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(54) **Title**: MODIFIED CELLS FOR IMMUNOTHERAPY

(57) **Abstract**: The present invention relates to engineered immune cells expressing antigen receptors, such as a T cell receptor (TCR) or a chimeric antigen receptor (CAR), as well as antibody targeting PD-Ll. Also provided are related nucleic acids, vectors, compositions and method for enhancing the immune response towards cancers and pathogens.

MODIFIED CELLS FOR IMMUNOTHERAPY

FIELD OF THE INVENTION

The present invention relates generally to engineered cells expressing an antigen receptor and an antibody blocking PD-L1 as well as methods of using the same for the treatment cancer and other disorders.

BACKGROUND

Immunotherapy with antigen- specific immune cells, such as T, NK cells or NKT cells offers a promising approach for the treatment of different types of diseases, e.g., cancers.

One therapeutic strategy is by engineering immune cells to express chimeric antigen receptors (CARs) which specifically target tumor cells and typically comprise an extracellular antigen recognition domain, a transmembrane domain, and an intracellular signaling domain derived from, for example, the T-cell receptor CD3-zeta chain. The signaling domain may be linked to one or more costimulatory molecule endodomains.

Still another approach is the transfer of antigen-specific T cell receptors (TCRs) with defined specificity into immune cells.

Cell-based immunotherapy has a major advantage over the currently available immunotherapies based on monoclonal antibodies, due to the potential of such genetically modified immune cells to traffic to sites of disease, to expand and to persist even after a single dose of administration.

Cancer cells utilize numerous pathways to escape the immune system. PD-L1 is often expressed on tumor cells and protects tumor cells from T cell-mediated destruction by binding PD-1. Up-regulated levels of PD-L1 correlate with increased tumor aggressiveness and an increased risk of death.

Animal studies demonstrated that blocking of the PD-L1:PD-1 interaction via monoclonal antibodies improves T cell activation and reduces tumor progression. In a clinical setting, monoclonal antibodies that block either PD-1 or PD-L1 have demonstrated impressive activity across a broad set of cancer subtypes, even at advanced and metastatic stages of disease (Maute et al (2015), PNAS, 24; 112(47): E6506–E6514).

Therefore, a therapeutic approach using antigen-specific immune cells in combination with anti-PD-L1 antibodies is promising. Particularly attractive is the generation of immune cells expressing both, an antigen receptor such as a TCR and/or a CAR and an antibody against PD-L1, as secretion of the antibody at the site of action of the immune cell would protect it

from inactivation. Moreover, such localized antibody delivery treatment approach has advantages over a systemic approach as to amount of drug to be administered as well as potential side-effects.

WO2014134165 describes the co-expression of a chimeric antigen receptor (CAR) and an
5 anti-mouse PD-1 scFv in mouse T cells. The scFv was derived from the Armenian hamster anti-PD-1 antibody clone J43 as described in US7,858,746. The scFv construct was cloned into a retroviral backbone expressing a CAR targeting human CD19 or human MUC-CD. Primary murine T cells were transduced with the respective constructs.

Both Suarez E. et al, Oncotarget. 2016 Jun 7; 7(23): 34341–34355 and WO2016100985
10 describe armored CAR T cells targeting human anti-carbonic anhydrase IX (CAIX) and secreting human anti-PD-L1 antibodies. Local antibody delivery at the tumor site led to marked inhibition of immune checkpoint pathways. In a murine model, tumor growth diminished 5 times and tumor weight reduced 50–80% when compared with the anti-CAIX CAR T cells alone. The antibody was of IgG1 isotype and therefore capable of mediating
15 ADCC, which led to human NK cells recruitment to the tumor site in the vivo model.

However, as a therapeutic has yet to be commercialized, there is still a significant unmet medical need to provide effective combination therapies involving immune cells expressing both antigen receptors and antibodies against immune checkpoint inhibitors.

20 SUMMARY OF THE INVENTION

Thus, in one aspect, the invention provides for an engineered cell expressing:

i) an antigen receptor, and
ii) an antibody that binds and blocks PD-L1.

Such antibody may be a humanized antibody or a fully human antibody. In some
25 embodiments, the antibody may comprise at least one CDR sequence as set forth in SEQ ID NOs.: 3, 4, 5, 6, 7 and 8 or variants thereof.

