strategy for flow-cytometry-based killing assay against target cell lines (a) or primary tumor samples (b).

Figure 10 – *In vivo* characterization of HuJovi-1 CAR and KFN CAR. (a) Schematic of NSG model with 2.5e6 HPB-ALL cells/animal (n = 6/group). (b) Median total flux radiance of HPB-ALL TRBC2 tumor burden (from a). Two-way ANOVA with Dunnett's post test against KFN CAR, *** p < 0.001, **** p < 0.0001. (c) Kaplan-Meier survival curve (cut-off 1e9 p/s/cm²/sr) (from a). Log-rank test *** p < 0.001. (d) (clockwise) HPB-ALL count in CAR treated mice; % of TRBC2 expressing cells in residual HPB-ALL population; human T cell count (CD3⁺) in treated mice; % RQR8⁺ (CAR) T cells in CD3⁺ population. One-way ANOVA with Dunnett's post-test. * P < 0.05, ** P < 0.01, **** P < 0.0001. (e) Schematic of NSG model with 5e6 Jurkat TRBC1 or 1e6 Jurkat TRBC2 cells/animal (n = 6/group). (f) (left) Median total radiance of Jurkat TRBC1 tumor burden. Two-way ANOVA with Dunnett's post-test against HuJovi-1 CAR, ** p < 0.01. (right) Kaplan-Meier survival curve (cut-off 1e9 p/s/cm²/sr). # 1 mouse from aCD19 CAR at d34 and remaining mice from the non-transduced group at d55, sacrificed due to xenogeneic graft versus host disease. Log-rank test ** p < 0.01. (g) (left) Median total radiance of Jurkat TRBC2 tumor burden. Two-way ANOVA with Dunnett's post-test against KFN CAR, **** p < 0.0001. (right) Kaplan-Meier survival curve (cut-off 1e9 p/s/cm²/sr). Log-rank test *** p < 0.001.

Figure 11 – Bioluminescent imaging for NSG HPB-ALL *in vivo* model. Total radiance bioluminescent imaging (BLI) of NSG mice engrafted with HPB-ALL TRBC2 cell line and treated with KFN-CD28stk-CD28TM-CD28z, HuJovi1-CD8stk-TyrpTM-CD28z, aCD19 CAR T cells or non-transduced PBMC, at 5e6 CAR T cells/mouse.

Figure 12 – Analysis of NSG HPB-ALL *in vivo* model. (a) Individual mouse total radiance BLI of HPB-ALL TRBC2⁺ tumor burden post CAR-T cells (n = 6). (b) HPB-ALL count in CAR-treated mice (top left), % of TRBC2 expressing cells in residual HPB-ALL population (top right), human T cell count (CD3⁺) in treated mice (bottom left), % RQR8⁺ (CAR)-T cells in CD3⁺ population (bottom right). One-way ANOVA with Dunnett's post-test. * p < 0.05, ** p < 0.01, **** p < 0.0001.

Figure 13 – Bioluminescent imaging for NSG Jurkat TRBC1/TRBC2 *in vivo* model. Total radiance BLI of NSG mice engrafted with Jurkat TRBC1 (left) or Jurkat TRBC2 (right) cell lines

and treated with KFN-CD28stk-CD28TM-CD28z, HuJovi1-CD8stk-TyrpTM-CD28z, aCD19 CAR-T cells or non-transduced PBMC, at 5e6 CAR T cells/mouse.

Figure 14 – Analysis of NSG Jurkat TRBC1/TRBC2 *in vivo* model. (a) Individual mouse total radiance BLI of Jurkat TRBC1⁺ tumor burden post CAR-T cells (n = 6). # xeno-GvHD event. (b) Individual mouse total radiance BLI of Jurkat TRBC2⁺ tumor burden post CAR-T cells (n = 6).

DETAILED DESCRIPTION OF THE INVENTION

TCR β CONSTANT REGION (TRBC)

The T-cell receptor (TCR) is expressed on the surface of T lymphocytes and is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. When the TCR engages with antigenic peptide and MHC (peptide/MHC), the T lymphocyte is activated through a series of biochemical events mediated by associated enzymes, co-receptors, specialized adaptor molecules, and activated or released transcription factors.

The TCR is a disulfide-linked membrane-anchored heterodimer normally consisting of the highly variable alpha (α) and beta (β) chains expressed as part of a complex with the invariant CD3 chain molecules. T-cells expressing this receptor are referred to as $\alpha\beta$ (or $\alpha\beta$) T-cells (~95% total T-cells). A minority of T-cells express an alternate receptor, formed by variable gamma (γ) and delta (δ) chains, and are referred to as $\gamma\delta$ T-cells (~5% total T cells).

Each α and β chain is composed of two extracellular domains: Variable (V) region and a Constant (C) region, both of Immunoglobulin superfamily (IgSF) domain forming antiparallel β -sheets. The constant region is proximal to the cell membrane, followed by a transmembrane region and a short cytoplasmic tail, while the variable region binds to the peptide/MHC complex (see Figure 1). The constant region of the TCR consists of short connecting sequences in which a cysteine residue forms disulfide bonds, which forms a link between the two chains.

The variable domains of both the TCR α -chain and β -chain have three hypervariable or complementarity determining regions (CDRs). The variable region of the β -chain also has an additional area of hypervariability (HV4), however, this does not normally contact antigen and is therefore not considered a CDR.

The TCR also comprises up to five invariant chains γ, δ, ϵ (collectively termed CD3) and ζ . The CD3 and ζ subunits mediate TCR signalling through specific cytoplasmic domains which interact with second-messenger and adapter molecules following the recognition of the

antigen by $\alpha\beta$ or $\gamma\delta$. Cell-surface expression of the TCR complex is preceded by the pair-wise assembly of subunits in which both the transmembrane and extracellular domains of TCR α and β and CD3 γ and δ play a role.

TCRs are therefore commonly composed of the CD3 complex and the TCR α and β chains, which are in turn composed of variable and constant regions (Figure 1).

The locus (Chr7:q34) which supplies the TCR β -constant region (TRBC) has duplicated in evolutionary history to produce two almost identical and functionally equivalent genes: TRBC1 and TRBC2 (Figure 2), which differ by only 4 amino acid in the mature protein produced by each (Figure 3). Each TCR will comprise, in a mutually exclusive fashion, either TRBC1 or TRBC2 and as such, each $\alpha\beta$ T-cell will express either TRBC1 or TRBC2, in a mutually exclusive manner.

The present inventors have previously determined that, despite the similarity between the sequence of the TRBC1 and TRBC2, it is possible to discriminate between them. The inventors have also previously determined that amino acid sequences of TRBC1 and TRBC2 can be discriminated whilst *in situ* on the surface of a cell, for example a T-cell (WO2015/132598).

CHIMERIC ANTIGEN RECEPTOR (CAR)

The term “chimeric antigen receptor” or “CAR” or “chimeric T cell receptor” or “artificial T cell receptors” or “chimeric immunoreceptors”, as used herein, refers to a chimeric type I trans-membrane protein which connects an extracellular antigen-recognising domain (binder) to an intracellular signalling domain (endodomain). The binder is typically a single-chain variable fragment (scFv) derived from a monoclonal antibody (mAb), but it can be based on other formats which comprise an antigen binding site. A spacer domain is usually necessary to separate the binder from the membrane and to allow it a suitable orientation. A common spacer domain used is the Fc of IgG1. More compact spacers can suffice e.g. the stalk from CD8 α and even just the IgG1 hinge alone, depending on the antigen. A trans-membrane domain anchors the protein in the cell membrane and connects the spacer to the endodomain.

Early CAR designs had endodomains derived from the intracellular parts of either the γ chain of the Fc ϵ R1 or CD3 ζ . Consequently, these first generation receptors transmitted immunological signal 1, which was sufficient to trigger T-cell killing of cognate target cells but failed to fully activate the T-cell to proliferate and survive. To overcome this limitation, compound endodomains have been constructed: fusion of the intracellular part of a T-cell co-stimulatory molecule to that of CD3 ζ results in second-generation receptors which can transmit

an activating and co-stimulatory signal simultaneously after antigen recognition. The co-stimulatory domain most commonly used is that of CD28. This supplies the most potent co-stimulatory signal – namely immunological signal 2, which triggers T-cell proliferation. Some receptors have also been described which include TNF receptor family endodomains, such as the closely related OX40 and 4-1BB which transmit survival signals. Even more potent third-generation CARs have now been described which have endodomains capable of transmitting activation, proliferation and survival signals.

When the CAR binds the target-antigen, this results in the transmission of an activating signal to the T-cell it is expressed on. Thus the CAR directs the specificity and cytotoxicity of the T cell towards tumour cells expressing the targeted antigen.

CARs typically therefore comprise: (i) an antigen-binding domain; (ii) a spacer; (iii) a transmembrane domain; and (iii) an intracellular domain which comprises or associates with a signalling domain (see Figure 4).

A CAR may have the general structure:

Antigen-binding domain – spacer domain – transmembrane domain – intracellular signalling domain (endodomain).

ANTIGEN BINDING DOMAIN

The term “antigen-binding domain”, as used herein, refers to region of a CAR which binds to the target. Suitably, the antigen-binding domain may be based on an antibody. For example, the antigen-binding domain may comprise a VH and VL domain, the variable regions of each pair of light and heavy chains of the antibody, i.e. the VL and VH domains, respectively, which form its binding site. They are characterised by the same general structure constituted by relatively preserved regions called frameworks (FR) joined by three hypervariable regions called complementarity determining regions (CDR) (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, 5th Ed., NIH Publication No. 91-3242, Bethesda, MD.; Chothia & Lesk, 1987, J Mol Biol 196:901-17). The term “complementarity determining region” or “CDR”, as used herein, refers to the region within an antibody that complements an antigen’s shape. Thus, CDRs determine the protein’s affinity (roughly, binding strength) and specificity for specific antigens. The CDRs of the two chains of each pair are aligned by the framework regions, acquiring the function of binding a specific epitope.

The antigen-binding domain may comprise an scFv.

ANTI-TRBC1 ANTIGEN-BINDING DOMAIN

The present CAR may comprise a humanised anti-TRBC1 antigen-binding domain which has a variable heavy chain (VH) and a variable light chain (VL) which comprise the following complementarity determining regions (CDRs):

- 5 VH CDR1: GYTFTGY (SEQ ID No: 10)
- VH CDR2: NPYNDD (SEQ ID No: 2)
- VH CDR3: GAGYNFDGAYRFFDF (SEQ ID No: 11)
- VL CDR1: RSSQRLVHSNGNTYLH (SEQ ID No: 4)
- VL CDR2: RVSNRFP (SEQ ID No: 5)
- 10 VL CDR3: SQSTHVPYT (SEQ ID No: 6)

The antigen-binding domain may comprise human framework regions, or human framework regions with one or more mutations. For example, the framework region(s) may comprise one or more substitutions compared to the human framework region sequence. The substitutions may be “back-mutations” where one or more amino acids are substituted with the equivalent residue from the murine antibody sequence. The murine antibody variable heavy chain (VH) sequence is shown below as SEQ ID No: 28 and the variable light chain (VL) sequence shown as SEQ ID No: 29. In both sequences, the CDR sequences are underlined.

SEQ ID No: 28 (murine Jovi-1 VH)

EVRLQQSGPDLIKPGASVKMSCKASGYTFTGYVMHWWKQRPGQGLEWIGFINPYNDDIQS

20 NERFRGKATLTSDKSSTTAYMELSSLTSEDSAVYYCARGGAGYNFDGAYRFFDFFWGQGTTLTVSS

SEQ ID No: 29 (murine Jovi-1 VL)

DVVMTQSPLSLPVSLGDQASISCRRSSQRLVHSNGNTYLHWYLQKPGQSPKLLIYRVSNRFP

GVPDRFSGSGSGTDFTLKISRVEAEDLGIYFCSQSTHVPYTFGGGTKLEIKR

25 A humanised VH sequence comprising the murine JOVI-1 CDRs shown as SEQ ID No: 10, 2 and 11 may comprise 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or 1 mutations compared to the wild-type human framework region sequence.

A humanised VL sequence comprising the murine JOVI-1 CDRs shown as SEQ ID Nos: 4, 5 and 6 may comprise 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or 1 mutation compared to the wild-type human framework region sequence.

30

The VH sequence may comprise JOVI-1 VH CDRs with the human framework H-AF062256. This sequence is shown as SEQ ID No: 12. The CDR sequences are underlined.

SEQ ID No: 12 (Humanised Jovi-1 H-AF062256 framework)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWRQAPGQGLEWMGFINPYNDDIQ
 SNERFRGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGAGYNFDGAYRFFDFWGQGT
 MVTVSS

- 5 The VH sequence may comprise JOVI-1 VH CDRs with the human framework H-EF177999. This sequence is shown as SEQ ID No: 30. The CDR sequences are underlined.

SEQ ID No: 30 (Humanised Jovi-1 H-EF177999 framework)

EVQLVESGAIEVKKPGASVKVSCKASGYTFTGYVMHWRQAPGQGLEWMGFINPYNDDIQ
 SNERFRGRVTMTRDTSTSTVYMELSSLRSEDVAVYYCARGAGYNFDGAYRFFDFWGQGT
 10 LVTVSS

The VH sequence may comprise JOVI-1 VH CDRs with the human framework H-KF688165. This sequence is shown as SEQ ID No: 31. The CDR sequences are underlined.

SEQ ID No: 31 (Humanised Jovi-1 H-KF688165 framework)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWRQAPGQGLEWMGFINPYNDDIQ
 15 SNERFRGRVTMTRDTSISTAYMEVSSLTSDDAIYYCARGAGYNFDGAYRFFDFWGQGT
 VTVSS

- The VH sequence may comprise the sequence shown as SEQ ID No: 12, 30 or 31 with one or more mutations, such as back-mutations. The VH sequence may comprise 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or 1 mutation compared to the wild-type human framework
 20 region sequence.

The VL sequence may comprise JOVI-1 VL CDRs with the human framework 3aaz. This sequence is shown as SEQ ID No: 8. The CDR sequences are underlined.

SEQ ID No: 8

DIVMTQSPLSLPVTPGEPASISCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVSNRFP
 25 GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPYTFGQGTKLEIKR

The VL sequence may comprise the sequence shown as SEQ ID No: 8 with one or more mutations, such as back-mutations. The VL sequence may comprise 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, 2 or 1 mutation compared to the wild-type human framework region sequence.

- 30 The anti-TRBC1 antigen-binding domain may comprise:

- a) a VH domain which comprises the sequence shown as SEQ ID No: 12, or a variant thereof having at least 80% sequence identity; and
- b) a VL domain which comprises the sequence shown as SEQ ID No: 8, or a variant thereof having at least 80% sequence identity.

5 The anti-TRBC1 antigen-binding domain may comprise:

- a) a VH domain which comprises the sequence shown as SEQ ID No: 12; and
- b) a VL domain which comprises the sequence shown as SEQ ID No: 8.

The VH and VL domain may be joined by a linker, such as to form an scFv. The linker may comprise the sequence GGGGSGGGGSGGGGS (SEQ ID No: 32).

10 The anti-TRBC1 antigen-binding domain may comprise an scFv having the amino acid sequence shown as SEQ ID No: 13. The CDR sequences are underlined.

SEQ ID No: 13 (scFv)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWWRQAPGQGLEWMGFINPYNDDIQ
 SNERFRGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGAGYNFDGAYRFFDFWGQGT
 15 MVTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTPGEPASISCRSSQRLVHSNGNTYL
HWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTH
VPYTFGGGTKLEIKR

The anti-TRBC1 antigen-binding domain may comprise the sequence shown as SEQ ID No: 13, or a variant thereof having at least 80% sequence identity.

20 The anti-TRBC1 antigen-binding domain may comprise or consist of the sequence shown as SEQ ID No: 13.

The anti-TRBC1 antigen-binding domain may consist of the sequence shown as SEQ ID No: 13, or a variant thereof having at least 80% sequence identity.

A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99%
 25 sequence identity to SEQ ID No: 12, 8, or 13, provided that the antigen-binding domain effectively binds TRBC1. For example, the variant antigen-binding domain may bind TRBC1 with essentially the same affinity and/or specificity as an antigen-binding domain having the sequence set out as SEQ ID No: 13.

ANTI-TRBC2 ANTIGEN-BINDING DOMAIN

The present CAR may comprise a humanised anti-TRBC2 antigen-binding domain which has a variable heavy chain (VH) and a variable light chain (VL) which comprise the following complementarity determining regions (CDRs):

- 5 VH CDR1: GYKFTGF (SEQ ID No: 1)
- VH CDR2: NPYNDD (SEQ ID No: 2)
- VH CDR3: GNGYNFDGAYRFFDF (SEQ ID No: 3)
- VL CDR1: RSSQRLVHSNGNTYLH (SEQ ID No: 4)
- VL CDR2: RVSNRFP (SEQ ID No: 5)
- 10 VL CDR3: SQSTHVPYT (SEQ ID No: 6)

The VH sequence may comprise VH CDRs having SEQ ID No: 1 to 3 with the human framework H-AF062256. This sequence is shown as SEQ ID No: 7. The CDR sequences are underlined.

SEQ ID No: 7 (H-AF062256 framework)

- 15 QVQLVQSGAEVKKPGASVKVSCKASGYKFTGFV¹MHWRQAPGQGLEWMGF²INPYNDD³IQ
SNERFRGRVTMT⁴RDTSISTAYMEL⁵SRLRSD⁶DAVYYCARGNGYNFDGAYRFFDF⁷WGQGT
M⁸TVSS

- 20 The VL sequence may comprise VL CDRs having SEQ ID No: 4 to 6 with the human framework 3aaz. This sequence is shown as SEQ ID No: 8. The CDR sequences are underlined.

SEQ ID No: 8 (3aaz framework)

DIVMTQSPLSLPVTPGEPASISCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVS¹NRFP
GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPYT²FGQGTKLEIKR

The anti-TRBC2 antigen-binding domain may comprise:

- 25 a) a VH domain which comprises the sequence shown as SEQ ID No: 7; and
- b) a VL domain which comprises the sequence shown as SEQ ID No: 8.

The anti-TRBC2 antigen-binding domain may comprise:

- a) a VH domain which comprises the sequence shown as SEQ ID No: 7, or a variant thereof having at least 80% sequence identity; and
- 30 b) a VL domain which comprises the sequence shown as SEQ ID No: 8, or a variant thereof having at least 80% sequence identity.

The VH and VL domain may be joined by a linker, such as to form an scFv. The linker may comprise the sequence GGGGSGGGGSGGGGS (SEQ ID No: 32).

The anti-TRBC2 antigen-binding domain may comprise an scFv having the amino acid sequence shown as SEQ ID No: 9. The CDR sequences are underlined.

5 SEQ ID No: 9 (scFv)

QVQLVQSGAEVKKPGASVKVSCKASGYKFTGFVMHWWRQAPGQGLEWMGFINPYNDDIQ
 SNERFRGRVTMTRDTSISTAYMELSRLRSDDTAVYYCARGNGYNFDGAYRFFDFWGQGT
 MVTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTGPGEPAISCRSSQRLVHSNGNTYL
 HWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTH
 10 VPYTFGQGTKLEIKR

The anti-TRBC2 antigen-binding domain may comprise the sequence shown as SEQ ID No: 9, or a variant thereof having at least 80% sequence identity.

The anti-TRBC2 antigen-binding domain may consist of the sequence shown as SEQ ID No: 9, or a variant thereof having at least 80% sequence identity.

15 A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID No: 7, 8, or 9, provided that the antigen-binding domain effectively binds TRBC2. For example, the variant antigen-binding domain may bind TRBC2 with essentially the same affinity and/or specificity as an antigen-binding domain having the sequence set out as SEQ ID No: 9.

20 The anti-TRBC2 antigen-binding domain may consist of the sequence shown as SEQ ID No: 9.

In some embodiments, the anti-TRBC2 antigen-binding domain comprises:

- a) a VH domain which comprises the sequence shown as SEQ ID No: 12, and
- b) a VL domain which comprises the sequence shown as SEQ ID No: 8,

25 wherein the VH domain comprises the mutations T28K, Y32F and A100N, and further comprises at least one mutation selected from:

a) in the VH domain:

- V2K, V2R,
- Y27F, Y27M, Y27N, Y27W,
- G31K, G31R, G31SR98K,
- Y102F, Y102L,

30

- N103A, N103E, N103F, N103H, N103L, N103M, N103Q, N103S, N103W, N103Y,
- A107S,

and

5 b) in the VL domain:

- N35M, N35F, N35Y, N35K, N35R, and
- R55K.

Suitably, a T28K mutation as described herein may be substituted with a T28R mutation.

SIGNAL PEPTIDE

10 The CAR of the present invention may comprise a signal peptide so that when the CAR is expressed inside a cell, such as a T-cell, the nascent protein is directed to the endoplasmic reticulum and subsequently to the cell surface, where it is expressed.

15 The core of the signal peptide may contain a long stretch of hydrophobic amino acids that has a tendency to form a single alpha-helix. The signal peptide may begin with a short positively charged stretch of amino acids, which helps to enforce proper topology of the polypeptide during translocation. At the end of the signal peptide there is typically a stretch of amino acids that is recognised and cleaved by signal peptidase. Signal peptidase may cleave either during or after completion of translocation to generate a free signal peptide and a mature protein. The free signal peptides are then digested by specific proteases.

20 The signal peptide may be at the amino terminus of the molecule.

The signal peptide may comprise the SEQ ID No: 33 to 36 or a variant thereof having 5, 4, 3, 2 or 1 amino acid mutations (insertions, substitutions or additions) provided that the signal peptide still functions to cause cell surface expression of the protein.

SEQ ID No: 33: MGWSCIIILFLVATATGVHS

25 The signal peptide of SEQ ID No: 33 is derived from Mouse Ig heavy chain.

SEQ ID No: 34: MGTSLLCWMALCLLGADHADG

The signal peptide of SEQ ID No: 34 is compact and highly efficient. It is predicted to give about 95% cleavage after the terminal glycine, giving efficient removal by signal peptidase.

SEQ ID No: 35: MSLPVTALLLPLALLLHAARP

The signal peptide of SEQ ID No: 35 is derived from IgG1.

SEQ ID No: 36: MAVPTQVLGLLLLWLT DARC

The signal peptide of SEQ ID No: 36 is derived from CD8.

In some embodiments, the CAR comprises the signal peptide SEQ ID No: 33.