Said antibody preferably binds to an epitope on PD-L1 such that PD-L1 interaction with both CD80 and PD-1 is inhibited. Further to PD-1, PD-L1 binds to CD80, a membrane receptor expressed on T cells and B cells, although the binding affinity of PD-L1 for CD80
30 is much lower than for PD-1. PD-L1 binding to either PD-1 or CD80 transmits inhibitory signals to T-lymphocytes, suppressing T-cell migration, proliferation and secretion of cytotoxic mediators, and reducing tumor cell killing. However, while PD-1/PD-L1 interaction drives T cell exhaustion, PD-L1/CD80 interaction drives T cell anergy. These are distinct processes as exhaustion is progressive over a period of weeks or months and depends

on the chronic antigen stimulus, while anergy is induced rapidly after antigen stimulation in the absence of appropriate costimulation.

The cell is preferably a therapeutic cell. Suitable cells may e.g. be a T cell, a Natural Killer T (NKT) cell, a natural killer (NK) cell, a human embryonic stem cell, or a hematopoietic stem cell (HSC) or induced pluripotent stem cells (iPS). Provided herein are engineered immune cells expressing an antigen receptor and an antibody.

In one embodiment, the cell is T cell. Such T cell may be a cytotoxic T lymphocyte (CTL), a regulatory T lymphocyte, an inflammatory T- lymphocytes, a helper T-lymphocyte, or a gamma-delta T cell.

In one embodiment, the cell is a NKT cell.

In one embodiment, the cell is of human in origin. In one embodiment, the cell is autologous; in one embodiment, the cell is allogenic.

In one embodiment, the antigen receptor is specific to a cancer antigen, for example a antigen which is only expressed on a cancer cell or is upregulated on a cancer cell.

In one embodiment, the antigen receptor is T cell receptor, for example a native T cell receptor (for example stimulated to be specific to antigen or selected for its specificity to antigen) or an engineered T cell receptor.

In one embodiment, the antigen receptor is a chimeric antigen receptor (CAR).

In one embodiment, the antigen receptor is membrane anchored.

In one embodiment, an immune cell according to the present disclosure may comprise a TCR and a CAR, for example a native TCR and a CAR (in particular specific to cancer antigen). Alternatively, the immune cell of the present disclosure (such as an NK cell) may comprise an engineered TCR and a CAR.

Exemplary engineered immune cells described herein show enhanced tumor-killing activity compared to engineered immune cells expressing the antigen receptor only. For example, immune cells encoding both an antigen receptor and an anti-PD-L1 antibody can be effective in killing cancer cells for longer periods of time than corresponding cells encoding only an antigen receptor. Thus, cells according to the present disclosure are less susceptible to exhaustion, especially in the tumor microenvironment.

Further provided are a nucleic acid encoding the antigen receptor and the antibody as described herein, as well as vectors comprising such nucleic acid.

Also provided are methods of generating an immune cell as described herein, comprising the steps of:

(a) Providing an immune cell,

(b) Introducing into said cell at least one nucleic acid encoding said antigen receptor and at least one nucleic acid encoding said antibody; and

(c) Expressing said nucleic acids by said cell.

Further provided are pharmaceutical compositions comprising

i) an effective amount of the engineered immune cell or of the expression vector described herein, and

ii) a pharmaceutically acceptable excipient.

Also provided are the engineered immune cells, the expression vector or the pharmaceutical composition as described herein for use in therapy. Further provided are methods of treating a subject in need thereof comprising:

(a) Providing the recombinant immune cell as described herein;

(b) Administrating said immune cells to said subject.

The present invention provides substantial benefits over alternatives such as the combined use of an engineered immune cell and a purified checkpoint inhibitor antibody. In attempting to use an engineered immune cell in combination with a checkpoint inhibitor antibody, the skilled practitioner will recognize that the timing of antibody delivery is a crucial factor. Furthermore, the local concentration of the antibody is also important. The antibody offering protective function should be present when cell contact is established between the antigen receptor expressing cells (effector cells) and their target cells, e.g., at the tumor site. For the medical practitioner, it is practically impossible to determine the precise timing of systemic antibody administration in a traditional antibody formulation, for example by infusion, to provide the antibody at the requisite cancer location. Furthermore, full-length antibodies are generally not able to cross the blood brain barrier. Thus, systemic delivery of a traditional (i.e., full-length) antibody formulation will not usually reach cancers in the brain. Thus, the antibody is administered systemically in high doses, thereby increasing the possibility of side-effects. In contrast, the instant therapeutic approach offers a controlled release of the

antibody at the site of action, thereby improving anti-tumor efficacy therapy and decreasing the likelihood of side effects for the subject.

The therapy or method of treatment of the invention may be in combination with one or more therapies selected from the group of antibody therapy, chemotherapy, cytokine therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

Further provided are kits for treatment of cancer, pathogen infection, and/or an autoimmune disorder comprising the engineered immune cell or the expression vector as described herein, and written instructions for use.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the design of the scFv anti-PD-L1 retroviral construct SFG.scFv.anti-PD-L1(I)eGFP having 8510 bp. The NcoI restriction site at 5' end, the SphI restriction site at 3' end and the TGA stop codon were added to the scFv anti-PD-L1 DNA by PCR. PCR product was cloned into the retroviral vector SFG(I)eGFP to obtain the final vector SFG.scFv.anti-PD-L1(I)eGFP. The reporter gene eGFP expressed upon IRES is used to assess the transduction efficiency. LTR: long terminal repeat; SD: splicing donor; PS: packaging signal; TGG: truncated gagpol; SA: splicing acceptor; SP: signal peptide; VL: variable light chain of the scFv; L: linker; VH: variable heavy chain of the scFv; IRES: internal ribosomal entry site; eGFP: enhanced green fluorescent protein.

Figure 2 shows that CD4 and CD8 T cells could be transduced with the scFv anti-PD-L1 retroviral construct without alterations of the CD4/CD8 ratio. Transduction efficiency of the SFG.scFv.anti-PD-L1(I)eGFP vector is shown as percentage of CD4 (Figure 2 A) or CD8 (Figure 2 B) T cells expressing eGFP. Figure 2 C: CD4/CD8 ratio of T cells. Mean and s.d are shown (n=4 independent experiments) *P<0.1 by paired t test. NT: Non Transduced T cells - PD-L1: T cells transduced only with SFG.scFv.anti-PD-L1(I)eGFP vector; CAR.28 PD-L1: T cells co-transduced with SFG.scFv.anti-PD-L1(I)eGFP vector and GD2.CAR encoding the CD28 endodomain; CAR.BB PD-L1: T cells co-transduced with SFG.scFv.anti-PD-L1(I)eGFP vector and GD2.CAR encoding the 4-1BB endodomain.

Figure 3 shows that T cells co-expressed GD2.CAR and eGFP anti-PD-L1 upon double retroviral transduction. Figure 3 A: Transduction efficiency of the SFG.scFv.anti-PD-L1(I)eGFP vector. Shown is the percentage of eGFP positive T cells. Figure 3 B: Transduction efficiency of GD2.CAR encoding either the CD28 or the 4-1BB endodomains.

Figure 3 C: Expression of GD2.CAR shown as RFI (Relative Fluorescence Intensity). Figure 3 D: Percentage of T cells co-transduced with the GD2.CAR and the scFv anti-PD-L1 retroviral vectors. Figure 3 E: Representative plots of T cells at day 10 after the T cell initiation. Mean and s.d are shown (n=6 independent experiments) *P<0.1, **P<0.01 by paired t test. NT: Non Transduced T cells, CAR.28: T cells transduced only with GD2.CAR encoding the CD28 endodomain; CAR.BB: T cells transduced only with GD2.CAR encoding the 4-1BB endodomain; - PD-L1: T cells transduced only with SFG.scFv.anti-PD-L1(I)eGFP vector; CAR.28 PD-L1: T cells co-transduced with SFG.scFv.anti-PD-L1(I)eGFP vector and GD2.CAR encoding the CD28 endodomain; CAR.BB PD-L1: T cells co-transduced with SFG.scFv.anti-PD-L1(I)eGFP vector and GD2.CAR encoding the 4-1BB endodomain;. Taken together these results indicate that T cells can be co-transduced with SFG.scFv.anti-PD-L1(I)eGFP and GD2.CAR vectors and that the transduction with SFG.scFv.anti-PD-L1(I)eGFP vector does not alter the expression level of GD2.CAR.