5 SPACER DOMAIN

CARs comprise a spacer sequence to connect the antigen-binding domain with the transmembrane domain and spatially separate the antigen-binding domain from the endodomain. A flexible spacer allows the antigen-binding domain to orient in different directions to facilitate binding.

- 10 In the CAR of the present invention, the spacer sequence may, for example, comprise an IgG1 hinge, a human CD8 stalk or the mouse CD8 stalk, or a human CD28 stalk or the mouse CD28 stalk. The spacer may alternatively comprise an alternative linker sequence which has similar length and/or domain spacing properties as an IgG1 hinge or a CD8 or CD28 stalk.

- 15 The CAR of the present invention may comprise a sequence selected from the sequences shown as SEQ ID Nos: 14 to 16, or a variant thereof having at least 80% sequence identity.

SEQ ID No: 14 (human IgG1 hinge)

EPKSPDKTHTCPPCP

SEQ ID No: 15 (human CD8 stalk)

TTTPAPRPPTPAPTASQPLSLRPEACRPAAGGAVHTRGLDFACDI

- 20 SEQ ID No: 16 (human CD28 stalk)

KIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKP

In some embodiments, the CAR comprises the spacer domain SEQ ID No: 14, or a variant thereof having at least 80% sequence identity.

- 25 In some embodiments, the CAR comprises the spacer domain SEQ ID No: 15, or a variant thereof having at least 80% sequence identity.

In some embodiments, the CAR comprises the spacer domain SEQ ID No: 16, or a variant thereof having at least 80% sequence identity.

In some embodiments, the CAR comprises the spacer domain SEQ ID No: 14.

In some embodiments, the CAR comprises the spacer domain SEQ ID No: 15.

In some embodiments, the CAR comprises the spacer domain SEQ ID No: 16.

A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of SEQ ID No: 14 to 16, provided that the spacer domain is able to orient the antigen-binding domain to facilitate binding.

5 TRANSMEMBRANE DOMAIN

The transmembrane domain is the sequence of the CAR that spans the membrane.

A transmembrane domain may be any protein structure which is thermodynamically stable in a membrane. This is typically an alpha helix comprising of several hydrophobic residues. The transmembrane domain of any transmembrane protein can be used to supply the transmembrane portion of the invention. The presence and span of a transmembrane domain of a protein can be determined by those skilled in the art using the TMHMM algorithm (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>). Further, given that the transmembrane domain of a protein is a relatively simple structure, i.e. a polypeptide sequence predicted to form a hydrophobic alpha helix of sufficient length to span the membrane, an artificially designed TM domain may also be used (US 7052906 B1 describes synthetic transmembrane components).

The transmembrane domain may be derived from CD28 or TYRP-1, which give good receptor stability.

The CAR of the present invention may comprise a sequence selected from the sequences shown as SEQ ID NOs: 17 and 18, or a variant thereof having at least 80% sequence identity.

20 SEQ ID No: 17 (TYRP-1 transmembrane domain)

IIAIAVVGALLLVALIFGTASYLI

SEQ ID No: 18 (CD28 transmembrane domain)

FWWLVVGGVLACYSLVTVAFIIFWV

25 In some embodiments, the CAR comprises the transmembrane domain SEQ ID No: 17, or a variant thereof having at least 80% sequence identity.

In some embodiments, the CAR comprises the transmembrane domain SEQ ID No: 18, or a variant thereof having at least 80% sequence identity.

In some embodiments, the CAR comprises the transmembrane domain SEQ ID No: 17.

In some embodiments, the CAR comprises the transmembrane domain SEQ ID No: 18.

A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID No: 17 and 18, provided that the transmembrane domain is thermodynamically stable in a membrane.

ENDODOMAIN

- 5 The endodomain is the signal-transmission portion of the CAR. After antigen recognition, receptors cluster, native CD45 and CD148 are excluded from the synapse and a signal is transmitted to the cell. The most commonly used endodomain component is that of CD3ζ which contains 3 ITAMs. This transmits an activation signal to the T cell after antigen is bound. CD3ζ may not provide a fully competent activation signal and additional co-stimulatory
- 10 signalling may be needed. Examples of co-stimulatory domains include the endodomains from CD28, OX40, 4-1BB, CD27, and ICOS, which can be used with CD3ζ to transmit a proliferative/survival signal.

In an embodiment, at least one co-stimulatory endodomain is used with CD3ζ. In a particular embodiment, the co-stimulatory endodomain is selected from the group consisting of the

15 endodomains from CD28 and 4-1BB.

SEQ ID No: 37 (CD28 endodomain)

RSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRS

SEQ ID No: 38 (4-1BB endodomain)

KRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCEL

- 20 Other endodomains are known in the art, for example, OX40, CD27, and ICOS.

In another embodiment, at least two co-stimulatory endodomains are used with CD3ζ. In a particular embodiment, the two co-stimulatory endodomain are selected from the group consisting of the endodomains from CD28, OX40, 4-1BB, CD27, and ICOS, in any combination and order. Particularly suitable combinations include the endodomains from

25 CD28 and CD3ζ, the endodomains of 4-1BB and CD3ζ, the endodomains from CD28, OX40 and CD3ζ, and the endodomains from CD28, 4-1BB and CD3ζ.

SEQ ID No: 39 (OX40 endodomain)

RDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI

SEQ ID No: 40 (CD3ζ endodomain)

- 30 RRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

In some embodiments, the CAR has an intracellular T-cell signalling domain (endodomain) comprising the sequence shown as SEQ ID No: 19, 20 or 21, or a variant thereof having at least 80% sequence identity.

SEQ ID No: 19 (41BB-CD3zeta endodomain)

5 KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRRVKFSRSADAPAYQQGQ
NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No: 20 (CD28-CD3zeta endodomain)

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQN
10 QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMK
GERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No: 21 (CD28-CD3zeta endodomain)

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRRVKFSRSADAPAYQQGQ
NQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGM
15 KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

In some embodiments, the CAR has an intracellular T-cell signalling domain (endodomain) comprising the sequence shown as SEQ ID No: 19, or a variant thereof having at least 80% sequence identity.

In some embodiments, the CAR has an intracellular T-cell signalling domain (endodomain)
20 comprising the sequence shown as SEQ ID No: 20, or a variant thereof having at least 80% sequence identity.

In some embodiments, the CAR has an intracellular T-cell signalling domain (endodomain) comprising the sequence shown as SEQ ID No: 21, or a variant thereof having at least 80% sequence identity.

25 A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to any one of SEQ ID No: 37 to 40 and 19 to 21, provided that the sequence provides an effective intracellular T cell signalling domain.

In some embodiments, the CAR has a transmembrane and intracellular T-cell signalling domain (endodomain) comprising the sequence shown as SEQ ID NO: 43 to 45, or a variant
30 thereof having at least 80% sequence identity.

SEQ ID No: 43 (comprising CD28 transmembrane domain and CD28 and CD3 ζ endodomains)

FWVLVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPR
DFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPP
R

- 5 SEQ ID No: 44 (comprising TYRP-1 transmembrane domain and 4-1BB and CD3ζ endodomains)

IIAIAVVGALLLVALIFGTASYLIKRGKRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGC
ELRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQE
GLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

- 10 SEQ ID No: 45 (comprising TYRP-1 transmembrane domain and CD28 and CD3ζ endodomains)

IIAIAVVGALLLVALIFGTASYLIRSKRSRLLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAY
RSRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPR

- 15 A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 43 to 45, provided that the sequence provides an effective transmembrane domain and an effective intracellular T cell signalling domain.

The person skilled in the art would understand that linkers may be used between some or all of the active domains. These are known in the art. Examples include:

- 20 Linker: SDPA (SEQ ID No: 41)

Linker: SDP

Linker: KDPK (SEQ ID No: 42)

In the first aspect of the invention, the CAR has the general structure:

- 25 Anti-TRBC2 antigen-binding domain – CD28 stalk – CD28 transmembrane domain –
CD28-CD3zeta endodomain

Suitably, the present invention further provides a CAR with the following general structure:

Anti-TRBC2 antigen-binding domain – IgG1 hinge domain – TYRP-1 transmembrane domain – CD28-CD3zeta endodomain

Suitably, the present invention also provides a CAR with the following general structure:

Anti-TRBC2 antigen-binding domain – CD8 stalk – TYRP-1 transmembrane domain – CD28-CD3zeta endodomain

The CAR may comprise an amino acid sequence selected from the group consisting of SEQ ID No: 22, 23 and 24, or a variant with at least 80% sequence identity.

5 SEQ ID No: 22 (KFN-cd28stk-CD28TM-CD28z)

MGWSCIIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYKFTGFVMHWVRQAP
GQGLEWMGFINPYNDDIQSNERFRGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARGNG
YNFDGAYRFFDFWGQGTMTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTGPGEPA
10 SCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISR
VEAEDVGVYYCSQSTHVPYTFGQGTKLEIKRSDPAKIEVMYPPPYLDNEKSNGTIIHVKGKH
LCPSPLFPGPSKPFWWLVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPG
PTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPR

15 SEQ ID No: 23 (KFN-hinge-TyrpTM-CD28z)

MGWSCIIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYKFTGFVMHWVRQAP
GQGLEWMGFINPYNDDIQSNERFRGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARGNG
YNFDGAYRFFDFWGQGTMTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTGPGEPA
20 SCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISR
VEAEDVGVYYCSQSTHVPYTFGQGTKLEIKRSDPAEPKSPDKTHTCPPCPKDPKIIAIAVVG
ALLLVALIFGTASYLIRSKRSRLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRRVKF
SRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No: 24 (KFN-CD8stk-TyrpTM-CD28z)

25 MGWSCIIILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYKFTGFVMHWVRQAP
GQGLEWMGFINPYNDDIQSNERFRGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCARGNG
YNFDGAYRFFDFWGQGTMTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTGPGEPA
SCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISR
VEAEDVGVYYCSQSTHVPYTFGQGTKLEIKRSDPTTTPAPRPPTPAPTASQPLSLRPEACR
30 PAAGGAVHTRGLDFACDIIIAIAVVGALLLVALIFGTASYLIRSKRSRLHSDYMNMTPRRPGP
TRKHYQPYAPPRDFAAYRSRRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPR

In some embodiments, the CAR comprises or consists of SEQ ID No: 22, or a variant with at least 80% sequence identity. For example, the CAR may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 22.

5 In some embodiments, the CAR comprises or consists of SEQ ID No: 23, or a variant with at least 80% sequence identity.

In some embodiments, the CAR comprises or consists of SEQ ID No: 24, or a variant with at least 80% sequence identity.

In some embodiments, the CAR comprises or consists of SEQ ID No: 22.

In some embodiments the CAR comprises SEQ ID No: 22.

10 In some embodiments, the CAR consists of SEQ ID No: 22.

In some embodiments, the CAR comprises or consists of SEQ ID No: 23.

In some embodiments, the CAR comprises or consists of SEQ ID No: 24.

A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 22 to 24, provided that the CAR is able to i) bind TRBC2 and
15 ii) induce T cell signalling.

In some embodiments, the CAR has the general structure:

Anti-TRBC1 antigen-binding domain – IgG1 hinge domain – TYRP-1 transmembrane domain – 41BB-CD3zeta endodomain

In some embodiments, the CAR has the general structure:

20 Anti-TRBC1 antigen-binding domain – CD8 stalk – TYRP-1 transmembrane domain – CD28-CD3zeta endodomain

In some embodiments, the CAR has the general structure:

Anti-TRBC1 antigen-binding domain – CD28 stalk – CD28 transmembrane domain – CD28-CD3zeta endodomain

25 The CAR may comprise an amino acid sequence selected from the group consisting of SEQ ID No: 25, 26 and 27, or a variant with at least 80% sequence identity.

SEQ ID No: 25 (Jovi1-hinge-TyrpTM-41BBz)

MGWSCILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWVRQAP
 GQGLEWMGFINPYNDDIQSNERFRGRVTMTRDTSISTAYMELSRRLSDDTAVYYCARGAG
 YNFDGAYRFFDFWQGQTMVTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTGPGEPA
 SCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISR
 5 VEAEDVGVYYCSQSTHVPYTFGQGTKLEIKRSDPAEPKSPDKTHTCPPCPKDPKIIAIAVVG
 ALLLVALIFGTASYLIKRGGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELRRVK
 FSRADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNEL
 QKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

SEQ ID No: 26 (Jovi1-CD8stk-TyrpTM-CD28z)

10 MGWSCILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWVRQAP
 GQGLEWMGFINPYNDDIQSNERFRGRVTMTRDTSISTAYMELSRRLSDDTAVYYCARGAG
 YNFDGAYRFFDFWQGQTMVTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTGPGEPA
 SCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISR
 VEAEDVGVYYCSQSTHVPYTFGQGTKLEIKRSDPTTTPAPRPPTPAPTASQPLSLRPEACR
 15 PAAGGAVHTRGLDFACDIIIAIAVVGALLLVALIFGTASYLIRSKRSRLHSDYMNMTPRRPGP
 TRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
 GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
 DTYDALHMQALPPR

SEQ ID No: 27 (Jovi1-cd28stk-CD28TM-CD28z)

20 MGWSCILFLVATATGVHSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYVMHWVRQAP
 GQGLEWMGFINPYNDDIQSNERFRGRVTMTRDTSISTAYMELSRRLSDDTAVYYCARGAG
 YNFDGAYRFFDFWQGQTMVTVSSGGGGSGGGGSGGGGSDIVMTQSPLSLPVTGPGEPA
 SCRSSQRLVHSNGNTYLHWYLQKPGQSPRLLIYRVSNRFPGVDPDRFSGSGSGTDFTLKISR
 VEAEDVGVYYCSQSTHVPYTFGQGTKLEIKRSDPAKIEVMYPPPYLDNEKSNGTIIHVKGKH
 25 LCPSPLFPGPSKPFWWLVVGGVLACYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPG
 PTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRR
 GRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
 DTYDALHMQALPPR

The CAR may comprise an amino acid sequence selected from the group consisting of SEQ
 30 ID No: 26 and 27, or a variant with at least 80% sequence identity.

In some embodiments, the CAR comprises or consists of SEQ ID No: 25, or a variant with at
 least 80% sequence identity. For example, the CAR may have at least 80%, 85%, 90%, 95%,
 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 25.

In some embodiments, the CAR comprises or consists of SEQ ID No: 26, or a variant with at least 80% sequence identity.

In some embodiments, the CAR comprises or consists of SEQ ID No: 27, or a variant with at least 80% sequence identity.

- 5 In some embodiments, the CAR comprises or consists of SEQ ID No: 25.

In some embodiments the CAR comprises SEQ ID No: 25.

In some embodiments, the CAR consists of SEQ ID No: 25.

In some embodiments, the CAR comprises or consists of SEQ ID No: 26.

In some embodiments, the CAR comprises or consists of SEQ ID No: 27.

- 10 A variant sequence may have at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO: 25 to 27, provided that the CAR is able to i) bind TRBC1 and ii) induce T cell signalling.

Variants of the above amino acid sequences may also be used in the present invention, provided that the resulting CAR binds TRBC1 or TRBC2 and does not significantly cross-react.

- 15 Typically, such variants have a high degree of sequence identity with one of the sequences specified above.

Methods of alignment of sequences for comparison are well known in the art.

- The NCBI Basic Local Alignment Search Tool (BLAST) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, Md.) and on the internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

- Typically, variants may contain one or more conservative amino acid substitutions compared to the original amino acid or nucleic acid sequence. Conservative substitutions are those substitutions that do not substantially affect or decrease the affinity of a CAR to bind TRBC1 or TRBC2.

- Functionally similar amino acids which may be exchanged by way of conservative substitution are well known to one of ordinary skill in the art. The following six groups are examples of amino acids that are considered to be conservative substitutions for one another: 1) Alanine (A), Serine (S), Threonine (T); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N),

Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

NUCLEIC ACID

The present invention further provides a nucleic acid encoding a CAR as defined above.

- 5 As used herein, the terms “polynucleotide”, “nucleotide”, and “nucleic acid” are intended to be synonymous with each other.

It will be understood by a skilled person that numerous different polynucleotides and nucleic acids can encode the same polypeptide as a result of the degeneracy of the genetic code. In addition, it is to be understood that skilled persons may, using routine techniques, make
10 nucleotide substitutions that do not affect the polypeptide sequence encoded by the polynucleotides described here to reflect the codon usage of any particular host organism in which the polypeptides are to be expressed.

Nucleic acids according to the invention may comprise DNA or RNA. They may be single-stranded or double-stranded. They may also be polynucleotides which include within them
15 synthetic or modified nucleotides. A number of different types of modification to oligonucleotides are known in the art. These include methylphosphonate and phosphorothioate backbones, addition of acridine or polylysine chains at the 3' and/or 5' ends of the molecule. For the purposes of the use as described herein, it is to be understood that the polynucleotides may be modified by any method available in the art. Such modifications
20 may be carried out in order to enhance the in vivo activity or life span of polynucleotides of interest.

The terms “variant”, “homologue” or “derivative” in relation to a nucleotide sequence include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence.

- 25 The present invention also provides a nucleic acid construct which comprises a first nucleic acid encoding a CAR as defined above; and a second nucleic acid encoding a suicide gene.

Suitable suicide genes for use in a CAR-expressing cell of the invention include RQR8, which is described in WO2013/153391; and RapCasp9, which is described in WO2016/135470.

In the nucleic acid construct described above, the first and second nucleic acid sequences
30 may be in either order.

VECTOR

The present invention also provides a vector, or kit of vectors, which comprises one or more nucleic acid sequence(s) or nucleic acid construct(s) of the invention. Such a vector may be used to introduce the nucleic acid sequence(s) or construct(s) into a host cell, for example, so that it expresses a CAR having an antigen-binding domain according to the invention.

- 5 The vector may, for example, be a plasmid or a viral vector, such as a retroviral vector or a lentiviral vector, or a transposon-based vector or synthetic mRNA.

The vector may be capable of transfecting or transducing a T cell or a NK cell.

CELL

- 10 The present invention also relates to a cell, such as an immune cell, comprising a CAR according to the invention.

The present invention also provides a composition comprising a plurality of cells according to the invention.

The cell(s) may comprise a nucleic acid, a nucleic acid construct or a vector of the present invention.

- 15 The cell(s) may be a cytolytic immune cell, such as a T-cell or a natural killer (NK) cell.

T cell may be T cells or T lymphocytes which are a type of lymphocyte that play a central role in cell-mediated immunity. They can be distinguished from other lymphocytes, such as B cells and natural killer cells (NK cells), by the presence of a T-cell receptor (TCR) on the cell surface. There are various types of T cell, as summarised below.

- 20 Helper T helper cells (TH cells) assist other white blood cells in immunologic processes, including maturation of B cells into plasma cells and memory B cells, and activation of cytotoxic T cells and macrophages. TH cells express CD4 on their surface. TH cells become activated when they are presented with peptide antigens by MHC class II molecules on the surface of antigen presenting cells (APCs). These cells can differentiate into one of several subtypes,
25 including TH1, TH2, TH3, TH17, Th9, or TFH, which secrete different cytokines to facilitate different types of immune responses.

- Cytolytic T cells (TC cells, or CTLs) destroy virally infected cells and tumor cells, and are also implicated in transplant rejection. CTLs express the CD8 at their surface. These cells recognize their targets by binding to antigen associated with MHC class I, which is present on
30 the surface of all nucleated cells. Through IL-10, adenosine and other molecules secreted by

regulatory T cells, the CD8⁺ cells can be inactivated to an anergic state, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis.

Memory T cells are a subset of antigen-specific T cells that persist long-term after an infection has resolved. They quickly expand to large numbers of effector T cells upon re-exposure to their cognate antigen, thus providing the immune system with “memory” against past infections. Memory T cells comprise three subtypes: central memory T cells (TCM cells) and two types of effector memory T cells (TEM cells and TEMRA cells). Memory cells may be either CD4⁺ or CD8⁺. Memory T cells typically express the cell surface protein CD45RO.

Regulatory T cells (Treg cells), formerly known as suppressor T cells, are crucial for the maintenance of immunological tolerance. Their major role is to shut down T cell mediated immunity toward the end of an immune reaction and to suppress autoreactive T cells that escaped the process of negative selection in the thymus.

Two major classes of CD4⁺ Treg cells have been described – naturally occurring Treg cells and adaptive Treg cells.

Naturally occurring Treg cells (also known as CD4⁺CD25⁺FoxP3⁺ Treg cells) arise in the thymus and have been linked to interactions between developing T cells with both myeloid (CD11c⁺) and plasmacytoid (CD123⁺) dendritic cells that have been activated with TSLP. Naturally occurring Treg cells can be distinguished from other T cells by the presence of an intracellular molecule called FoxP3. Mutations of the FOXP3 gene can prevent regulatory T cell development, causing the fatal autoimmune disease IPEX.

Adaptive Treg cells (also known as Tr1 cells or Th3 cells) may originate during a normal immune response.

Gamma delta T cells ($\gamma\delta$ T cells) are T cells that have a TCR that comprised of one γ (gamma) chain and one δ (delta) chain. Gamma delta T cells are typically less common than $\alpha\beta$ T cells. In humans, in 95% of T cells the TCR consists of an alpha (α) chain and a beta (β) chain (encoded by TRA and TRB, respectively). However, in about 5% of T cells the TCR consists of gamma and delta (γ/δ) chains (encoded by TRG and TRD, respectively). Gamma delta T cells are abundant in the gut mucosa. Examples of gamma delta cells include V γ 9V δ 2 T cells.

$\gamma\delta$ TCRs are MHC independent and may detect markers of cellular stress expressed by tumours. The $\gamma\delta$ TCR may be capable of binding to a phosphoantigen/butyrophilin 3A1 complex; major histocompatibility complex class I chain-related A (MICA); major histocompatibility complex class I chain-related B (MICB); NKG2D ligand 1-6 (ULBP 1-6);

CD1c; CD1d; endothelial protein C receptor (EPCR); lipohexapeptides; phycoreythrins or histidyl-tRNA-synthase.

Natural killer T (NKT) cells are a heterogeneous group of T cells that share properties of both T cells and natural killer cells. Many of these cells recognize the non-polymorphic CD1d molecule, an antigen-presenting molecule that binds self and foreign lipids and glycolipids.

Invariant natural killer T (iNKT) cells, also known as type I or classical NKT cells, are a distinct population of T cells that express an invariant $\alpha\beta$ T-cell receptor (TCR) and a number of cell surface molecules in common with natural killer (NK) cells. NKT cells express a restricted TCR repertoire that, in humans, is composed of a $V\alpha 24$ - $J\alpha 18$ TCR α chain preferentially coupled with a $V\beta 11$ TCR β chain. Unlike conventional T cells, which mostly recognise peptide antigens presented by MHC molecules, iNKT cells recognise glycolipid antigens presented by the non-polymorphic MHC class I-like molecule, CD1d.