Figure 4 shows that transduction with the scFv anti-PD-L1 did not affect T cell proliferation. Fold increase of T cells calculated as number of T cells at day 6 (Figure 4 A) and at day 12 (Figure 4 B) after activation divided by number of T cells at 2 days before transduction. Mean and s.d are shown (n=6 independent experiments). NT: Non Transduced T cells, CAR.28: T cells transduced only with GD2.CAR encoding the CD28 endodomain; CAR.BB: T cells transduced only with GD2.CAR encoding the 4-1BB endodomain; - PD-L1: T cells transduced only with SFG.scFv.anti-PD-L1(I)eGFP vector; CAR.28 PD-L1: T cells co-transduced with SFG.scFv.anti-PD-L1(I)eGFP vector and GD2.CAR encoding the CD28 endodomain; CAR.BB PD-L1: T cells co-transduced with SFG.scFv.anti-PD-L1(I)eGFP vector and GD2.CAR encoding the 4-1BB endodomain;

Figure 5 shows that expression of the anti-PD-L1 scFv did not affect T cell subset compositions. Figure 5 A: Scheme of T lymphocyte subpopulations determined by the expression of CD62L, CD45RA and CD95 [Naïve (TN) CD62L+CD45RA+CD95-, Stem Cell Memory (TSCM) CD62L+CD45RA+CD95+, Central Memory (TCM) CD62L+CD45RA-, Effector Memory (TEM) CD62L-CD45RA-, Effector T cell (T_{EFF}) CD62L-CD45RA+]. T cell subset compositions (Figure 5 B), percentage of CD27 (Figure 5 C), CD28 (Figure 5 D) and PD-1 (Figure 5 E) positive cells 10 days after stimulation with immobilized anti-CD3/CD28. Mean and s.d. are shown (n=4 independent experiments).

Figure 6 shows that the anti-PD-L1 scFv is released by transduced T cells. Figure 6 A: Quantification of anti-PD-L1 scFv released from Non-transduced (NT) and anti-PD-L1 scFv transduced T cells (scFv PD-L1) in T cell medium with 10% FBS. T cells were seeded and

activated with immobilized anti-CD3/CD28 antibodies. The supernatant was collected after 18 hours and anti-PD-L1 scFv quantified by a specific sandwich ELISA. Figure 6 B: The anti-PD-L1 scFv secreted by transduced T cells binds PD-L1. The cell culture supernatants of non-transduced (NT) and anti-PD-L1 transduced T cells (scFv PD-L1) were tested for
5 their binding to recombinant human PD-L1. Anti-PD-L1 scFv produced in *E. coli* was used as a reference control (scFv control).

Figure 7 shows that GD2.CAR T cells with the 4-1BB endodomain transduced with the anti-PD-L1 scFv show better killing of tumor cells in the second cycle of co-culture compared to GD2.CAR T cells with the 4-1BB endodomain that are not secreting the scFV anti-PD-L1..
10 Figure 7 A shows that in the first cycle (7 days) of antigen stimulation GD2.CAR-Ts encoding 4-1BB efficiently eliminated tumor cells and the presence of the secreted anti-PD-L1 scFv does not show any impairment of the GD2.CAR-T cytotoxic function. During the second cycle (14 days) GD2.CAR T cells with the 4-1BB endodomain transduced with the anti-PD-L1 scFv showed enhanced killing of tumor cells than the GD2.CAR T cells without
15 the anti-PD-L1 scFv. Representative plots of T cells at the end of the first (7 days of culture; Figure 7 B) and second cycle of co-culture (14 days of culture; Figure 7 C) (T cells identifies as CD3+ and tumor cells CHLA-255 identified as GD2+ cells, respectively). E:T ratio 1:5. CHLA: tumor cells, T cells: T cells alone with no tumor cells, Tc NT: Non Transduced T cells, Tc PD-L1: T cells transduced only with SFG.scFv.anti-PD-L1(I)eGFP vector, Tc
20 CAR-41BB: T cells transduced only with GD2.CAR encoding the 4-1BB endodomain, Tc CAR-41BB PD-L1: T cells co-transduced with SFG.scFv.anti-PD-L1(I)eGFP vector and GD2.CAR encoding the 4-1BB endodomain.,

Figure 8 shows that in the first cycle (7 days) of antigen stimulation GD2.CAR-Ts encoding 4-1BB efficiently release IFNgamma and the presence of the secreted anti-PD-L1 scFv does
25 not show any impairment of the GD2.CAR-T function (IFNgamma release). During the second cycle (14 days) GD2.CAR T cells with the 4-1BB endodomain transduced with the anti-PD-L1 scFv showed enhanced release of IFNgamma compared to GD2.CAR T cells without the anti-PD-L1 scFv IFNgamma ELISA assay to quantify the IFNgamma produced in the first 24 hours after the first (7 days of culture; Figure 8 A) and second (14 days of
30 culture; Figure 8 B) tumor specific stimulation.