The cell of the invention may be any of the T cell types mentioned above.

The cell may be a Natural Killer cell (or NK cell). NK cells are a type of cytolytic cell that form part of the innate immune system. NK cells provide rapid responses to innate signals from virally infected cells in an MHC independent manner.

NK cells (belonging to the group of innate lymphoid cells) are defined as large granular lymphocytes (LGL) and constitute the third kind of cells differentiated from the common lymphoid progenitor generating B and T lymphocytes. NK cells are known to differentiate and mature in the bone marrow, lymph node, spleen, tonsils and thymus where they then enter into the circulation.

The cells of the invention may be any of the cell types mentioned above. In an embodiment, the cell of the invention is a T cell. In another embodiment, the cell of the invention is an NK cell.

Cells according to the invention may either be created *ex vivo* either from a patient's own peripheral blood (1st party), or in the setting of a haematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an unconnected donor (3rd party).

Alternatively, cells expressing a CAR according to the invention may be derived from *ex vivo* differentiation of inducible progenitor cells or embryonic progenitor cells to cytolytic cells.

Alternatively, an immortalized T-cell line which retains its lytic function and could act as a therapeutic may be used.

In all these embodiments, CAR cells are generated by introducing DNA or RNA coding for the CAR by one of many means including transduction with a viral vector, transfection with DNA or RNA.

5 The CAR-expressing cell of the invention may be an *ex vivo* cell from a subject. The cell may be from a peripheral blood mononuclear cell (PBMC) sample. The cell, in particular a cytolytic cell, may be activated and/or expanded prior to being transduced with nucleic acid encoding a CAR according to the invention, for example by treatment with an anti-CD3 monoclonal antibody.

10 The cell of the invention may be made by a method which comprises a step of transducing or transfecting a cell with a nucleic acid encoding the CAR or vector which comprises a nucleic acid sequence encoding the CAR.

The cell composition of the invention may be made by a method which comprises a step of transducing or transfecting a sample of cells with a nucleic acid encoding the CAR or vector which comprises a nucleic acid sequence encoding the CAR.

15 The method for making a cell or cell composition of the invention may further comprise a step of isolating the cell(s) from a cell-containing sample from a subject or from other sources listed above, prior to the transduction or transfection step. Where the cell is a cytolytic cell, the sample is a cytolytic cell-containing sample from the subject. The cell or cell composition may be isolated from a cell-containing sample on the basis of its expression of TRBC1 or TRBC2.
20 For example, a TRBC1-positive T cell depletion step at the beginning of the manufacturing process may be used to isolate TRBC2-positive cells for expression of an anti-TRBC1 CAR. Conversely, a TRBC2-positive T cell depletion step at the beginning of the manufacturing process may be used to isolate TRBC1-positive cells for expression of an anti-TRBC2 CAR.

25 The cells may then be purified, for example, selected on the basis of expression of the antigen-binding domain of the antigen-binding polypeptide.

In one embodiment, the cell comprising an anti-TRBC2 CAR according to the invention is a TRBC1+ cell.

In one embodiment, the cell comprising an anti-TRBC1 CAR according to the invention is a TRBC2+ cell.

30 The term “subject” or “individual”, as used in the context of the present invention, refers to members of mammalian species, preferably a male or female human being of any age or race.

The present invention also provides a kit which comprises a cell comprising a CAR according to the invention.

PHARMACEUTICAL COMPOSITION

The present invention also relates to a pharmaceutical composition containing a therapeutic
5 entity such as a nucleic acid, vector, CAR-expressing cell, or plurality of cells of the present invention.

The pharmaceutical composition may additionally comprise a pharmaceutically acceptable carrier, diluent or excipient. The pharmaceutical composition may optionally comprise one or more further pharmaceutically active polypeptides and/or compounds. Such a formulation
10 may, for example, be in a form suitable for intravenous infusion.

The present invention provides a pharmaceutical composition for use as a medicament.

The method of the present invention may comprise the step of administering the therapeutic entity in the form of a pharmaceutical composition. The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and
15 standard pharmaceutical practice. The pharmaceutical compositions may comprise as (or in addition to) the carrier, excipient or diluent, any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilising agent(s), and other carrier agents.

T-CELL LYMPHOMA AND/OR LEUKAEMIA

The present invention provides a nucleic acid, a vector, a cell, or a composition of the invention
20 for use in medicine.

The present invention provides a method for treating a T-cell lymphoma or leukaemia in a subject, comprising the step of administering nucleic acid, a vector, a cell, or a composition of the invention to a subject, wherein the malignant T-cells express either TRBC1 or TRBC2. The administration step may be in the form of a pharmaceutical composition as described
25 above.

The invention provides a nucleic acid, a vector, a cell, or a composition of the invention for use in the treatment of a T-cell lymphoma or leukaemia, wherein the malignant T-cells express either TRBC1 or TRBC2.

The invention provides the use of nucleic acid, a vector, a cell, or a composition of the invention
30 in the manufacture of a medicament for treating a T-cell lymphoma or leukaemia, wherein the malignant T-cells express either TRBC1 or TRBC2.

A method for treating a T-cell lymphoma and/or leukaemia relates to the therapeutic use of the nucleic acid, vector, cell, or composition of the invention. Herein the nucleic acid, vector, cell, or composition of the invention may be administered to a subject having an existing T-cell lymphoma and/or leukaemia in order to lessen, reduce or improve at least one symptom associated with the disease and/or to slow down, reduce or block the progression of the disease.

The method for preventing a T-cell lymphoma and/or leukaemia relates to the prophylactic use of the nucleic acid, vector, cell, or composition of the present invention. Herein such nucleic acid, vector, cell, or composition may be administered to a subject who has not yet contracted the T-cell lymphoma and/or leukaemia and/or who is not showing any symptoms of the T-cell lymphoma and/or leukaemia to prevent or impair the cause of the disease or to reduce or prevent development of at least one symptom associated with the disease. The subject may have a predisposition for, or be thought to be at risk of developing, the T-cell lymphoma and/or leukaemia.

The method may involve the steps of:

- (i) isolating a cytotoxic cell-containing sample;
- (ii) transducing or transfecting such cell with a nucleic acid sequence or vector provided by the present invention; and
- (iii) administering the cell from (ii) to a subject.

The cytotoxic cell-containing sample may be isolated from the subject or from other sources, for example as described above. The cytotoxic cell, such as a T or NK, may be isolated from a subject's own peripheral blood (1st party), or in the setting of a haematopoietic stem cell transplant from donor peripheral blood (2nd party), or peripheral blood from an unconnected donor (3rd party).

These therapeutic applications will comprise the administration of a therapeutically effective amount of the nucleic acid, vector, cell, or composition of the present invention.

The term "therapeutically effective amount", as used herein, refers to the amount of the nucleic acid, vector, cell, or composition of the present invention which is required to achieve an appreciable prevention, cure, delay, reduction of the severity of, or amelioration of one or more symptoms of either a TRBC1 or a TRBC2 positive T-cell lymphoma and/or leukaemia.

The method of the present invention may be used for the treatment of any lymphoma and/or leukaemia associated with the clonal expansion of a cell expressing a T-cell receptor (TCR)

comprising TRBC1. As such the present invention relates to a method for treating a disease which involves malignant T cells which express a TCR comprising a TRBC1.

The method of the present invention may be used for the treatment of any lymphoma and/or leukaemia associated with the clonal expansion of a cell expressing a T-cell receptor (TCR) comprising TRBC2. As such the present invention relates to a method for treating a disease which involves malignant T cells which express a TCR comprising a TRBC2.

The term 'malignant' is used herein according to its standard meaning to refer to a cell which is not self-limited in its growth, may be capable of invading into adjacent tissues and may be capable of spreading to distant tissue. As such the term 'malignant T cell' is used herein to refer to a clonally expanded T cell in the context of a lymphoma or leukaemia.

As stated above, each $\alpha\beta$ T-cell expresses a TCR which comprises either TRBC1 or TRBC2. In a clonal T-cell disorder, such as a T-cell lymphoma or leukaemia, malignant T-cells derived from the same clone will all express either TRBC1 or TRBC2.

Methods to determine whether a T cell malignancy is TRBC1- or TRBC2-positive are known by the skilled person and include polymerase chain reaction (PCR), sequencing, next-generation sequencing (NGS), Western blotting, flow cytometry, fluorescent microscopy, and immunohistochemistry (IHC).

Once the TRBC expressed by a malignant T-cell has been determined, the appropriate TRBC1 or TRBC2 selective agent (i.e. nucleic acid, vector, cell, or composition according to the invention) is administered to the subject. The 'appropriate TRBC selective agent' means that where the malignant T-cell is determined to express TRBC1, a TRBC1 selective agent is administered, whereas where the malignant T-cell is determined to express TRBC2, a TRBC2 selective agent is administered.

Thus the present method comprises the step of administering a TRBC1 or TRBC2 selective agent to the subject, wherein the agent causes selective depletion of the malignant T-cells, together with normal T-cells which express the same TRBC as the malignant T-cells, but does not cause significant depletion of normal T-cells expressing the other TRBC from the malignant T-cells.

Because the TRBC selective agent does not cause significant depletion of normal T-cells expressing the other TRBC from the malignant T-cells it does not cause depletion of the entire T-cell compartment. Retention of a proportion of the subject's T-cell compartment (i.e. T-cells which do not express the same TRBC as the malignant T-cell) results in reduced toxicity and reduced cellular and humoral immunodeficiency, thereby reducing the risk of infection.

Administration of a TRBC1 selective agent according to the method of the present invention may result in a 5, 10, 20, 50, 75, 90, 95 or 99% depletion, i.e. reduction in the number of T-cells expressing TRBC1.

5 Administration of a TRBC2 selective agent according to the method of the present invention may result in a 5, 10, 20, 50, 75, 90, 95 or 99% depletion, i.e. reduction in the number of T-cells expressing TRBC2.

10 The method of the present invention may be used to treat a T-cell lymphoma in which the malignant T-cell expresses a TCR comprising either TRBC1 or TRBC2. "Lymphoma" is used herein according to its standard meaning to refer to a cancer which typically develops in the lymph nodes, but may also affect the spleen, bone marrow, blood and other organs. Lymphoma typically presents as a solid tumour of lymphoid cells. The primary symptom associated with lymphoma is lymphadenopathy, although secondary (B) symptoms can include fever, night sweats, weight loss, loss of appetite, fatigue, respiratory distress and itching.

15 The method of the present invention may be used to treat a T-cell leukaemia in which the malignant T-cell expresses a TCR comprising either TRBC1 or TRBC2. "Leukaemia" is used herein according to its standard meaning to refer to a cancer of the blood or bone marrow.

The following is an illustrative, non-exhaustive list of diseases which may be treated by the method of the present invention.

20 PERIPHERAL T-CELL LYMPHOMA

Peripheral T-cell lymphomas are relatively uncommon lymphomas and account fewer than 10% of all non-Hodgkin lymphomas (NHL). However, they are associated with an aggressive clinical course and the causes and precise cellular origins of most T-cell lymphomas are still not well defined.

25 Lymphoma usually first presents as swelling in the neck, underarm or groin. Additional swelling may occur where other lymph nodes are located such as in the spleen. In general, enlarged lymph nodes can encroach on the space of blood vessels, nerves, or the stomach, leading to swollen arms and legs, to tingling and numbness, or to feelings of being full, respectively. Lymphoma symptoms also include nonspecific symptoms such as fever, chills, unexplained
30 weight loss, night sweats, lethargy, and itching.

The WHO classification utilizes morphologic and immunophenotypic features in conjunction with clinical aspects and in some instances genetics to delineate a prognostically and

therapeutically meaningful categorization for peripheral T-cell lymphomas (Swerdlow *et al.*; WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed.; Lyon: IARC Press; 2008). The anatomic localization of neoplastic T-cells parallels in part their proposed normal cellular counterparts and functions and as such T-cell lymphomas are associated with lymph nodes and peripheral blood. This approach allows for better understanding of some of the manifestations of the T-cell lymphomas, including their cellular distribution, some aspects of morphology and even associated clinical findings.

The most common of the T-cell lymphomas is peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) comprising 25% overall, followed by angioimmunoblastic T-cell lymphoma (AITL) (18.5%)

PERIPHERAL T-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED (PTCL-NOS)

PTCL-NOS comprises over 25% of all peripheral T-cell lymphomas and NK/T-cell lymphomas and is the most common subtype. It is determined by a diagnosis of exclusion, not corresponding to any of the specific mature T-cell lymphoma entities listed in the current WHO 2008. As such it is analogous to diffuse large B-cell lymphoma, not otherwise specified (DLBCL-NOS).

Most patients are adults with a median age of 60 and a male to female ratio 2:1. The majority of cases are nodal in origin, however, extranodal presentations occur in approximately 13% of patients and most commonly involve skin and gastrointestinal tract.

The cytologic spectrum is very broad, ranging from polymorphous to monomorphous. Three morphologically defined variants have been described, including lymphoepithelioid (Lennert) variant, T-zone variant and follicular variant. The lymphoepithelioid variant of PTCL contains abundant background epithelioid histiocytes and is commonly positive for CD8. It has been associated with a better prognosis. The follicular variant of PTCL-NOS is emerging as a potentially distinct clinicopathologic entity.

The majority of PTCL-NOS have a mature T-cell phenotype and most cases are CD4-positive. 75% of cases show variable loss of at least one pan T-cell marker (CD3, CD2, CD5 or CD7), with CD7 and CD5 being most often downregulated. CD30 and rarely CD15 can be expressed, with CD15 being an adverse prognostic feature. CD56 expression, although uncommon, also has negative prognostic impact. Additional adverse pathologic prognostic factors include a proliferation rate greater than 25% based on KI-67 expression, and presence of more than 70% transformed cells. Immunophenotypic analysis of these lymphomas has offered little insight into their biology.

ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA (AITL)

AITL is a systemic disease characterized by a polymorphous infiltrate involving lymph nodes, prominent high endothelial venules (HEV) and peri-vascular expansion of follicular dendritic cell (FDC) meshworks. AITL is considered as a *de-novo* T-cell lymphoma derived from $\alpha\beta$ T-cells of follicular helper type (TFH), normally found in the germinal centres.

AITL is the second most common entity among peripheral T-cell lymphoma and NK/T-cell lymphomas, comprising about 18.5% of cases. It occurs in middle aged to elderly adults, with a median age of 65 years old, and an approximately equal incidence in males and females. Clinically, patients usually have advanced stage disease, with generalized lymphadenopathy, hepatosplenomegaly and prominent constitutional symptoms. Skin rash with associated pruritus is commonly present. There is often polyclonal hypergammaglobulinemia, associated with autoimmune phenomena.

Three different morphologic patterns are described in AITL. The early lesion of AITL (Pattern I) usually shows preserved architecture with characteristic hyperplastic follicles. The neoplastic proliferation is localized to the periphery of the follicles. In Pattern II the nodal architecture is partially effaced with retention of few regressed follicles. The subcapsular sinuses are preserved and even dilated. The paracortex contains arborizing HEV and there is a proliferation of FDC beyond the B-cell follicle. The neoplastic cells are small to medium in size, with minimal cytologic atypia. They often have clear to pale cytoplasm, and may show distinct T-cell membranes. A polymorphous inflammatory background is usually evident.

Although AITL is a T-cell malignancy, there is a characteristic expansion of B-cells and plasma cells, which likely reflects the function of the neoplastic cells as TFH cells. Both EBV-positive and EBV-negative B-cells are present. Occasionally, the atypical B-cells may resemble Hodgkin/Reed–Sternberg-like cells morphologically and immunophenotypically, sometimes leading to a diagnostic confusion with that entity. The B-cell proliferation in AITL may be extensive and some patients develop secondary EBV-positive diffuse large B-cell lymphomas (DLBCL) or – more rarely – EBV-negative B-cell tumors, often with plasmacytic differentiation.

The neoplastic CD4-positive T-cells of AITL show strong expression of CD10 and CD279 (PD-1) and are positive for CXCL13. CXCL13 leads to an increased B-cell recruitment to lymph nodes via adherence to the HEV, B-cell activation, plasmacytic differentiation and expansion of the FDC meshworks, all contributing to the morphologic and clinical features of AITL. Intense PD-1-expression in the perifollicular tumor cells is particularly helpful in distinguishing AITL Pattern I from reactive follicular and paracortical hyperplasia.

The follicular variant of PTCL-NOS is another entity with a TFH phenotype. In contradistinction to AITL, it does not have prominent HEV or extra-follicular expansion of FDC meshworks. The neoplastic cells may form intrafollicular aggregates, mimicking B-cell follicular lymphoma, but also can have interfollicular growth pattern or involve expanded mantle zones. Clinically, the follicular variant of PTCL-NOS is distinct from AITL as patients more often present with early stage disease with partial lymph node involvement and may lack the constitutional symptoms associated with AITL.

ANAPLASTIC LARGE CELL LYMPHOMA (ALCL)

ALCL may be subdivided as ALCL-‘anaplastic lymphoma kinase’ (ALK)+ or ALCL-ALK-.

ALCL-ALK+ is one of the best-defined entities within the peripheral T-cell lymphomas, with characteristic “hallmark cells” bearing horseshoe-shaped nuclei and expressing ALK and CD30. It accounts for about 7% of all peripheral T-cell and NK-cell lymphomas and is most common in the first three decades of life. Patients often present with lymphadenopathy, but the involvement of extranodal sites (skin, bone, soft tissues, lung, liver) and B symptoms is common.

ALCL, ALK+ shows a wide morphologic spectrum, with 5 different patterns described, but all variants contain some hallmark cells. Hallmark cells have eccentric horseshoe- or kidney-shaped nuclei, and a prominent perinuclear eosinophilic Golgi region. The tumour cells grow in a cohesive pattern with predilection for sinus involvement. Smaller tumour cells predominate in the small cell variant, and in the lymphohistiocytic variant abundant histiocytes mask the presence of tumour cells, many of which are small.

By definition, all cases show ALK and CD30 positivity, with expression usually weaker in the smaller tumour cells. There is often loss of pan-T-cell markers, with 75% of cases lacking surface expression of CD3.

ALK expression is a result of a characteristic recurrent genetic alteration consisting of a rearrangement of *ALK* gene on chromosome 2p23 to one of the many partner genes, resulting in an expression of chimeric protein. The most common partner gene, occurring in 75% of cases, is Nucleophosmin (*NPM1*) on chromosome 5q35, resulting in t(2;5)(p23;q35). The cellular distribution of ALK in different translocation variants may vary depending on the partner gene.

ALCL-ALK- is included as a provisional category in the 2008 WHO classification. It is defined as a CD30 positive T-cell lymphoma that is morphologically indistinguishable from ALCL-ALK+ with a cohesive growth pattern and presence of hallmark cells, but lacking ALK protein

expression.

Patients are usually adults between the ages of 40 and 65, in contrast to ALCL-ALK+, which is more common in children and young adults. ALCL-ALK- can involve both lymph nodes and extranodal tissues, although the latter is seen less commonly than in ALCL-ALK+. Most cases of ALCL-ALK- demonstrate effacement of lymph node architecture by sheets of cohesive neoplastic cells with typical "hallmark" features. In contrast to the ALCL-ALK+, the small cell morphologic variant is not recognized.

Unlike its ALK+ counterpart, ALCL-ALK- shows a greater preservation of surface T-cell marker expression, while the expression of cytotoxic markers and epithelial membrane antigen (EMA) is less likely. Gene expression signatures and recurrent chromosomal imbalances are different in ALCL-ALK- and ALCL-ALK+, confirming that they are distinct entities at a molecular and genetic level.

ALCL-ALK- is clinically distinct from both ALCL-ALK+ and PTCL-NOS, with significant differences in prognosis among these three different entities. The 5 year overall survival of ALCL-ALK- is reported as 49% which is not as good as that of ALCL-ALK+ (at 70%), but at the same time it is significantly better than that of PTCL- NOS (32%).

ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA (EATL)

EATL is an aggressive neoplasm which thought to be derived from the intraepithelial T-cells of the intestine. Two morphologically, immunohistochemically and genetically distinct types of EATL are recognized in the 2008 WHO classification: Type I (representing the majority of EATL) and Type II (comprising 10–20% of cases).

Type I EATL is usually associated with overt or clinically silent gluten-sensitive enteropathy, and is more often seen in patients of Northern European extraction due to high prevalence of celiac disease in this population.

Most commonly, the lesions of EATL are found in the jejunum or ileum (90% of cases), with rare presentations in duodenum, colon, stomach, or areas outside of the gastrointestinal tract. The intestinal lesions are usually multifocal with mucosal ulceration. Clinical course of EATL is aggressive with most patients dying of disease or complications of disease within 1 year.

The cytological spectrum of EATL type I is broad, and some cases may contain anaplastic cells. There is a polymorphous inflammatory background, which may obscure the neoplastic component in some cases. The intestinal mucosa in regions adjacent to the tumour often shows features of celiac disease with blunting of the villi and increased numbers of

intraepithelial lymphocytes (IEL), which may represent lesional precursor cells.

By immunohistochemistry, the neoplastic cells are often CD3+CD4-CD8-CD7+CD5-CD56- β F1+, and contain cytotoxic granule-associated proteins (TIA-1, granzyme B, perforin). CD30 is partially expressed in almost all cases. CD103, which is a mucosal homing receptor, can be expressed in EATL.

Type II EATL, also referred to as monomorphic CD56+ intestinal T-cell lymphoma, is defined as an intestinal tumour composed of small- to medium-sized monomorphic T-cells that express both CD8 and CD56. There is often a lateral spread of tumour within the mucosa, and absence of an inflammatory background. The majority of cases express the $\gamma\delta$ TCR, however there are cases associated with the $\alpha\beta$ TCR.

Type II EATL has a more world-wide distribution than Type I EATL and is often seen in Asians or Hispanic populations, in whom celiac disease is rare. In individuals of European descent EATL, II represents about 20% of intestinal T-cell lymphomas, with a history of celiac disease in at least a subset of cases. The clinical course is aggressive.

HEPATOSPLENIC T-CELL LYMPHOMA (HSTL)

HSTL is an aggressive systemic neoplasm generally derived from $\gamma\delta$ cytotoxic T-cells of the innate immune system, however, it may also be derived from $\alpha\beta$ T-cells in rare cases. It is one of the rarest T-cell lymphomas, and typically affects adolescents and young adults (median age, 35 years) with a strong male predominance.

EXTRANODAL NK/T-CELL LYMPHOMA NASAL TYPE

Extranodal NK/T-cell lymphoma, nasal type, is an aggressive disease, often with destructive midline lesions and necrosis. Most cases are of NK-cell derivation, but some cases are derived from cytotoxic T-cells. It is universally associated with Epstein-Barr Virus (EBV).

CUTANEOUS T-CELL LYMPHOMA

The method of the present invention may also be used to treat cutaneous T-cell lymphoma.

Cutaneous T-cell lymphoma (CTCL) is characterised by migration of malignant T-cells to the skin, which causes various lesions to appear. These lesions change shape as the disease progresses, typically beginning as what appears to be a rash and eventually forming plaques and tumours before metastasizing to other parts of the body.

Cutaneous T-cell lymphomas include those mentioned in the following illustrative, non-exhaustive list; mycosis fungoides, pagetoid reticulosis, Sézary syndrome, granulomatous

slack skin, lymphomatoid papulosis, pityriasis lichenoides chronica, CD30+ cutaneous T-cell lymphoma, secondary cutaneous CD30+ large cell lymphoma, non-mycosis fungoides CD30-cutaneous large T-cell lymphoma, pleomorphic T-cell lymphoma, Lennert lymphoma, subcutaneous T-cell lymphoma and angiocentric lymphoma.

- 5 The signs and symptoms of CTCL vary depending on the specific disease, of which the two most common types are mycosis fungoides and Sézary syndrome. Classic mycosis fungoides is divided into three stages:

- Patch (atrophic or nonatrophic): Nonspecific dermatitis, patches on lower trunk and buttocks; minimal/absent pruritus;
- 10 - Plaque: Intensely pruritic plaques, lymphadenopathy; and
- Tumor: Prone to ulceration

Sézary syndrome is defined by erythroderma and leukemia. Signs and symptoms include edematous skin, lymphadenopathy, palmar and/or plantar hyperkeratosis, alopecia, nail dystrophy, ectropion and hepatosplenomegaly.

- 15 Of all primary cutaneous lymphomas, 65% are of the T-cell type. The most common immunophenotype is CD4 positive. There is no common pathophysiology for these diseases, as the term cutaneous T-cell lymphoma encompasses a wide variety of disorders.

- The primary etiologic mechanisms for the development of cutaneous T-cell lymphoma (ie, mycosis fungoides) have not been elucidated. Mycosis fungoides may be preceded by a T-cell-mediated chronic inflammatory skin disease, which may occasionally progress to a fatal lymphoma.
- 20

PRIMARY CUTANEOUS ALCL (C-ALCL)

- C-ALCL is often indistinguishable from ALC-ALK- by morphology. It is defined as a cutaneous tumour of large cells with anaplastic, pleomorphic or immunoblastic morphology with more than 75% of cells expressing CD30. Together with lymphomatoid papulosis (LyP), C-ALCL belongs to the spectrum of primary cutaneous CD30-positive T-cell lymphoproliferative disorders, which as a group comprise the second most common group of cutaneous T-cell lymphoproliferations after mycosis fungoides.
- 25

- The immunohistochemical staining profile is quite similar to ALCL-ALK-, with a greater proportion of cases staining positive for cytotoxic markers. At least 75% of the tumour cells should be positive for CD30. CD15 may also be expressed, and when lymph node involvement occurs, the differential with classical Hodgkin lymphoma can be difficult. Rare cases of ALCL-
- 30

ALK+ may present with localized cutaneous lesions, and may resemble C-ALCL.

T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

T-cell acute lymphoblastic leukaemia (T-ALL) accounts for about 15% and 25% of ALL in paediatric and adult cohorts respectively. Patients usually have high white blood cell counts and may present with organomegaly, particularly mediastinal enlargement and CNS involvement.

The method of the present invention may be used to treat T-ALL which is associated with a malignant T cell which expresses a TCR comprising TRBC1.

T-CELL PROLYMPHOCYTIC LEUKAEMIA

T-cell-prolymphocytic leukemia (T-PLL) is a mature T-cell leukaemia with aggressive behaviour and predilection for blood, bone marrow, lymph nodes, liver, spleen, and skin involvement. T-PLL primarily affects adults over the age of 30. Other names include T-cell chronic lymphocytic leukaemia, "knobby" type of T-cell leukaemia, and T-prolymphocytic leukaemia/T-cell lymphocytic leukaemia.

In the peripheral blood, T-PLL consists of medium-sized lymphocytes with single nucleoli and basophilic cytoplasm with occasional blebs or projections. The nuclei are usually round to oval in shape, with occasional patients having cells with a more irregular nuclear outline that is similar to the cerebriform nuclear shape seen in Sézary syndrome. A small cell variant comprises 20% of all T-PLL cases, and the Sézary cell-like (cerebriform) variant is seen in 5% of cases.

T-PLL has the immunophenotype of a mature (post-thymic) T-lymphocyte, and the neoplastic cells are typically positive for pan-T antigens CD2, CD3, and CD7 and negative for TdT and CD1a. The immunophenotype CD4+/CD8- is present in 60% of cases, the CD4+/CD8+ immunophenotype is present in 25%, and the CD4-/CD8+ immunophenotype is present in 15% of cases.

The TRBC1-positive or TRBC2-positive T-cell malignancy may be selected from peripheral T-cell lymphoma (PTCL), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), angio-immunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL), enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL), extranodal NK/T-cell lymphoma nasal type, cutaneous T-cell lymphoma, primary cutaneous ALCL, or T cell prolymphocytic leukaemia and T-cell acute lymphoblastic leukaemia.

ADMINISTRATION

The administration of the selective agent can be accomplished using any of a variety of routes that make the active ingredient bioavailable. For example, the agent can be administered by oral and parenteral routes, intraperitoneally, intravenously, subcutaneously, transcutaneously, 5 intramuscularly, via local delivery for example by catheter or stent.

Typically, a physician will determine the actual dosage which will be most suitable for an individual subject and it will vary with the age, weight and response of the particular patient. The dosage is such that it is sufficient to reduce or deplete the number of clonal T-cells expressing either TRBC1 or TRBC2.

10 This disclosure is not limited by the exemplary methods and materials disclosed herein, and any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of this disclosure. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, any nucleic acid sequences are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino 15 to carboxy orientation, respectively.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value 20 in that stated range is encompassed within this disclosure. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within this disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those 25 included limits are also included in this disclosure.

It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise.

The terms "comprising", "comprises" and "comprised of" as used herein are synonymous with "including", "includes" or "containing", "contains", and are inclusive or open-ended and do not 30 exclude additional, non-recited members, elements or method steps. The terms "comprising", "comprises" and "comprised of" also include the term "consisting of".

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that such publications constitute prior art to the claims appended hereto.

The invention will now be further described by way of Examples, which are meant to serve to assist one of ordinary skill in the art in carrying out the invention and are not intended in any way to limit the scope of the invention.

EXAMPLES

Anti-TRBC1 and anti-TRBC2 binders

A humanized (HuJovi-1) version of the known Jovi-1 (MuJovi-1) antibody has been previously described (WO2018/224844). The KFN anti-TRBC2 antibody has been previously described (WO2020/089644).

Example 1 – In vitro therapeutic efficacy of TCR β targeted CAR-T cells

KFN and HuJovi1 were tested in CARs with different spacers (IgG1 hinge, CD8 stalk and CD28 stalk), transmembrane domains (Tyrp and CD28) and endodomains (4-1BBz and CD28z) in a second-generation CAR architecture (Figure 5a, b). An anti-CD19 CAR (CD8stk-4-1BBz) was included as negative control.

TRBC1⁺ or TRBC2⁺ T cells isolated from PBMCs were transduced with KFN or HuJovi-1 CARs respectively. CAR expression was first determined by anti-idiotypic staining. CARs showed comparable surface expression levels between HuJovi-1 and KFN CARs, with the CD28stk-CD28TM CARs having the highest surface expression (Figure 6 and 7). Analysis at baseline showed more differentiation of KFN and HuJovi-1 CAR T cells compared with CD19 CAR T cells, perhaps due to residual TRBC2/TRBC1 T cells. Small differences in basal exhaustion were noted which correlated with presence of CD28 endodomain (Figure 8).

T cells transduced with the HuJovi-1 and KFN CARs were co-cultured with cell lines that either endogenously expressed TRBC1 (Jurkat and H9) or TRBC2 (HPB-ALL, T-ALL1 and HD-MAR2), or were engineered to express TRBC1, TRBC2 or TCR KO (Jurkat and HPB-ALL). The HuJovi-1 and KFN CARs killed TRBC1 or TRBC2-expressing cells at 1:8 effector: target (E:T) ratio respectively, with limited cross-reactivity towards the non-target cell lines (Figures 5c, 5d, 6c, 7d).

Among the architectures tested, the CD8stk-TyrpTM-CD28z and CD28z-CD28TM-CD28z showed selective killing, proliferation, the highest levels of selective cytokine release (e.g. IL-2, IL-4, IL17a, IFN γ and TNF α) and selective inflammatory mediators (Figures 5e, 5f, 6c and

7d). KFN-CD28stk-CD28TM-CD28z and HuJovi-1-CD8stk-TyrpTM-CD28z were selected for further study given their selectivity and potency.

Reverse killing (cytolysis of CAR T cells by cognate TCR T-cells) has been reported with CAR T cells directed against the TCR $\gamma\delta$. It was determined whether KFN or HuJovi-1 CARs were susceptible to reverse killing. KFN and HuJovi-1 CAR T cells were able to kill cognate healthy donor derived T cells (TRBC1⁺ and TRBC2⁺, respectively), showing efficient and specific killing of target cells with no indication of reverse kill even at low E:T ratio (Figures 6g, 6h, 9a). The CAR constructs were also tested against a number of patient-derived TRBC1 or TRBC2 T-PLL primary tumors. Both CAR-T products showed efficient and specific killing of the tumors carrying the respective TCR beta chain target (Figures 6i, 9b).

Example 2 – In vivo testing of TCR β targeted CAR T cells

To test the efficacy of CAR-T cells in mouse models of T cell malignancies, NSG mice were intravenously injected with Jurkat (TRBC1⁺ or TRBC2⁺) and HPB-ALL (TRBC2⁺) T cells, modified to express firefly luciferase (FLuc). Cells were stably engrafted in the bone marrow of all injected animals prior to administration of T cells expressing aTRBC2 CAR (KFN-CD28stk-CD28TM-CD28z) or aTRBC1 CAR (HuJovi-1-CD8stk-TyrpTM-CD28z). KFN CAR-T cells controlled tumor in the HPB-ALL TRBC2 and Jurkat TRBC2 models, in contrast to HuJovi-1 CAR and aCD19 CAR and non-transduced T cell controls (Figures 10 to 14). This response also translated into a survival advantage for KFN CAR-treated mice in both mouse models (Figures 10c, 10g). Analysis of bone marrow (BM) tumor content revealed significantly lower tumor burden in the HPB-ALL TRBC2 for KFN CAR at day 50 (Figure 10d), with only one animal in the HPB-ALL and Jurkat TRBC2 models showing expanding tumor cells by BLI (Figures 11 to 14). Conversely, the KFN CAR failed to control tumor growth in the Jurkat TRBC1 model (Figures 10d-f, 13, 14), where instead the HuJovi-1 CAR treated mice showed consistently low tumor burden and event-free survival for the duration of the observation period (Figures 10d-f, 14a). In this case, the HuJovi-1 CAR cohort showed significantly lower Jurkat TRBC1 tumor burden in the BM at day 66 compared to KFN CAR cohort (Figure 14b). aCD19 CAR treated mice demonstrated progression of both TRBC1⁺ and TRBC2⁺ engrafted tumors.

Materials and methods

Generation of TCR-KO cells by CRISPR/ Cas-9 Nicking Strategy – TCR negative versions of Jurkat and HBP-ALL cell lines were generated using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 genome engineering. TCR KO was confirmed by flow cytometry.

Flow cytometry – HPB-ALL TRBC2⁺ and the TRBC1⁺ or TCR KO HPB-ALL cells engineered from the TRBC2⁺ population via CRISPR/Cas9 homology-directed genome editing, were incubated (1×10^5 cells/well) with the test antibody. Protein-labelled cells were stained using anti-human IgG H+L Alexa fluor 647 conjugated antibody (Invitrogen). After washing off unbound antibodies, cells were stained with 7AAD viability dye (Biolegend). Stained samples were acquired using a MacsQuant10 instrument and analyzed on FlowJo software. Co-staining of human PBMCs was carried out using HuJovi-1 in human IgG1 Fc and biotinylated KFN in murine IgG2a Fc.

Retroviral supernatant production – 4.5×10^6 HEK-293T cells were transiently transfected with an RD114 envelope expression plasmid (RDF), a Gag-pol expression plasmid (PeqPam-env), and the transgene of interest expressed in a retroviral (SFG) vector plasmid at a ratio of 1:1.5:1.5 (total DNA=12.5 μ g). Transfections were performed with GeneJuice® (Millipore) according to the manufacturer's instructions and viral supernatants were harvested 48 hours post transfection and stored at -80°C.

Calculation of functional retroviral titers – Functional viral titers of retroviral supernatant were calculated using frozen supernatant on 293T cells, in the presence of 7 μ g/mL of Polybrene (Sigma-Aldrich), where 1×10^4 293T were seeded and then cultured at 37°C, 5% CO₂ for 72h. Transduced cells were identified by measuring for RQR8 expression. Viral titers were calculated with T cells that were less than 20% transduced.

Isolation of TRBC1⁺ and TRBC2⁺ primary T cells – Leucocyte cones of healthy donors were purchased from National Health Service Blood and Transplant (NHSBT, UK). Whole blood was extracted from each cone and diluted to 50 mL with sterile PBS. PBMCs were isolated by Ficoll gradient centrifugation. PBMCs were resuspended in cell separation buffer (StemCell) and incubated with biotinylated JOVI. Samples were centrifuged and then washed with separation buffer before following EasySep™ Release Human Biotin Positive Selection Kit (StemCell) protocol. The unbound (TRBC2⁺) fraction were harvested from the first incubation on the magnetic rack. The bound (TRBC1⁺) fraction was collected by following the protocol as stated. Isolation was confirmed via flow cytometry, staining with aCD3-PE/Cy7 and Streptavidin-APC.

Retroviral transduction of primary human T cells – Isolated TRBC1⁺ or TRBC2⁺ T cells were stimulated with TransAct (Miltenyi Biotec), IL-7 (Miltenyi Biotec) and IL-15 (Miltenyi Biotec). Twenty-four hours after, cells were collected, plated on retronectin-coated (Takara) 6-well plates in the presence of retroviral supernatant at an MOI of 1, and centrifuged for spinoculation. Transduction efficiency was determined on day 5 after transduction, and further

experiments were commenced on days 5–9 after transduction. CAR expression was assessed by staining with aCD3-PE/Cy7 (Biolegend) and QBend10 APC (R&D System).

Retroviral transduction of TCR KO cell line – TCR KO Jurkat and HPB ALL cells were collected, plated on retronectin-coated (Takara, T100B) 6-well plates in the presence of retroviral supernatant expressing TRBC2 or TRBC1 respectively. The plates were centrifuged for spinoculation. TRBC1 or TRBC2 expression was assessed by staining with in-house produced aTRBC1 (Jovi) or aTRBC2 (KFN).

Human CAR T cells FACS based cytotoxicity assay co-culture – Mock (Non-Transduced PBMCs) and CAR-transduced T cells were co-cultured with TRBC1⁺, TRBC2⁺ or TCR KO Jurkat or HBP-ALL, TRBC1⁺H9, TRBC2⁺ HD-MAR and T-ALL1 target cells. Target cells were labelled with CellTrace™ CFSE (ThermoFisher Scientific) following manufacturer instructions. Mock and CAR-transduced T cells were labelled with CellTrace™ Violet (ThermoFisher Scientific) following manufacturer instructions. Effector and target cells were mixed to reach an E:T ratio of 1:4, 1:8, 1:16 and 1:32. 72 hours after co-culture live cell data were collected via Flow cytometry using the MacsQuantX flow cytometer (Miltenyi). Data analysis was conducted using FlowJo v10 (Treestar). Percentage of live cells was calculated relative to the number of live target cells after co-culture with non-transduced T cells.

FACS based cytotoxicity assay co-culture with Autologous Healthy Primary T cells - Mock (Non-Transduced PBMCs) and CAR-transduced T cells were co-cultured with autologous TRBC1⁺ and TRBC2⁺ non transduced T cells. Target cells were labelled with CellTrace™ CFSE (ThermoFisher Scientific) following manufacturer's instructions. Effector mock and CAR-transduced T cells were labelled with CellTrace™ Violet (ThermoFisher Scientific) following manufacturer's instructions. Effector and target cells were mixed to reach an E:T ratio of 1:4, 1:1 and 4:1. 72 hours after co-culture live cell data were collected via Flow cytometry using the MacsQuantX flow cytometer (Miltenyi). Data analysis was conducted using FlowJo v10 (Treestar). Percentage of live cells was calculated relative to the number of live target cells after co-culture with non-transduced T cells.

FACS based cytotoxicity assay co-culture with Primary T-PLL tumor samples – Mock (Non-Transduced PBMCs) and CAR-transduced T cells were co-cultured with either TRBC1⁺ or TRBC2⁺ human primary T-PLL tumor. Human primary T-PLL tumors were obtained from patients with T-PLL at the University Hospital of Cologne. Target cells were then labelled with CellTrace™ CFSE (ThermoFisher Scientific) following manufacturer's instructions. Mock and CAR-transduced T cells were labelled with CellTrace™ Violet (ThermoFisher Scientific) following manufacturer's instructions. Effector and target cells were mixed to reach an E:T ratio of 1:4, 1:1 and 4:1. 72 hours after co-culture live cell data were collected via flow

cytometry using the MacsQuantX flow cytometer (Miltenyi). Data analysis was conducted using FlowJo v10 (Treestar). Percentage of live cells was calculated relative to the number of live target cells after co-culture with non-transduced T cells.

Analysis of cytokine production via Cytokine Bead Array – Supernatants from 72h co-culture were analyzed for multi-cytokine production utilizing LEGENDplex™ Human CD8/NK Panel (13-plex) (Biolegend) according to manufacturer's protocol. The samples were detected using LSRFortessa X20 (BD Biosciences) and analyzed using LEGENDplex™ Data Analysis Software Suite (Biolegend).

T cell Immunophenotyping – Mock and CAR-transduced T cells were co-cultured with TRBC1⁺, TRBC2⁺ or TCR KO Jurkat. Target cells were then labelled with CellTrace™ CFSE (ThermoFisher Scientific) following manufacturer instructions. Effector and target cells were mixed at 1:1 E:T ratio for 72h. Samples were harvested from cell culture plates and prepared for staining in 96-well plates (Greiner Bio-one). Cells were put through multiple rounds of staining, incubation and wash procedures in accordance with standard operating procedures, using a 16 color flow cytometry panel. Cells were finally fixed (eBiosciences) before being acquired on the LSRFortessa X20 (BD Biosciences). FMX controls (fluorescence minus multiple) were run alongside fully stained samples to assist in setting gates upon analysis.

Establishment of T cell acute lymphoblastic leukemia (T-ALL) xenograft mouse models – For the generation of the Jurkat T-ALL model 10 to 14-week-old female NSG mice (NOD scid gamma; NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ) (Charles River) received intravenous injections of 5 x 10⁶/mouse firefly luciferase-transduced TRBC1⁺ or 1 x 10⁶/mouse firefly luciferase-transduced TRBC2⁺ Jurkat cells at day -10 relative to CAR-T infusion. On day 0, mice (n=6/cohort) received intravenous infusions of either 1 x 10⁶ or 5 x 10⁶ transduced CAR-T cells. For the generation of the HPB-ALL T-ALL model 10 to 14-week-old female NSG mice (NOD SCID gamma; NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ) (Charles River) received intravenous injections of 2.5 x 10⁶/mouse luciferase-transduced HPB-ALL day -7 relative to CAR-T infusion. On day 0, mice (n=6/cohort) received intravenous infusions of 5 x 10⁶ transduced CAR-T cells. The mice were imaged bi-weekly for bioluminescence signal from tumor cells using the IVIS® system (IVIS, Xenogen Corporation, Alameda, CA) 10–15 minutes after 150 mg/kg D-luciferin (Xenogen) per mouse was injected intraperitoneally. Mice were euthanized if body weight loss ≥ 20%. Animals were sacrificed by Isoflurane inhalation followed by cervical dislocation. Bone marrow from lower limbs was collected at the time of euthanasia for tumor and CAR-T cell tracking.

Flow cytometry analysis of mouse bone marrow – Animals were sacrificed by Isoflurane inhalation followed by cervical dislocation. Bone marrow was collected by centrifugation of

lower limb bones, after the epiphyses were cut. Erythrocytes were lysed using 500 μ L of ACK lysis buffer (Sigma). Cells were strained through a 70 μ m cell strainer. 1×10^6 cells were aliquoted for phenotyping by flow cytometry. First, the Fc receptor was blocked using anti-CD32/CD16 (BioLegend) to avoid non-specific binding. Transduced CAR-T cell populations were identified based on the expression of CD45 (Biolegend), CD3 (Biolegend), CD4 (Biolegend), RQR8 (R&D System), CAR (aJOVI/KFN idotype) and the absence of CD11b (Biolegend). The identification of tumors was performed by the detection of mCLOVER and either TRBC1 or TRBC2. The samples were stained in 96 well plates and resuspended in 100 μ L of PBS. Flow cytometry was performed using the MacsQuantX flow cytometer (Miltenyi). Data analysis was conducted using FlowJo v10 (Treestar).