As used throughout the Figures “CAR.BB” refers to “CAR.41BB” as well as “CAR.4-1BB”.

DETAILED DESCRIPTION

Unless otherwise defined, all other scientific and technical terms used in the description, figures and claims have their ordinary meaning as commonly understood by one of ordinary skill in the art. Although similar or equivalent methods and materials to those described herein can be used in the practice or testing of the engineered cells, antibodies, antigen receptors, nucleic acids, vectors, compositions, methods and uses disclosed herein, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will prevail. The materials, methods, and examples are illustrative only and not intended to be limiting.

The term "engineered immune cell" as used herein refers to an immune cell which was genetically modified to express the proteins described herein.

As used herein, the term "antigen receptor" refers to a receptor that is capable of activating an immune cell in response to antigen binding. Exemplary antigen receptors may be endogenous (i.e., native) or recombinant T cell receptors (TCRs) or chimeric antigen receptors (CARs). TCRs are membrane-anchored heterodimeric proteins expressed on immune cells. Upon binding to antigenic molecules presented by antigen presenting cells, the immune cell is activated. Whereas some TCRs comprise variable alpha and beta chains, others comprise gamma and delta chains, the chains being expressed as part of a complex with the invariant CD3 chain molecules. Each of the alpha and beta chain may comprise a variable region and a constant region, both being located extracellulary, wherein each variable domain has three complementarity determining regions (CDRs) which enable binding of the TCR to the peptide/MHC complex. The variable region of the beta chain has an additional hypervariable region HV4 which typically does not contact antigen and is therefore not considered a CDR (see.e.g. Richman, S.A. et al, Mol Immunol. 2009; 46(5): 902-916).

CARs typically comprise an extracellular domain (ectodomain), a transmembrane domain and a cytoplasmic domain (endodomain). The ectodomain provides antigen recognition and is most commonly a scFv but other antibody formats may also be used. The scFv is connected via a spacer to the transmembrane domain, which is then connected to an endodomain. First generation CARs had a simple structured endodomain comprising CD3-zeta. Upon antigen binding, receptors cluster and an activation signal is transmitted to the cell. To increase the activation signal, second generation CARs further include a co-stimulatory domain, such as CD28, OX40 and/or 4-1BB, and third generation CARs

include two or more co-stimulatory domains (Maus MV et al (2014) Blood, 123: 2625-2635). Apart from CD3-zeta, other ITAM-containing domains have been explored including the Fc receptor for IgE-γ domain.

In some embodiments, binding of the antigen to the antigen receptor activates the immune
5 cell through induction of signal transduction or changes in protein expression in the immune cell which results in initiation of an immune response.

The term "endogenous" refers to a nucleic acid or a polypeptide that is normally expressed in a cell or tissue, absent recombinant engineering.

As used herein, "PD-L1" refers to the protein also known as "programmed cell death ligand
10 1," "cluster of differentiation 274 (i.e., CD274)" or "B7 homolog 1 (i.e., B7-H1)". The native protein comprises two extracellular domains, a transmembrane domain, and a cytoplasmic domain. The term encompasses full-length and/or unprocessed PD-L1 as well as any intermediate resulting from processing in the cell. PD-L1 can exist as a transmembrane protein or as a soluble protein; thus, the term as used herein may refer to the full length or
15 the extracellular domain of the protein. The term also encompasses naturally occurring variants of PD-L1, e.g., splice variants or allelic variants. The protein may additionally contain a tag, such as a his tag or Fc tag. The amino acid sequence of exemplary human full-length PD-L1 protein can e.g. be found under NCBI protein database accession number NP_054862.The term "hPD-L1" refers to human PD-L1 and comprises natural hPD-L1 and
20 recombinant human rhPD-L1. "rPD-L1" refers to recombinant PD-L1. Recombinant PD-L1 may or may not have an amino terminal methionine residue, depending upon the method by which it is prepared. "rhPD-L1" refers to recombinant human PD-L1. Likewise, PD-L1 may also be obtained by isolation from biological samples of human or non-human origin. rhPD-L1 may, e.g., be obtained from RnD Systems, USA, cat. no. 156-B7, or from Peprotech,
25 USA, cat. no. 310-35. "Monkey PD-L1" refers to PD-L1 of Rhesus macacque (Macaca mulatta). The amino acid sequence of exemplary monkey PD-L1 protein can e.g. be found under NCBI protein database accession number NP_001077358. Monkey PD-L1 may, e.g., be obtained from Sino Biological, China, cat. no. 90251-C02H. "Rat PD-L1" refers to PD-L1 of Rattus norvegicus (Norway rat). The amino acid sequence of exemplary rat PD-L1
30 protein can e.g. be found under NCBI protein database accession number NP_001178883 Rat PD-L1 may, e.g., be obtained from Sino Biological, China, cat. no. 80450-R02H. "Mouse PD-L1" refers to PD-L1 of Mus musculus. The amino acid sequence of exemplary mouse PD-L1 protein can e.g. be found under NCBI protein database accession number

NP_068693Mouse PD-L1 may, e.g., be obtained from Sino Biological, China, cat. no. 50010-M03H or from RnD Systems, USA, cat. no. 1019-B7-100.

"PD-1" is the programmed cell death protein 1, also known as CD279 is a cell surface receptor for PD-L1. PD-1 binds two ligands, PD-L1 and PD-L2. PD-1 is a transmembrane
5 protein including an extracellular domain followed by a transmembrane region and an intracellular domain. The term encompasses full-length and/or unprocessed PD-1 as well as any intermediate resulting from processing in the cell. PD-1 can exist as a transmembrane protein or as a soluble protein; thus, the term as used herein may refer to the full length or the extracellular domain of the protein. The term also encompasses naturally occurring
10 variants of PD-1, e.g., splice variants or allelic variants. The protein may additionally contain a tag, such as a his tag or Fc tag. The amino acid sequence of exemplary human PD-1 protein can e.g. be found under NCBI protein database accession number NP_005009 The term "hPD-1" refers to human PD-1 and comprises its natural form (hPD-1) as well as the recombinant human form (rhPD-1). "rPD-1" refers to recombinant PD-1.

15 "CD80" refers to the cluster of differentiation 80, also known as B7-1, B7.1, BB1, CD28LG, CD28LG1, LAB7. It is a membrane receptor for CD28 and CTLA-4 as well as PD-L1 and comprises extracellular domain followed by a transmembrane region and an intracellular domain. The term encompasses full-length and/or unprocessed CD80 as well as any intermediate resulting from processing in the cell. CD80 can exist as a transmembrane
20 protein or as a soluble protein; thus, the term as used herein may refer to the full length or the extracellular domain of the protein. The term also encompasses naturally occurring variants of CD80, e.g., splice variants or allelic variants. The protein may additionally contain a tag, such as a his tag or Fc tag. The amino acid sequence of exemplary human CD80 protein can e.g. be found under NCBI protein database accession number NP_005182,
25 CD80 may, e.g., be obtained from RnD Systems, USA, cat. no. 9050-B1-100.". The term "hCD80" refers to human CD80 and comprises its natural form (hCD80) as well as the recombinant human form (rhCD80). "rCD80" refers to recombinant CD80.

"PD-L2" refers to the protein also known as "Programmed cell death 1 ligand 2", "B7-DC", or "CD273" (cluster of differentiation 273). The term as used herein encompasses full-length
30 and/or unprocessed PD-L2 as well as any intermediate resulting from processing in the cell. PD-L2 can exist as a transmembrane protein or as a soluble protein; thus, the term as used herein may refer to the full length or the extracellular domain of the protein. The term also encompasses naturally occurring variants of PD-L2, e.g., splice variants or allelic variants. The protein may additionally contain a tag, such as a his tag or Fc tag. The amino acid