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Claims

1. A chimeric antigen receptor (CAR) comprising an anti-TRBC2 antigen-binding domain, a CD28 spacer, a CD28 transmembrane domain, and a CD28-CD3zeta endodomain.
- 5 2. A chimeric antigen receptor (CAR) comprising:
 - a. an anti-TRBC1 antigen-binding domain, an IgG1 hinge domain, a TYRP-1 transmembrane domain, and a 41BB-CD3zeta endodomain;
 - b. an anti-TRBC1 antigen-binding domain, a CD8 spacer, a TYRP-1 transmembrane domain, and a CD28-CD3zeta endodomain; or
 - 10 c. an anti-TRBC1 antigen-binding domain, a CD28 spacer, a CD28 transmembrane domain, and a CD28-CD3zeta endodomain.
3. The CAR according to claim 1, wherein the anti-TRBC2 antigen-binding domain has a variable heavy chain (VH) and a variable light chain (VL) which comprise the following complementarity determining regions (CDRs):
 - 15 a. VH CDR1: GYKFTGF (SEQ ID No: 1)
 - b. VH CDR2: NPYNDD (SEQ ID No: 2)
 - c. VH CDR3: GNGYNFDGAYRFFDF (SEQ ID No: 3)
 - d. VL CDR1: RSSQRLVHSNGNTYLH (SEQ ID No: 4)
 - 20 e. VL CDR2: RVSNRFP (SEQ ID No: 5)
 - f. VL CDR3: SQSTHVPYT (SEQ ID No: 6)
4. The CAR according to claim 3, wherein the anti-TRBC2 antigen-binding domain comprises:
 - 25 a. A VH domain comprising SEQ ID No: 7, or a sequence with at least 80% identity to SEQ ID No: 7, and
 - b. A VL domain comprising SEQ ID NO: 8, or a sequence with at least 80% identity to SEQ ID No: 8.
- 30 5. The CAR according to claim 4, wherein the anti-TRBC2 antigen-binding domain comprises SEQ ID No: 9, or a sequence with at least 80% identity to SEQ ID No: 9.
6. The CAR according to claim 2, wherein the anti-TRBC1 antigen-binding domain has a variable heavy chain (VH) and a variable light chain (VL) which comprise the following complementarity determining regions (CDRs):
 - 35 a. VH CDR1: GYTFTGY (SEQ ID No: 10)

- b. VH CDR2: NPYNDD (SEQ ID No: 2)
- c. VH CDR3: GAGYNFDGAYRFFDF (SEQ ID No: 11)
- d. VL CDR1: RSSQRLVHSNGNTYLH (SEQ ID No: 4)
- e. VL CDR2: RVSNRFP (SEQ ID No: 5)
- f. VL CDR3: SQSTHVPYT (SEQ ID No: 6)

7. The CAR according to claim 6, wherein the anti-TRBC1 antigen-binding domain comprises:

- a. A VH domain comprising SEQ ID No: 12, or a sequence with at least 80% identity to SEQ ID No: 12, and
- a. A VL domain comprising SEQ ID No: 8, or a sequence with at least 80% identity to SEQ ID No: 8.

8. The CAR according to claim 7, wherein the anti-TRBC1 antigen-binding domain comprises SEQ ID No: 13, or a sequence with at least 80% identity to SEQ ID No: 13.

9. The CAR according to any preceding claim, wherein the IgG1 hinge domain comprises SEQ ID No: 14, or a sequence with at least 80% identity to SEQ ID No: 14.

10. The CAR according to any of claims 1 to 8, wherein the CD8 spacer comprises SEQ ID No: 15, or a sequence with at least 80% identity to SEQ ID No: 15.

11. The CAR according to of claims 1 to 8, wherein the CD28 spacer comprises SEQ ID No: 16, or a sequence with at least 80% identity to SEQ ID No: 16.

12. The CAR according to any preceding claim, wherein the TYRP-1 transmembrane domain comprises SEQ ID No: 17, or a sequence with at least 80% identity to SEQ ID No: 17.

13. The CAR according to any of claims 1 to 11, wherein the CD28 transmembrane comprises SEQ ID No: 18, or a sequence with at least 80% identity to SEQ ID No: 18.

14. The CAR according to any preceding claim, wherein the 41BB-CD3zeta endodomain comprises SEQ ID No: 19, or a sequence with at least 80% identity to SEQ ID No: 19.

15. The CAR according to any of claims 1 to 13, wherein the CD28-CD3Zeta endodomain comprises SEQ ID No: 20 or SEQ ID No: 21, or a sequence with at least 80% identity to SEQ ID No: 20 or SEQ ID No: 21.

16. The CAR according to any preceding claim,

wherein the CAR has an anti-TRBC2 antigen-binding domain and comprises:

- a. an amino acid sequence having SEQ ID No: 22, or a sequence with at least 80% identity thereto,

or

wherein the CAR has an anti-TRBC1 antigen-binding domain and comprises:

- a. an amino acid sequence having SEQ ID No: 25, or a sequence with at least 80% identity thereto, or
- b. an amino acid sequence having SEQ ID No: 26, or a sequence with at least 80% identity thereto, or
- c. an amino acid sequence having SEQ ID No: 27, or a sequence with at least 80% identity thereto.

17. The CAR according to claim 16, which consists of SEQ ID No: 22, SEQ ID No: 25, SEQ ID No: 26, or SEQ ID No: 27, or a sequence with at least 80% identity thereto.

18. The CAR according to claim 17, which consists of SEQ ID No: 22, or a sequence with at least 80% identity thereto.

19. A nucleic acid which encodes a CAR according to any preceding claim.

20. A vector which comprises a nucleic acid according to claim 19.

21. A cell which comprises a CAR according to any one of claims 1 to 18, a nucleic acid according to claim 19 or a vector according to claim 20.

22. A composition comprising a plurality of cells according to claim 21.

23. A method of making a cell according to claim 21, which comprises the step of transducing or transfecting a cell with a nucleic acid according to claim 19 or a vector according to claim 20.

24. A method for making a cell composition according to claim 22, which comprises the step of transducing or transfecting a sample of cells from a subject *ex vivo* with a nucleic acid according to claim 19 or a vector according to claim 20.
- 5 25. A pharmaceutical composition which comprises a cell according to claim 21, or a cell composition according to claim 22, together with a pharmaceutically acceptable carrier, diluent or excipient.
- 10 26. A nucleic acid according to claim 19, a vector according to claim 20, a cell according to claim 21, a composition according to claim 22, or a pharmaceutical composition according to claim 25 for use in a method of treating T-cell lymphoma or leukaemia.
- 15 27. Use of a nucleic acid according to claim 19, a vector according to claim 20, a cell according to claim 21, a composition according to claim 22, or a pharmaceutical composition according to claim 25 for the manufacture of a medicament for the treatment of T-cell lymphoma or leukaemia.
- 20 28. A method for treating T-cell lymphoma or leukaemia, comprising administering a nucleic acid according to claim 19, a vector according to claim 20, a cell according to claim 21, a composition according to claim 22, or a pharmaceutical composition according to claim 25 to a subject.
- 25 29. The nucleic acid, vector, cell, composition or pharmaceutical composition for use according to claim 26, the use according to claim 27 or the method according to claim 28, wherein the T-cell lymphoma or leukaemia is selected from: peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS); angio-immunoblastic T-cell lymphoma (AITL), anaplastic large cell lymphoma (ALCL), enteropathy-associated T-cell lymphoma (EATL), hepatosplenic T-cell lymphoma (HSTL), extranodal NK/T-cell lymphoma nasal type, cutaneous T-cell lymphoma, primary cutaneous ALCL, T cell
30 polymphocytic leukaemia and T-cell acute lymphoblastic leukaemia.

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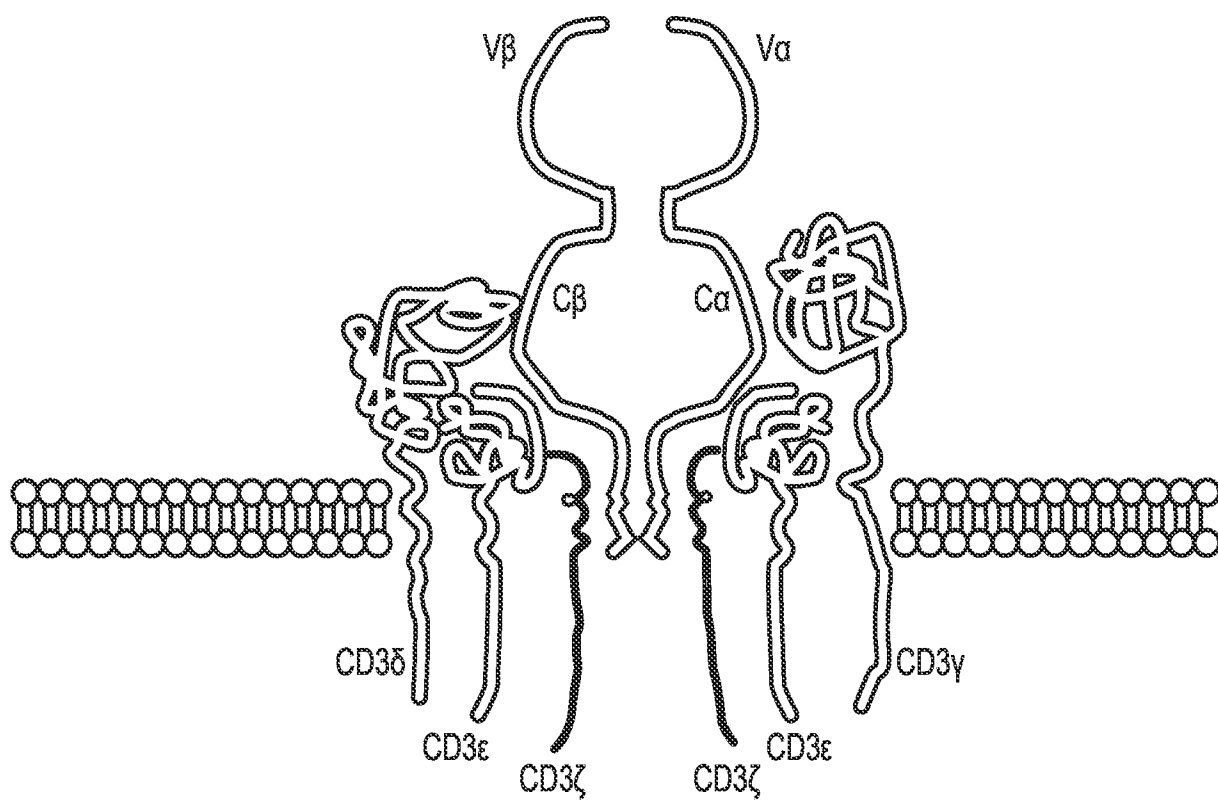


FIG. 1

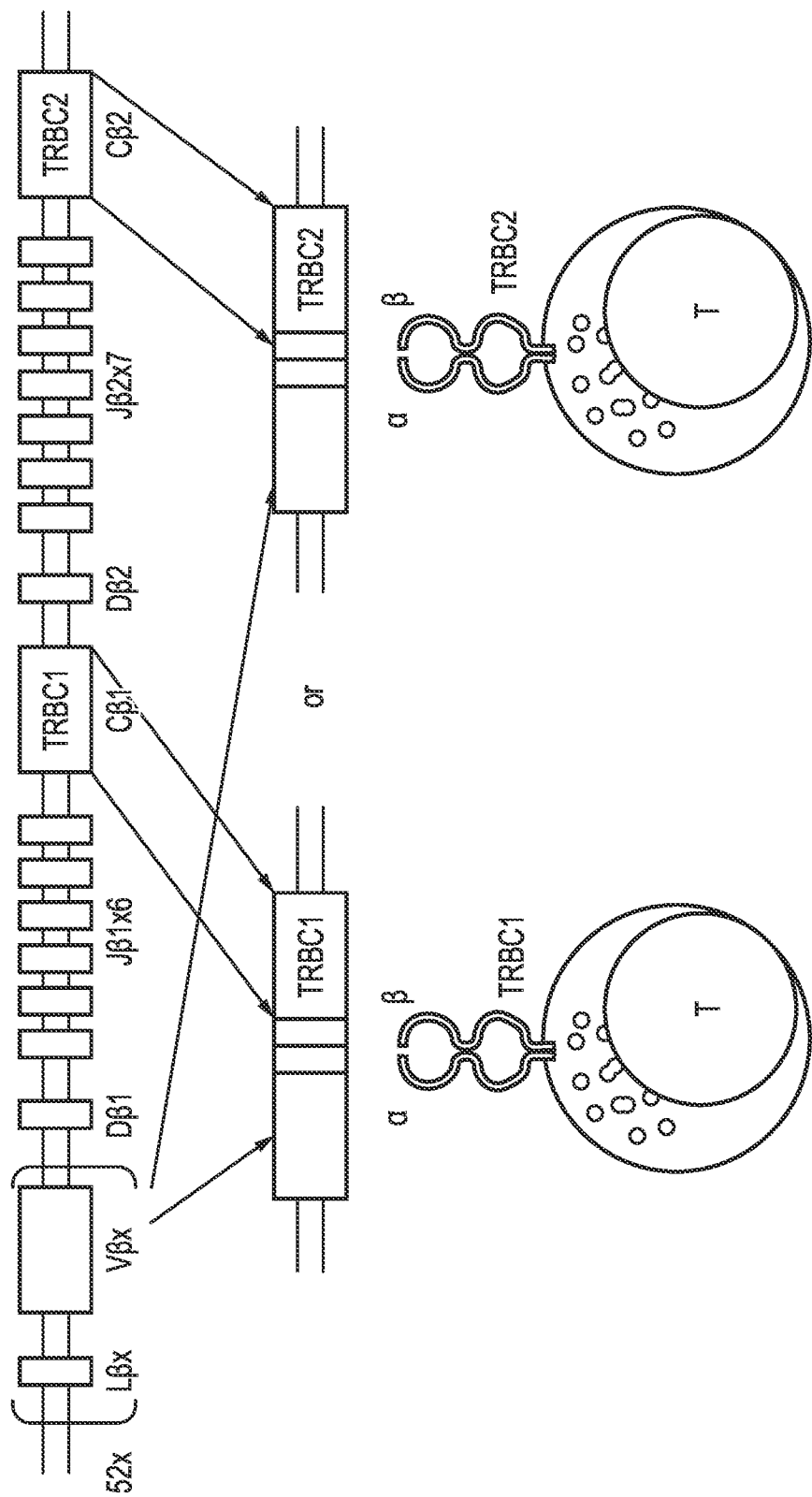


FIG. 2

TRBC1	1	DLNKVFPPEVAVFEPSEAEISHTQKATIVCIATGFTDPDHVELSWWVNGKEVHSGVSTDRQ
TRBC2	1	DLNKVFPPEVAVFEPSEAEISHTQKATIVCIATGFTDPDHVELSWWVNGKEVHSGVSTDRQ
TRBC1	61	PLKEQPALNDSRYCLSSRLRVSATFWONPRNHRCQVQFYGLSENDEWTOODRAKEVTOIV
TRBC2	61	PLKEQPALNDSRYCLSSRLRVSATFWONPRNHRCQVQFYGLSENDEWTOODRAKEVTOIV
TRBC1	121	SAEAWGRADCCGFTSVSYQQGVLSATIIYEIILIGKATIIYAVLVSALVLMAMVKRKD
TRBC2	121	SAEAWGRADCCGFTSESYQQGVLSATIIYEIILIGKATIIYAVLVSALVLMAMVKRKD

FIG. 3

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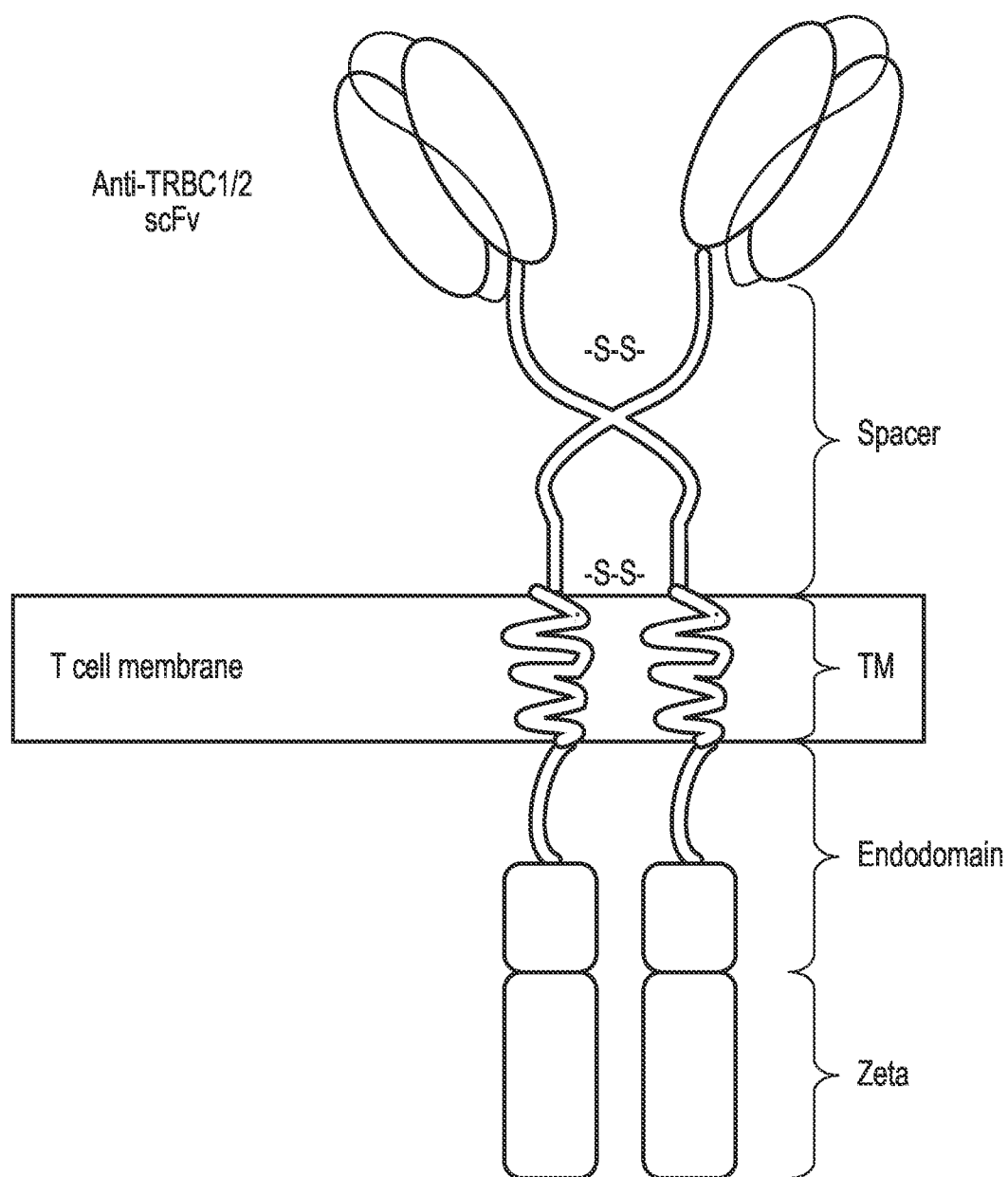
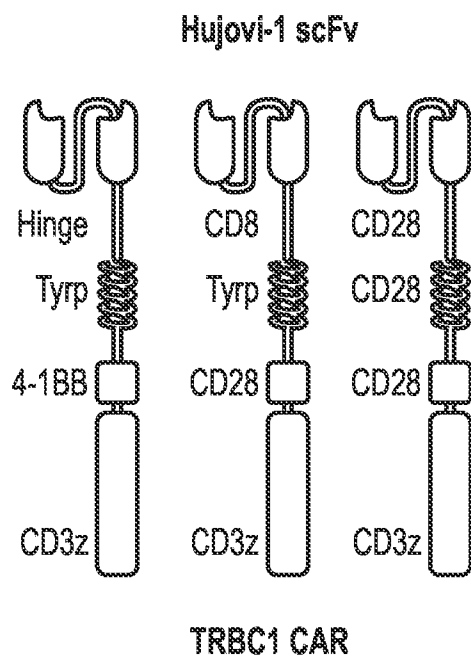


FIG. 4

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a



b

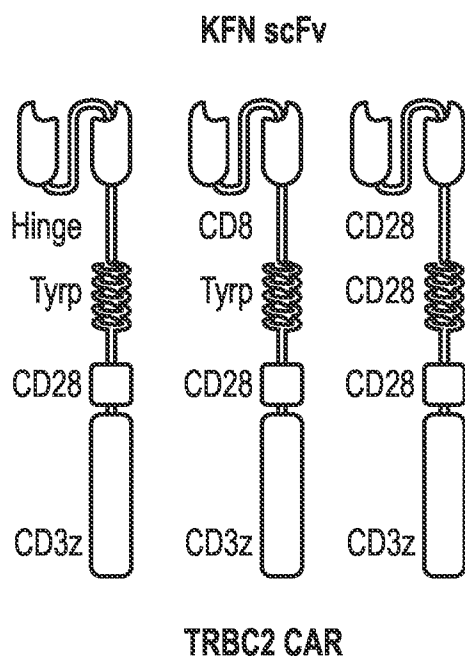


FIG. 5

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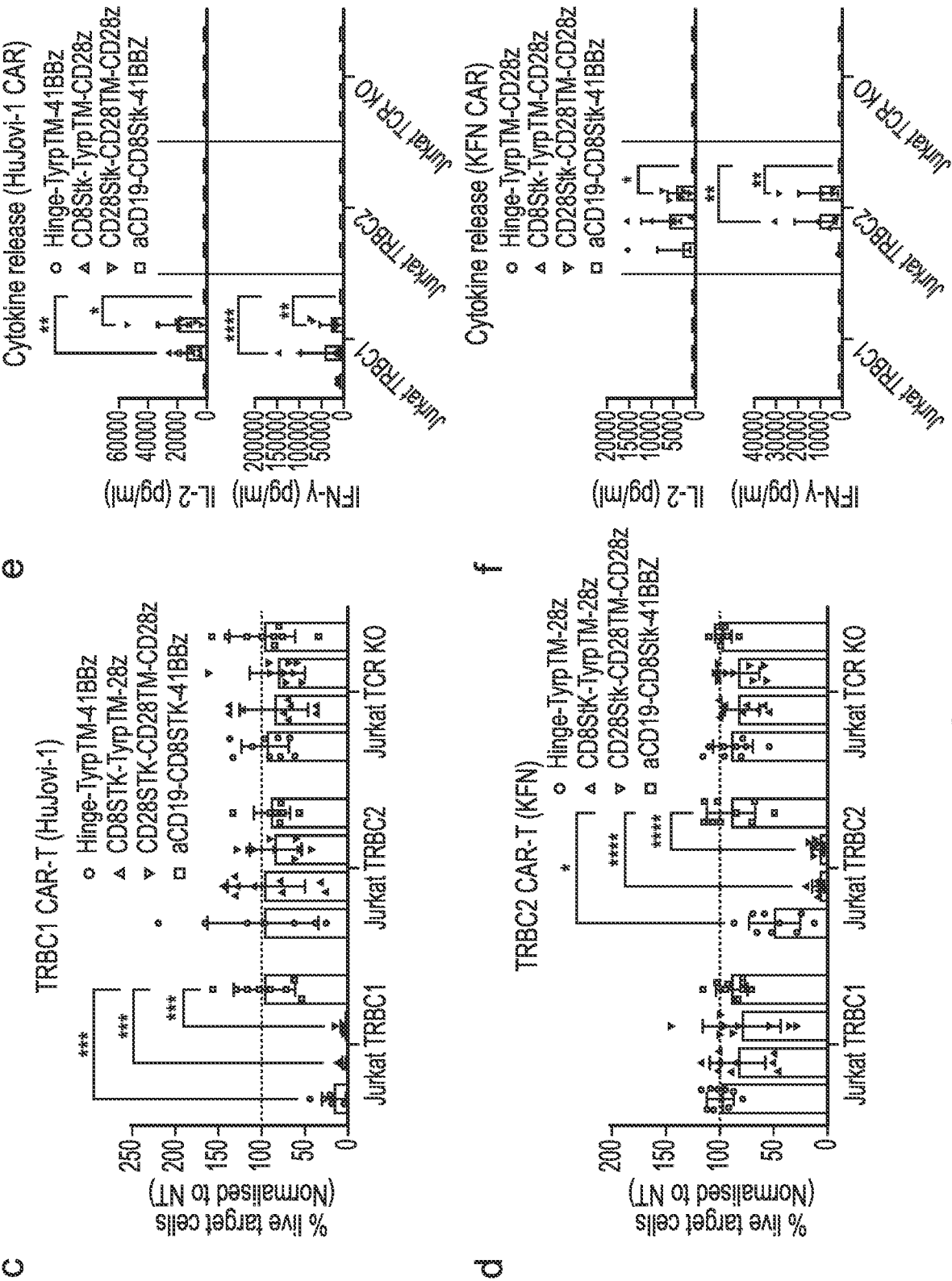


FIG. 5 (continued)

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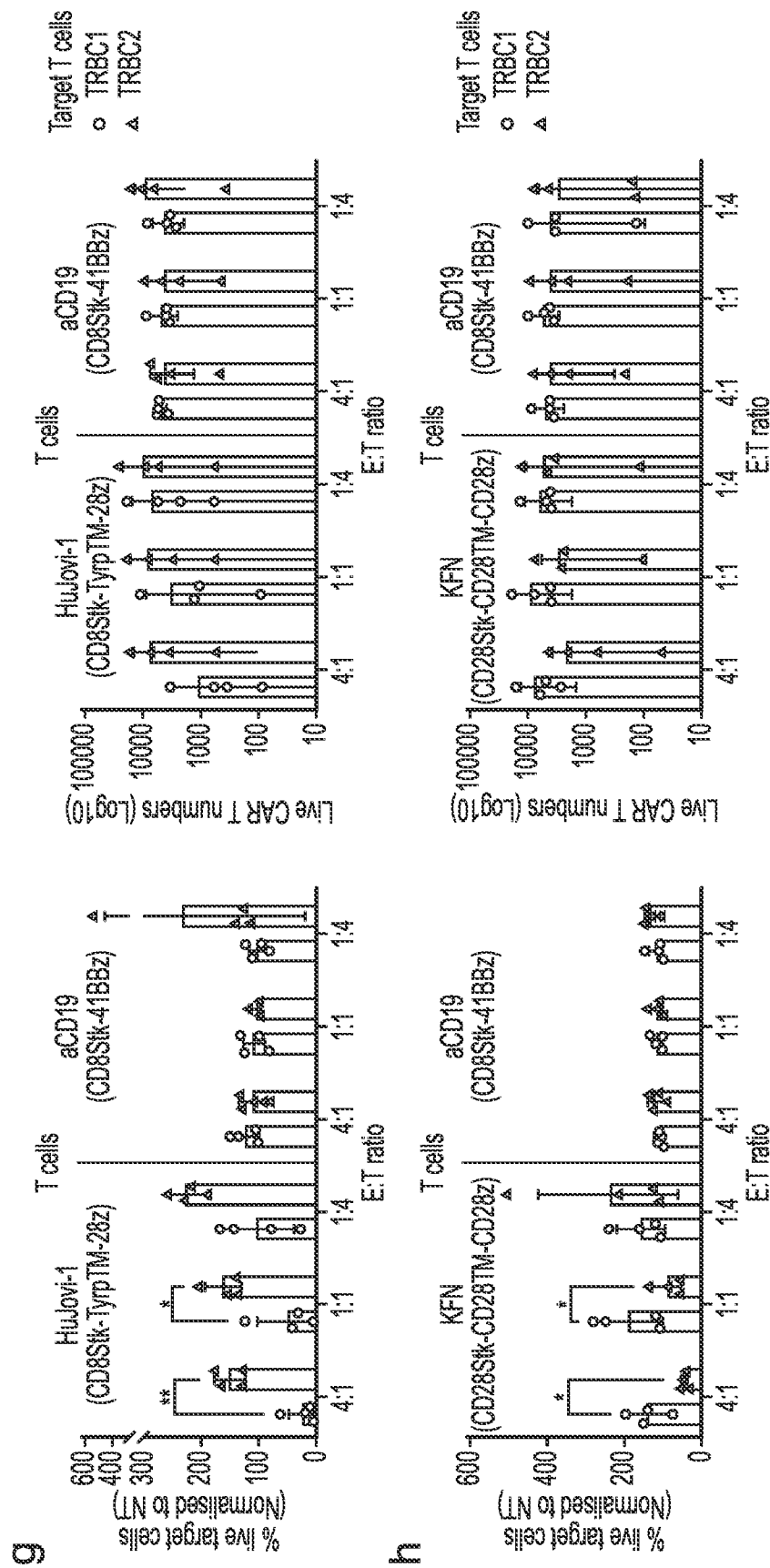


FIG. 5 (continued)

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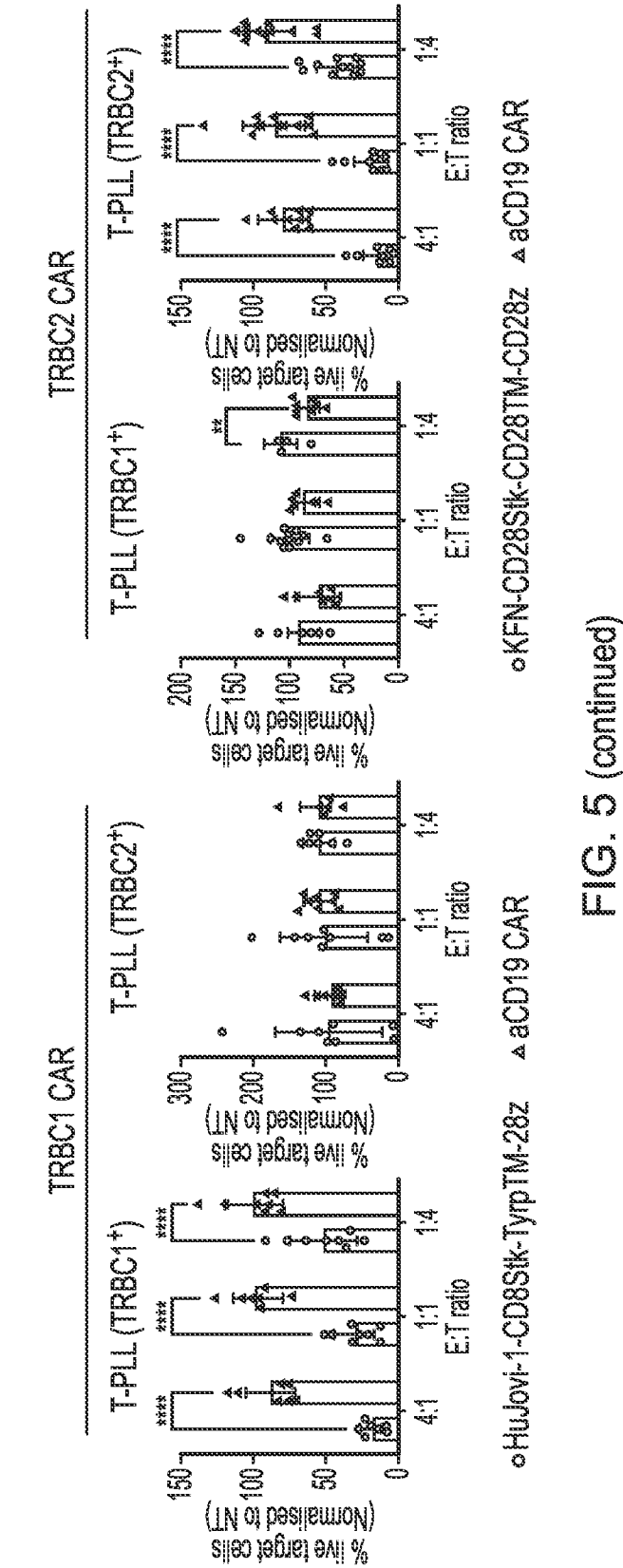
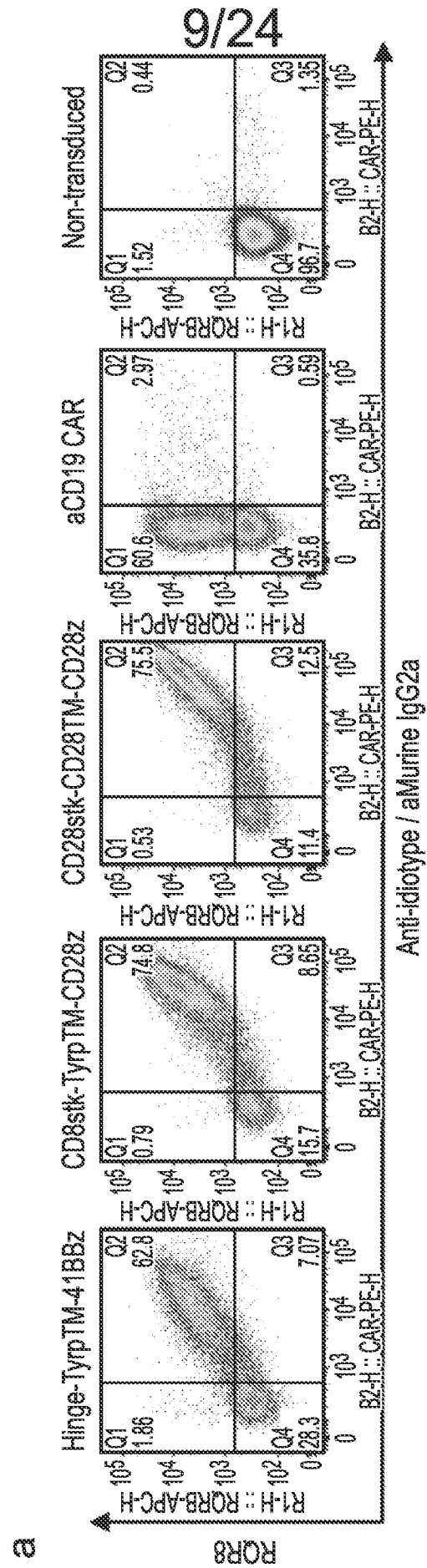


FIG. 5 (continued)





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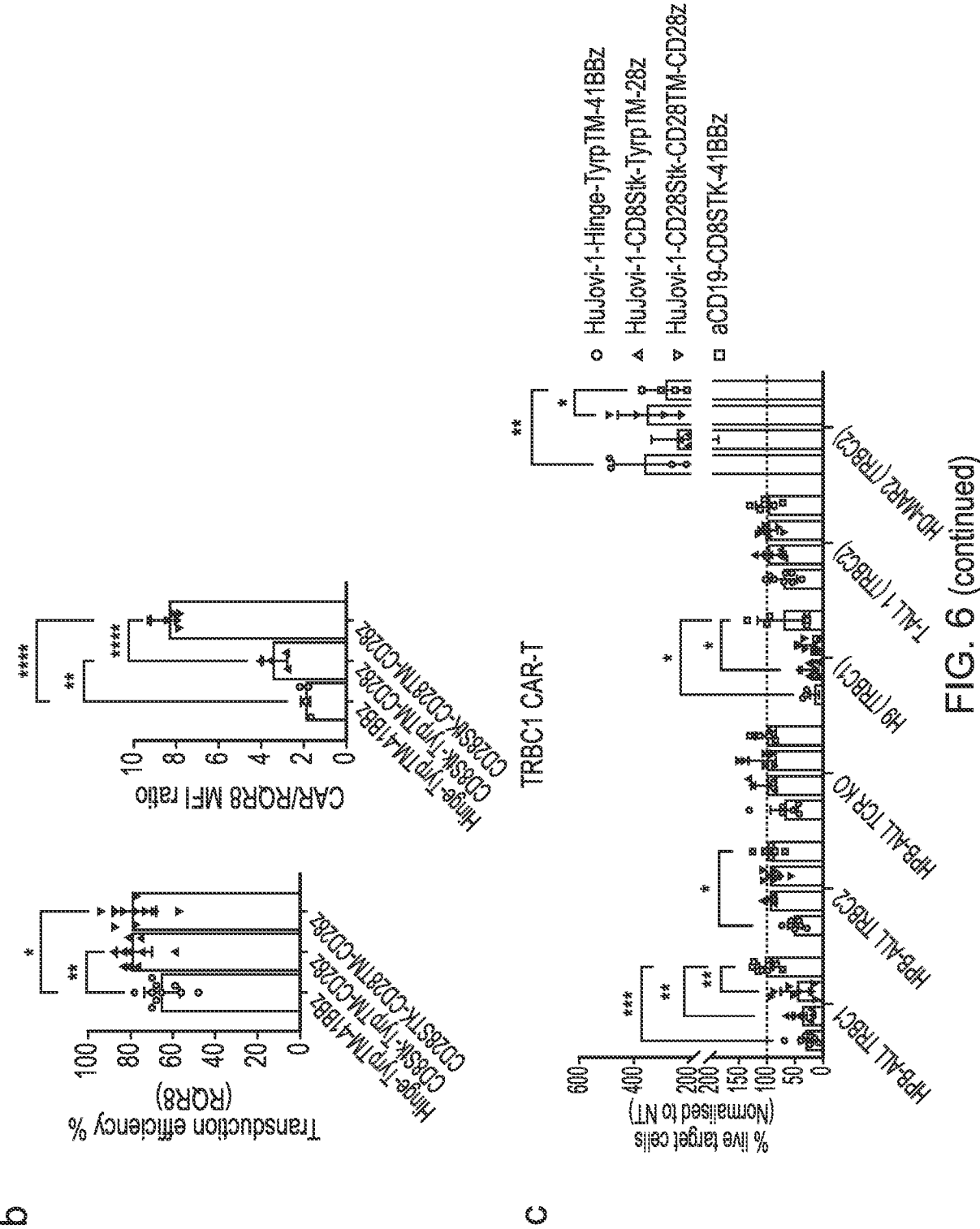


FIG. 6 (continued)

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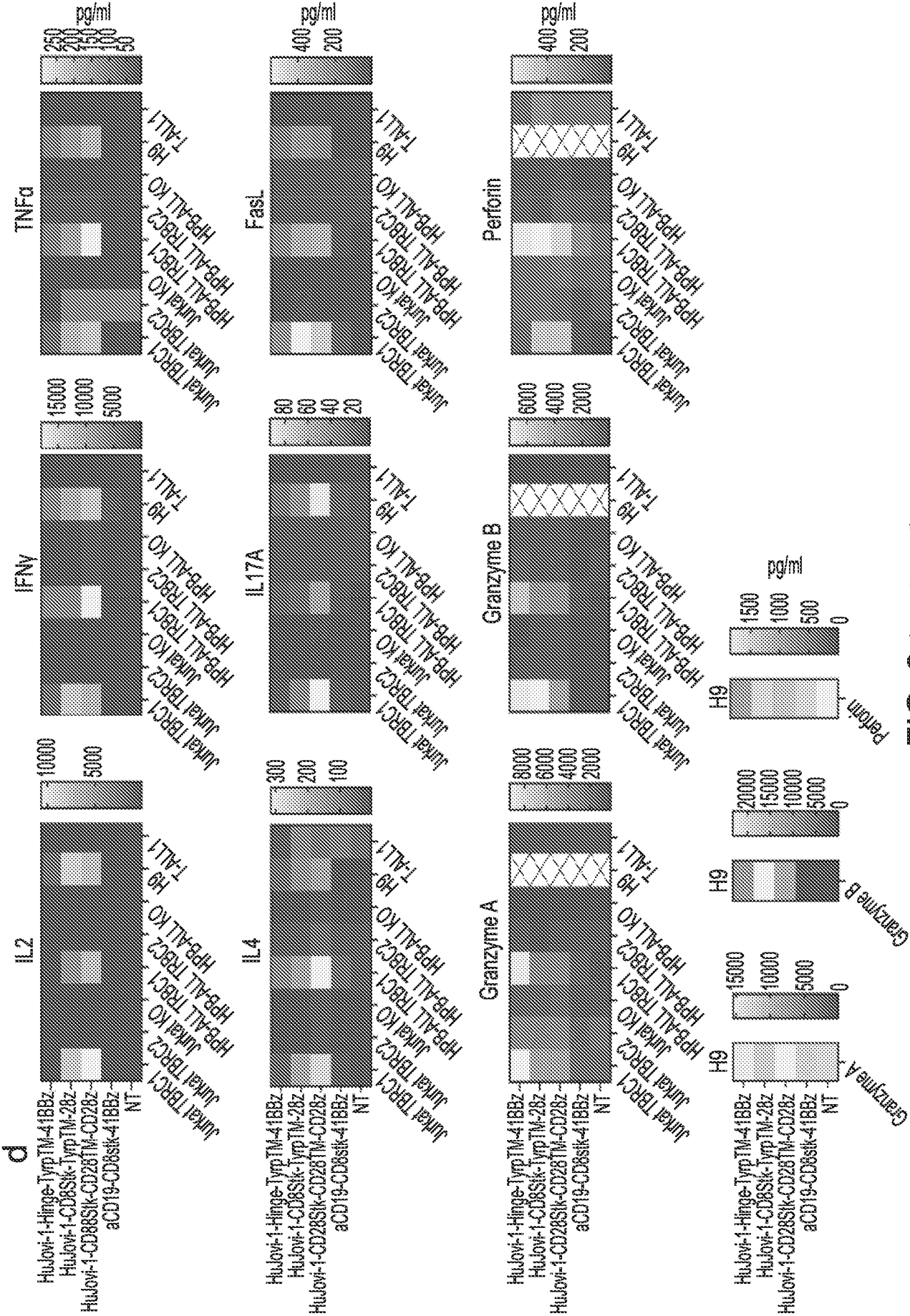


FIG. 6 (continued)

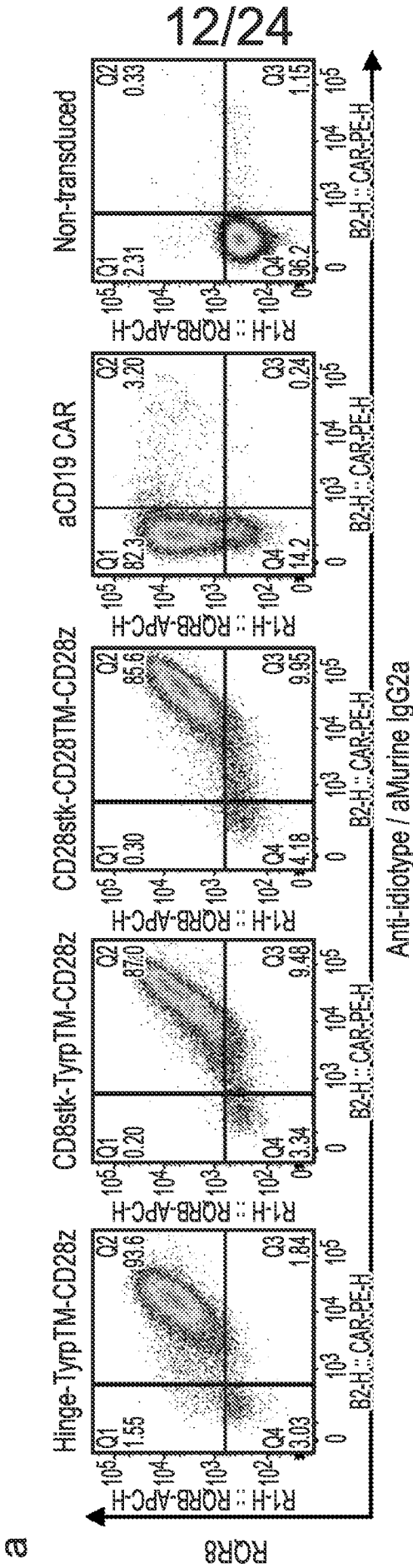
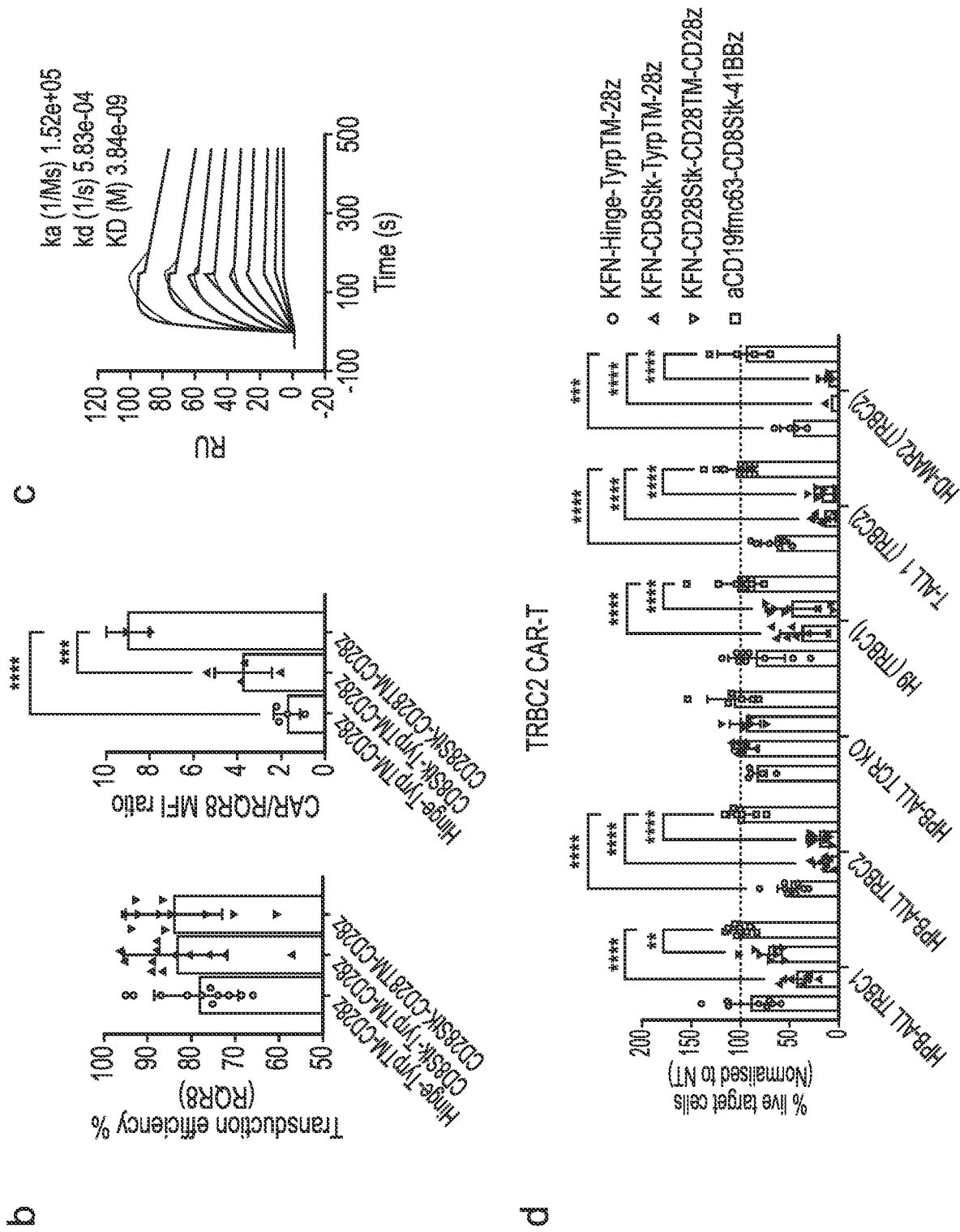


FIG. 7



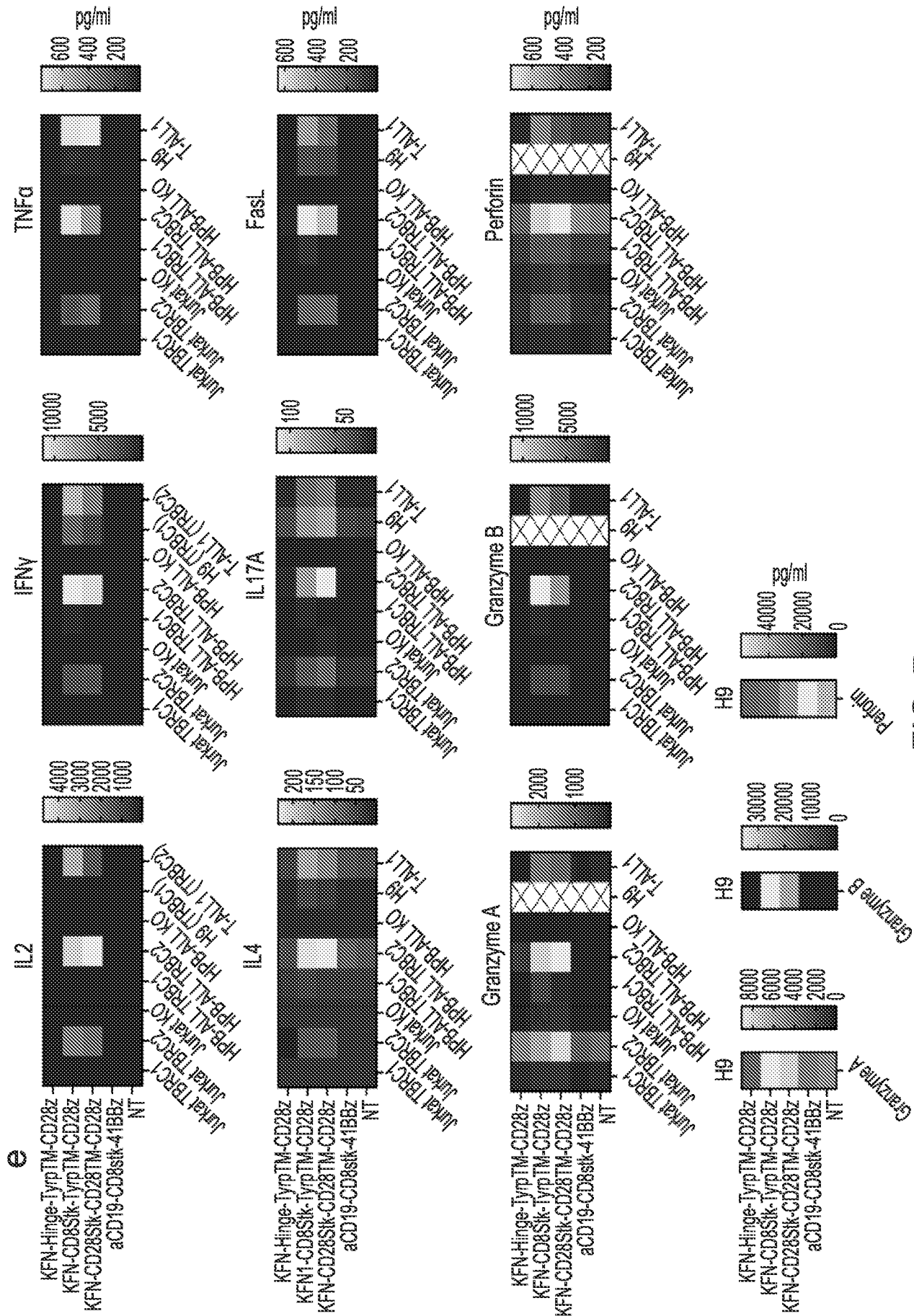


FIG. 7 (continued)

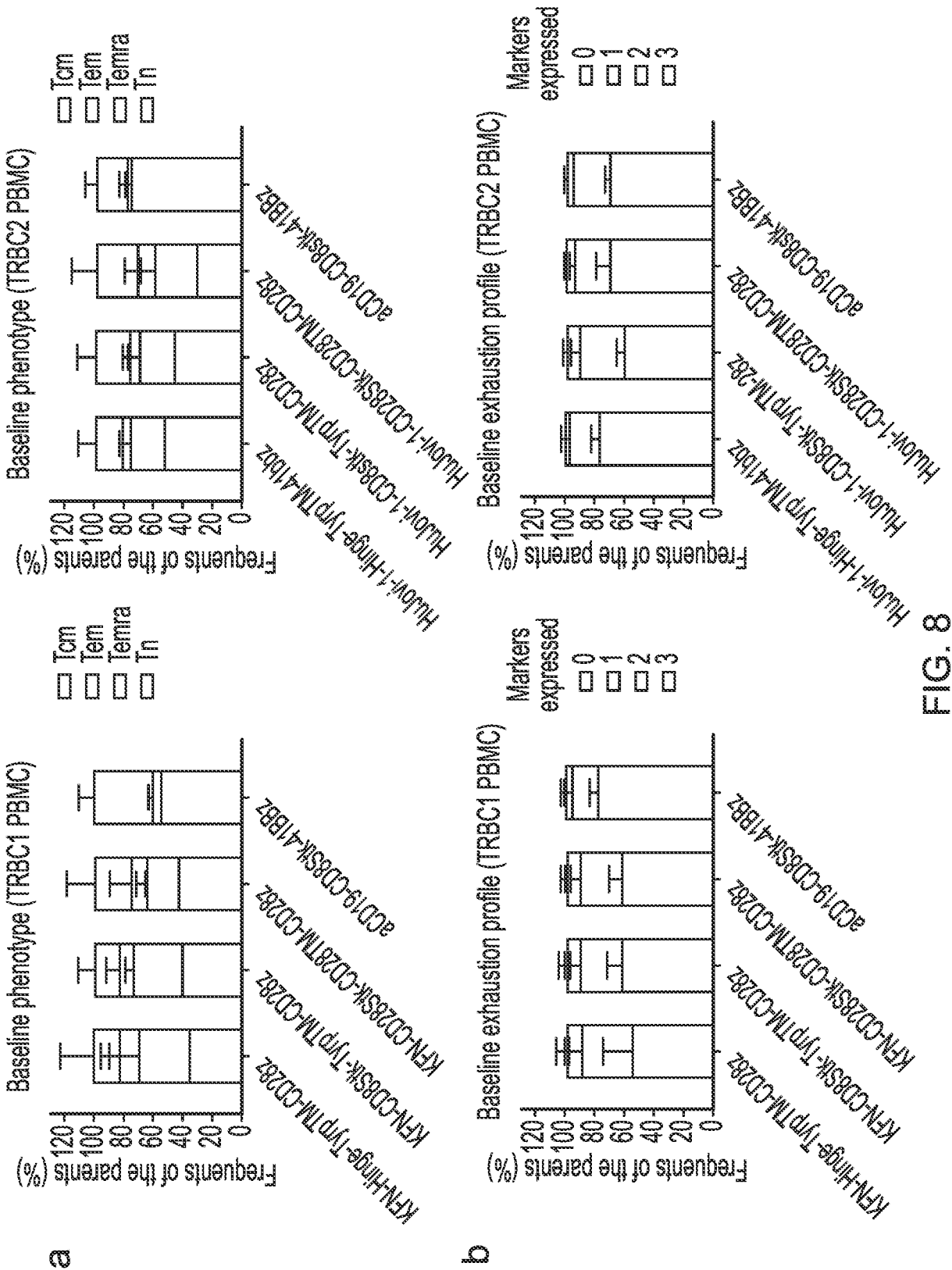


FIG. 8

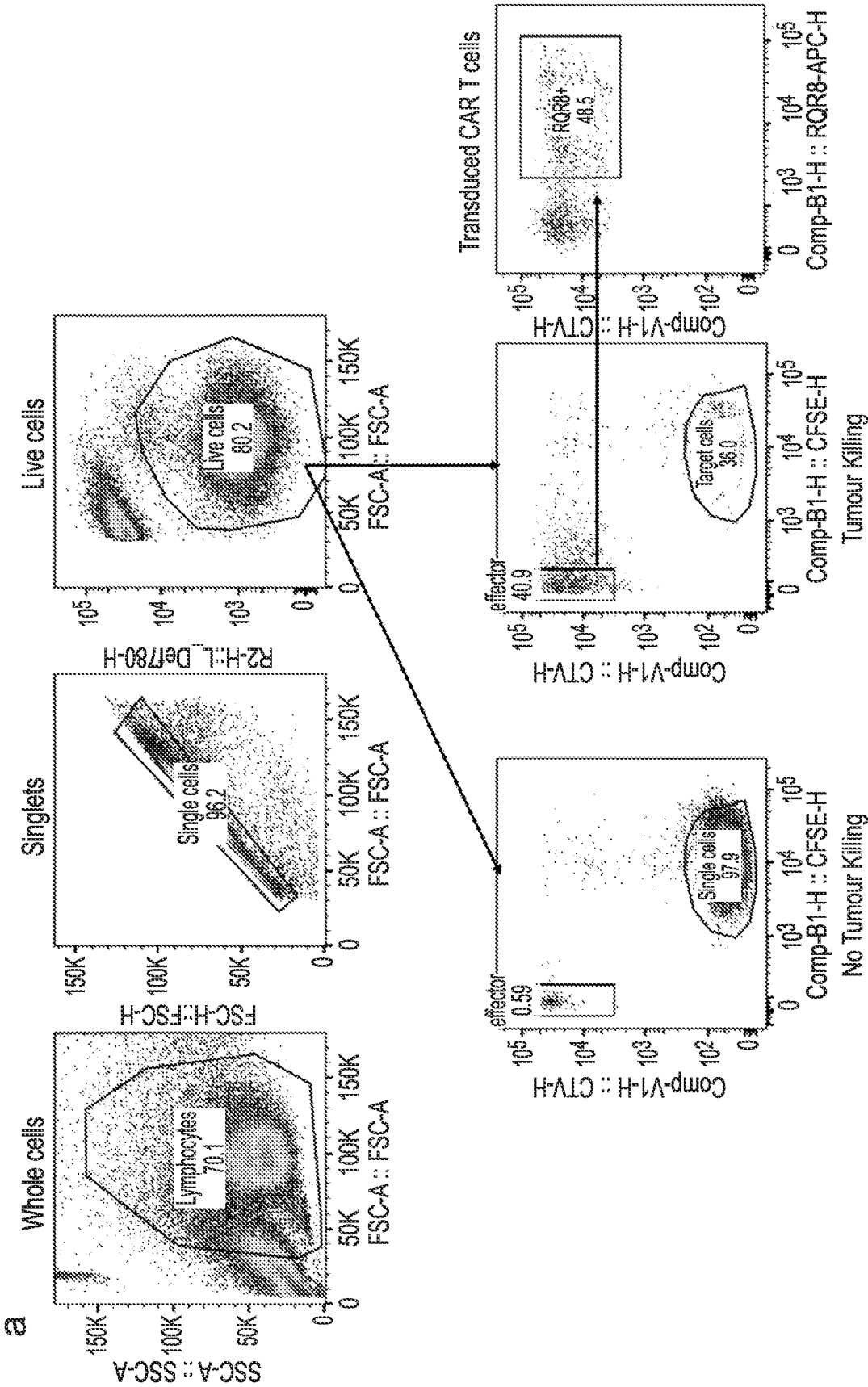


FIG. 9

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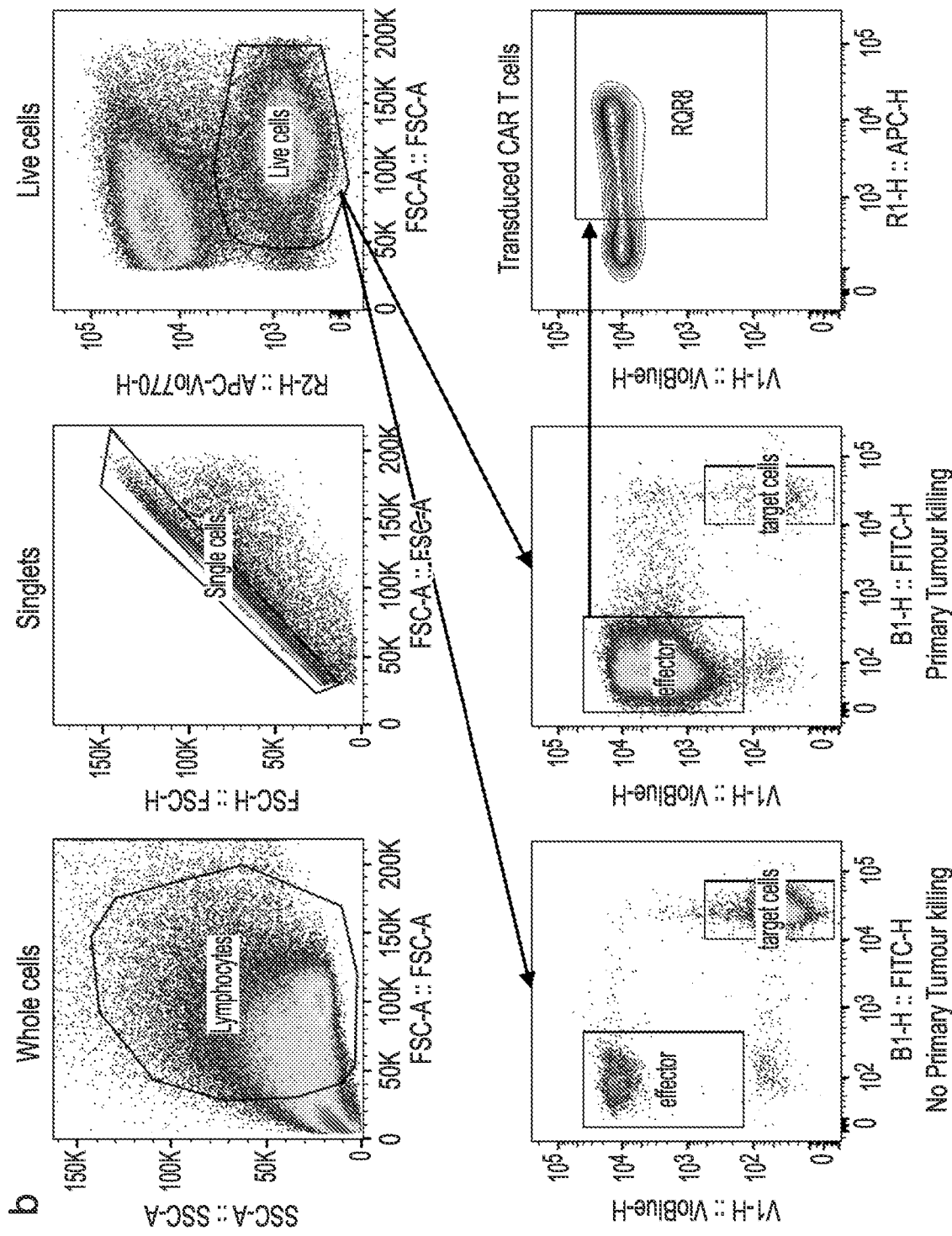


FIG. 9 (continued)

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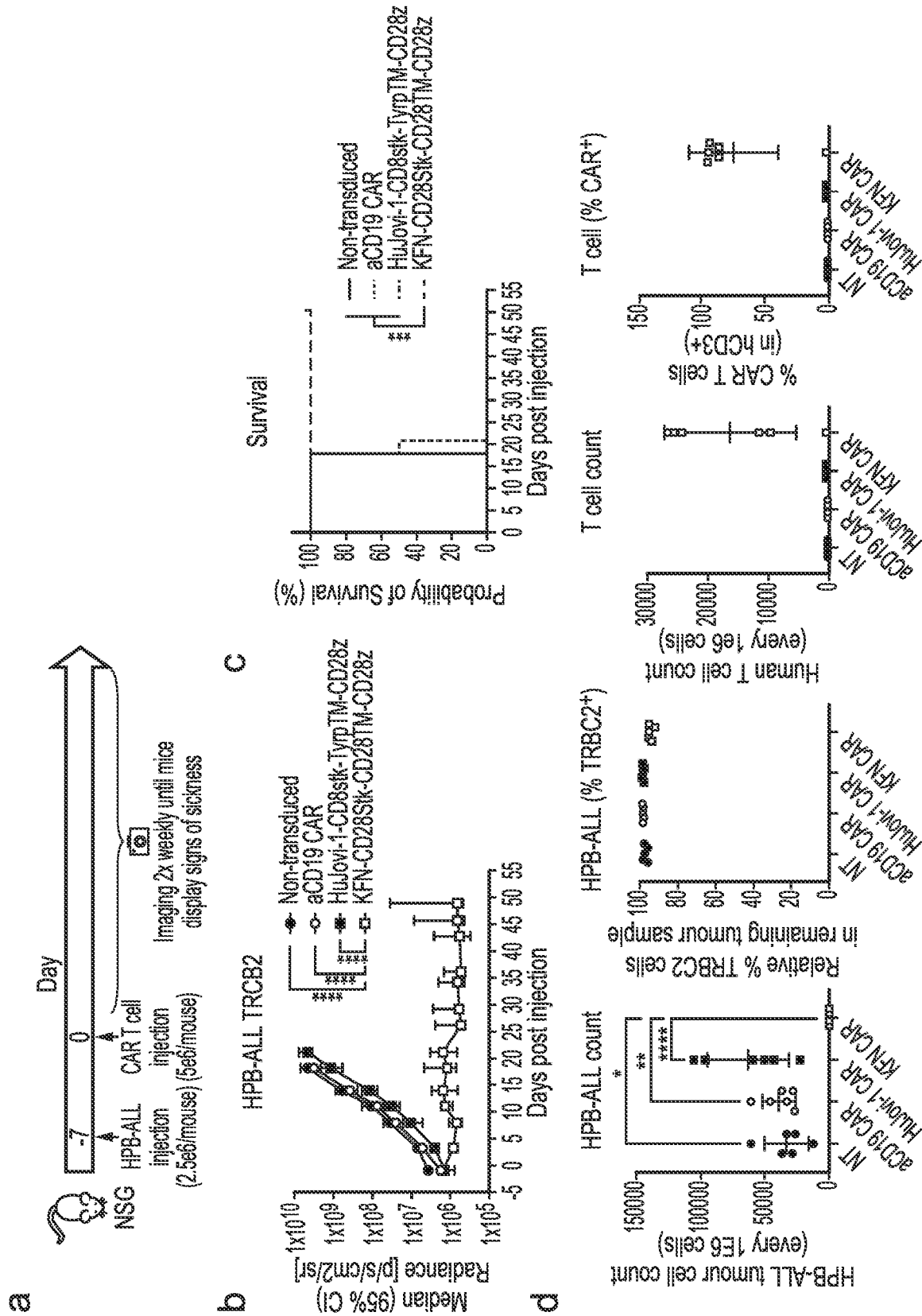


FIG. 10

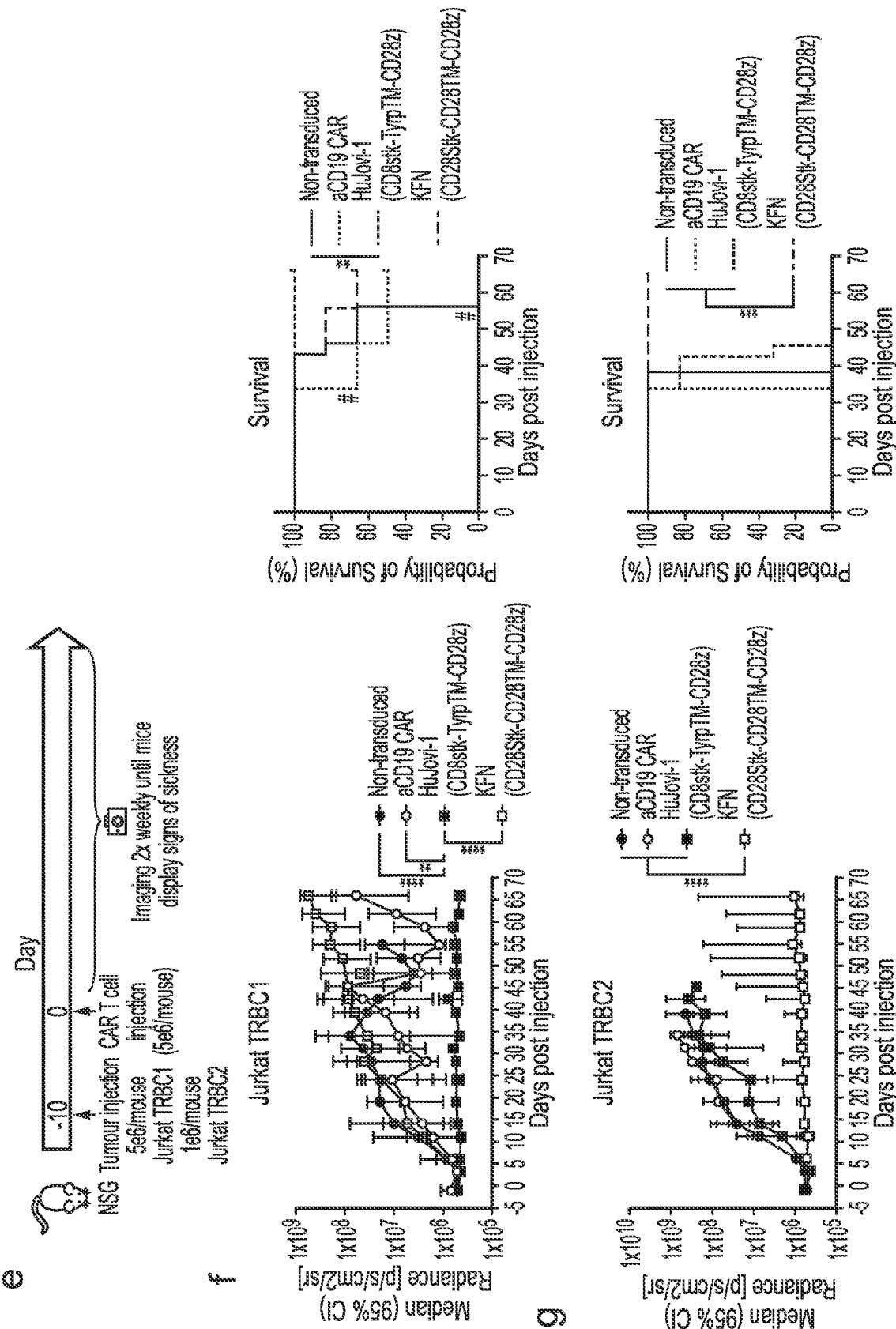


FIG. 10 (continued)

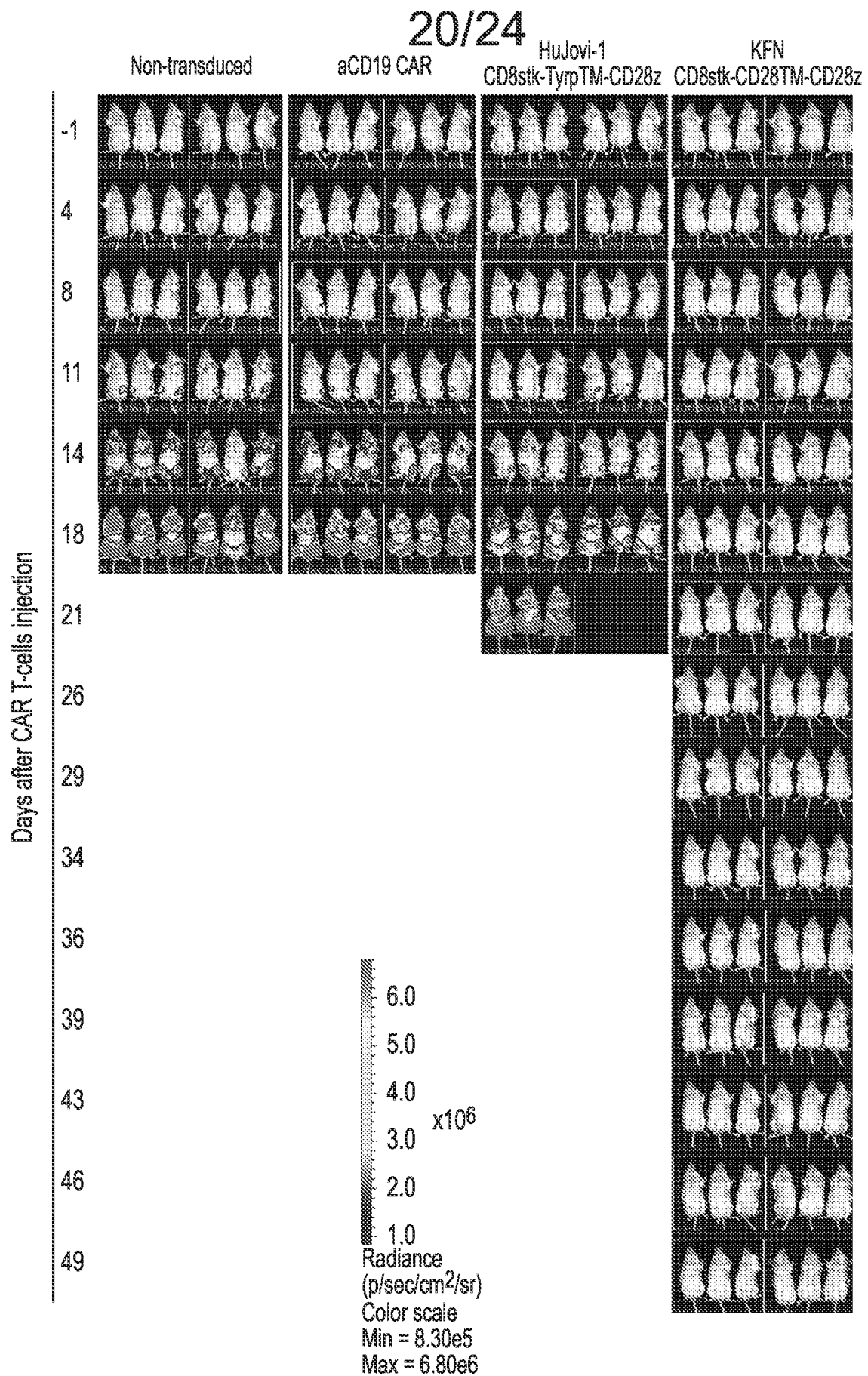


FIG. 11

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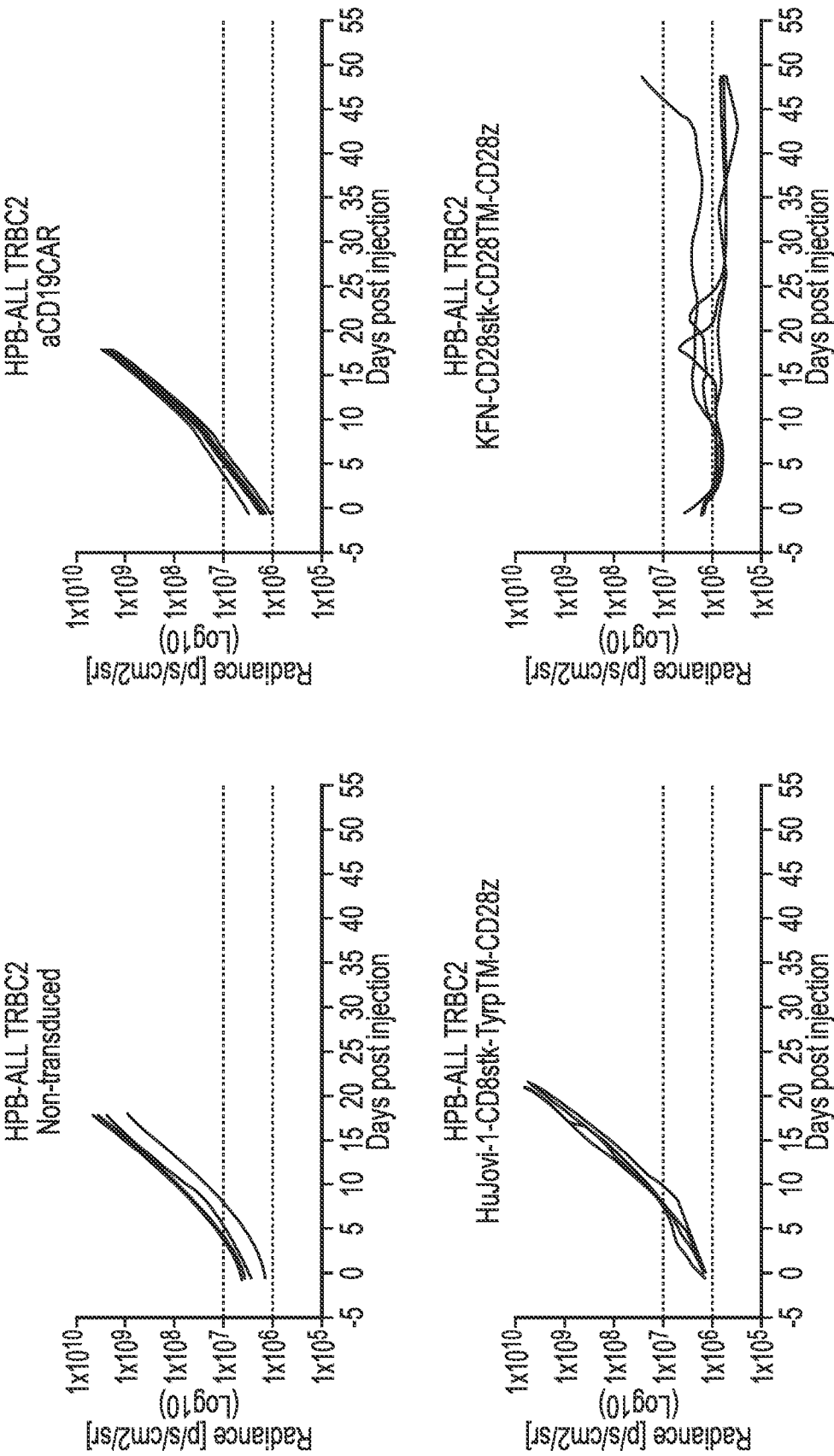


FIG. 12

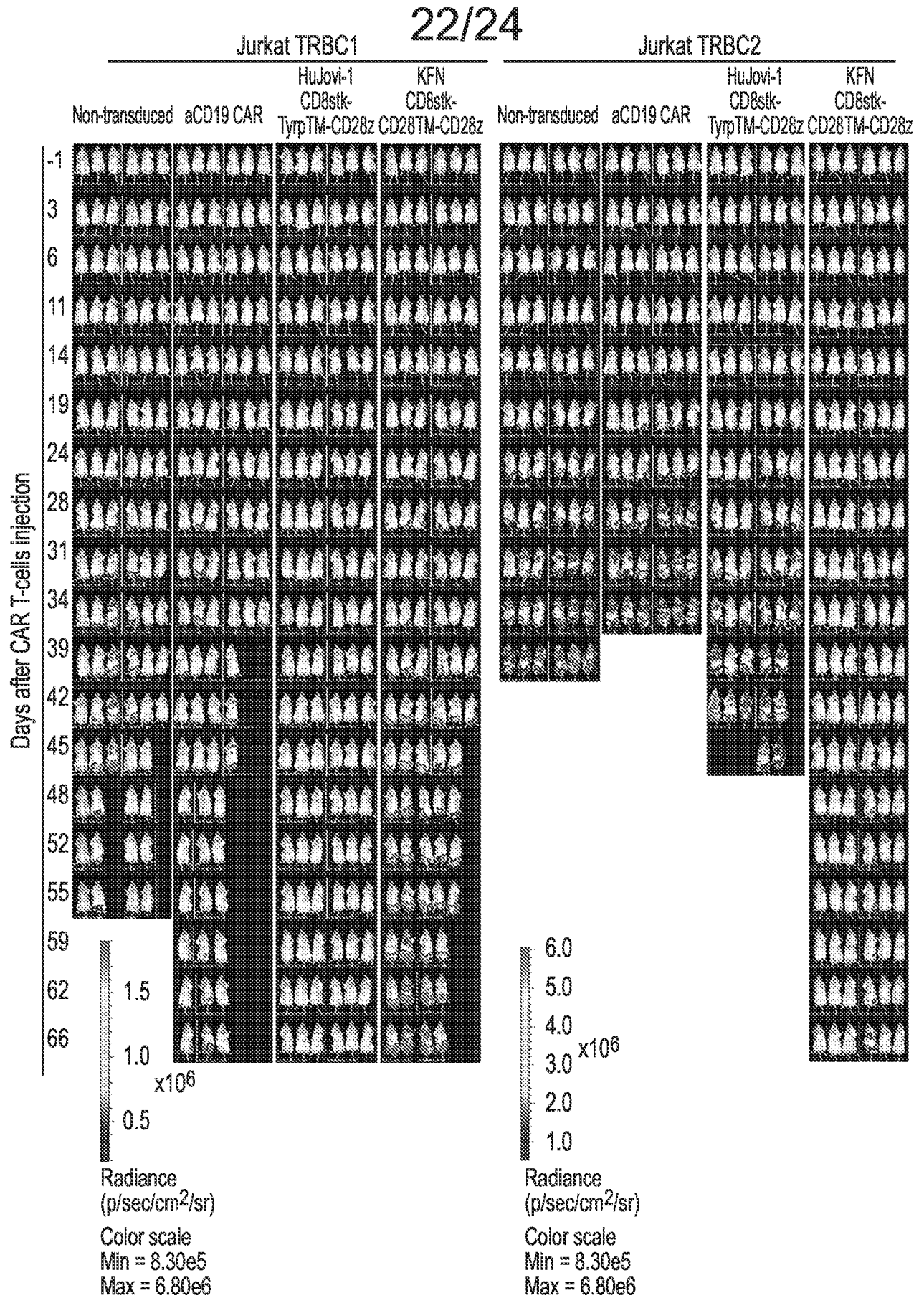
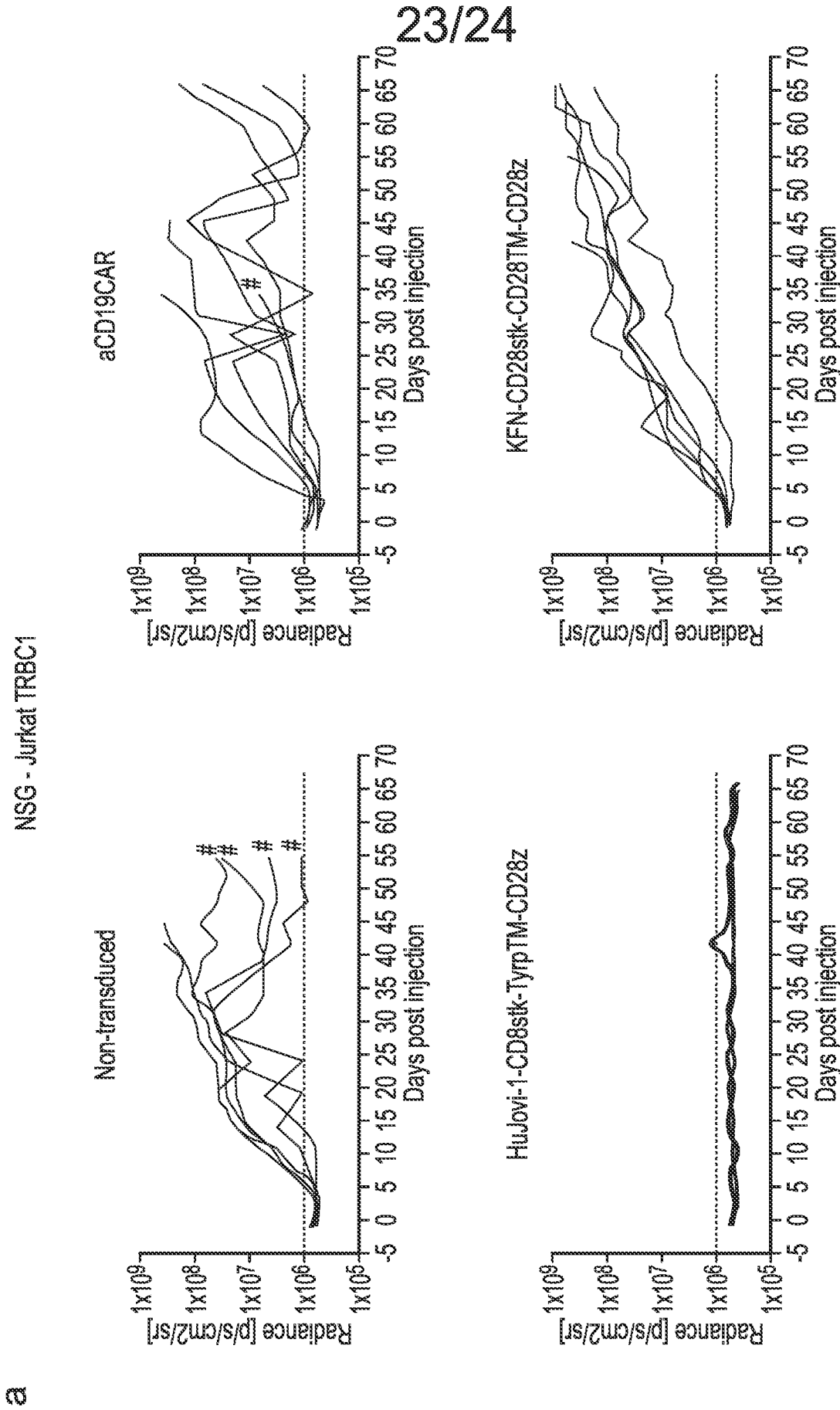


FIG. 13



b

NSG - Jurkat TRBC2

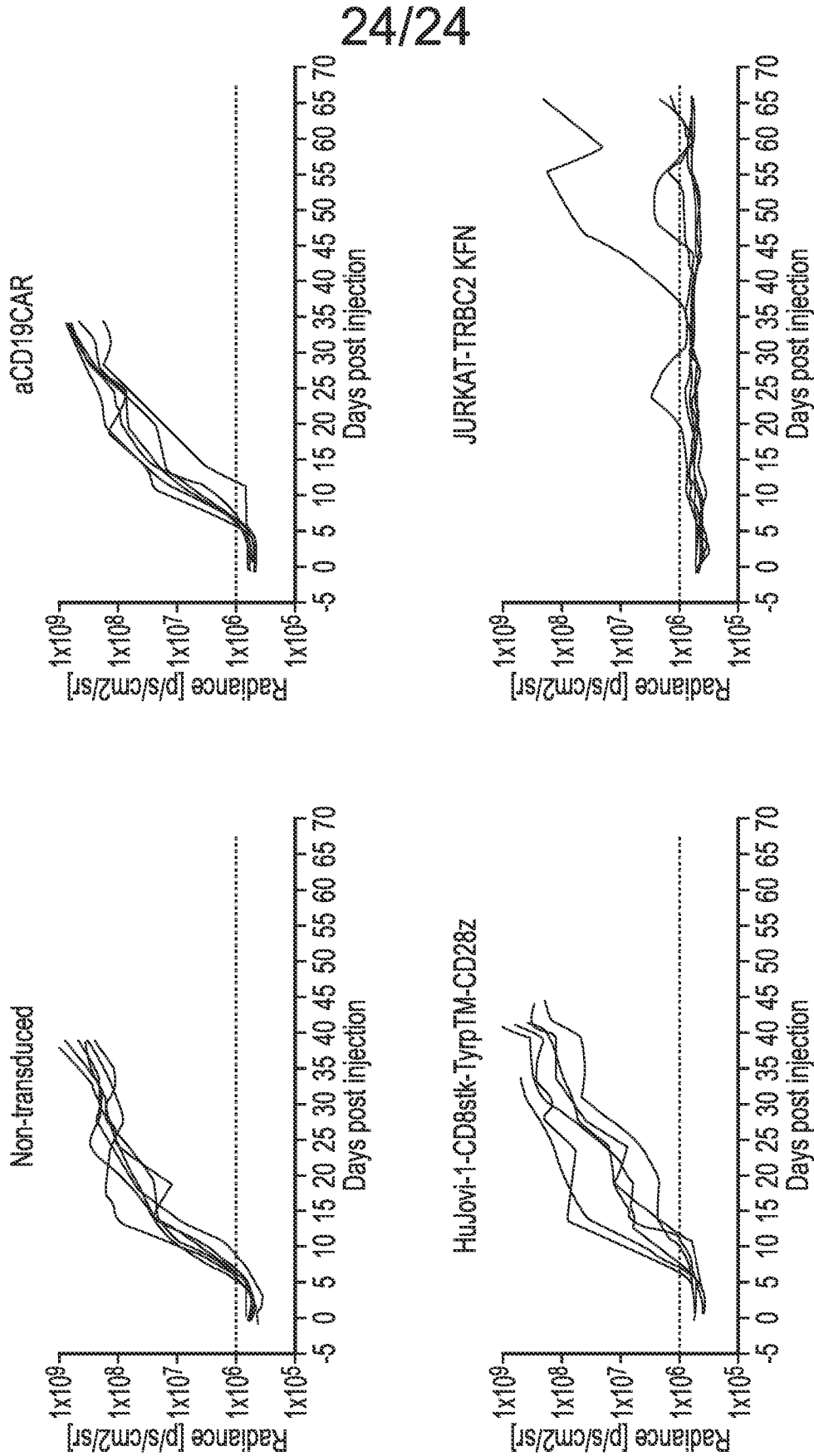


FIG. 14 (continued)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/051515

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2020/089644 A1 (AUTOLUS LTD [GB]) 7 May 2020 (2020-05-07) cited in the application page 4, line 23 - page 6, line 20 page 16, line 20 - page 22, line 20 page 22, line 33 - page 31, line 32 -----	1 - 29
X	WO 2018/224844 A1 (AUTOLUS LTD [GB]) 13 December 2018 (2018-12-13) cited in the application page 3, line 1 - page 7, line 26 page 8, line 1 - page 11, line 21 page 26, line 20 - page 38, line 18 -----	2, 6 - 29
A	US 2022/098300 A1 (CORDOBA SHAUN [GB] ET AL) 31 March 2022 (2022-03-31) paragraphs [0036], [0037], [0081] - [0086], [0089], [0188] - [0239] ----- - / - -	1 - 29



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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"&" document member of the same patent family

Date of the actual completion of the international search

14 October 2024

Date of mailing of the international search report

18/10/2024

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Page, Michael

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2024/051515

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2017/066827 A1 (PULÉ MARTIN [GB] ET AL) 9 March 2017 (2017-03-09) paragraphs [0003], [0004], [0023] - [0040], [0086] - [0131]; examples 4-6 -----	1 - 29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB2024/051515

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

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☐ forming part of the international application as filed.
 - b. ☒ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☒ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2024/051515

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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