sequence of exemplary human full-length PD-L2 protein can e.g. be found under NCBI protein database accession number NP_079515. PD-L2 may, e.g., be obtained from RnD Systems, USA, cat. no. 1224-PL. The term “rhPD-L2” refers to recombinant human PD-L2. "B7-H3" refers to the protein also known as CD276 (Cluster of Differentiation 276). The term as used herein encompasses full-length and/or unprocessed B7-H3 as well as any intermediate resulting from processing in the cell. B7-H3 can exist as a transmembrane protein or as a soluble protein; thus, the term as used herein may refer to the full length or the extracellular domain of the protein. The term also encompasses naturally occurring variants of B7-H3, e.g., splice variants or allelic variants. The protein may additionally contain a tag, such as a his tag or Fc tag. The amino acid sequence of exemplary human full-length B7-H3 protein can e.g. be found under NCBI protein database accession number NP_079516. B7-H3 may, e.g., be obtained from RnD Systems, USA, cat. no. 1027-B3. The term “rhB7-H3” refers to recombinant human B7-H3.

T cell exhaustion as employed herein is a state of T cell dysfunction that arises during many chronic viral infections, autoimmunity and cancer. It is characterized by poor effector function, sustained expression of inhibitory receptors and a transcriptional state which is distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of infectious conditions and tumors, i.e., in particular in chronic environment.

Because anti-tumor T cells are persistently exposed to antigen in the tumor microenvironment, they are particularly susceptible to exhaustion. Exhaustion is a likely mechanism contributing to T cell dysfunction in cancer patients. Accordingly, exhausted T cells have been reported for melanoma patients as well as patients with ovarian cancer and hepatocellular carcinoma. Exhausted T cells express multiple inhibitory receptors including PD-1 and LAG-3, and progressively lose cytotoxic and proliferative potential. Ultimately, they may be driven to apoptosis. Expression of high levels of inhibitory receptors, includes programmed cell death protein 1 (PD-1), lymphocyte activation gene 3 protein (LAG-3), T-cell immunoglobulin domain and mucin domain protein 3 (TIM-3), cytotoxic T lymphocyte antigen-4 (CTLA-4), band T lymphocyte attenuator (BTLA) and T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT). The other principal characteristic of exhausted T cells is the progressive loss of their ability to express effector cytokines. Typically, interleukin-2 (IL-2) production and ex vivo killing capacity are lost at the early stage of exhaustion. At the intermediate stage, tumor necrosis factor-alpha (TNF-alpha) production is lost. Finally, at the advanced stage of exhaustion, interferon-gamma (IFN-gamma) and granzyme B (GzmB) production are lost. The first evidence connecting

exhausted T cells with tumor microenvironment was that programmed cell death ligand 1 was overexpressed. See for example the review T-cell exhaustion in the tumor microenvironment, Jiang et al., Cell Death and Disease (2015) 6, e1792.

Whereas T cell exhaustion is a result of chronic over-stimulation, T cell anergy typically refers to a hyporesponsive state which is induced by triggering the TCR either (i) without adequate concomitant co-stimulation through CD28 or (ii) in the presence of high co-inhibitory molecule signaling. As a result thereof, IL-2 is not effectively transcribed, but anergy-associated genes such as GRAIL are expressed instead which contribute to impaired TCR signaling via negative feedback.

The term “isolated” indicates that matter such as a peptide, a nucleic acid molecule or a cell has been removed from its normal physiological environment, e.g. a natural source, or that a peptide or nucleic acid is synthesized. Use of the term “isolated” indicates that a naturally occurring sequence has been removed from its normal cellular (e.g., the chromosomal or cellular) environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. “Isolated” in reference to a polypeptide or nucleic acid molecule means a polymer of amino acids (2 or more amino acids) or nucleotides coupled to each other, including a polypeptide or nucleic acid molecule that is isolated from a natural source or that is synthesized. The term “isolated” does not imply that the sequence is the only amino acid chain or nucleotide chain present, but that it is essentially free of e.g. non-amino acid material and/or non-nucleic acid material, respectively, naturally associated with it. An "isolated cell" refers to a cell that is separated from the molecular and/or cellular components that naturally accompany the cell.

A “variant” refers to an amino acid or nucleic acid sequence which differs from the parental sequence by virtue of addition (including insertions), deletion, modification and/or substitution of one or more amino acid residues or nucleobases while retaining at least one desired activity of the parent sequence disclosed herein. In the case of antibodies such desired activity may include specific antigen binding. Similarly, a variant nucleic acid sequence may be modified when compared to the parent sequence by virtue of addition, deletion and/or substitution of one or more nucleobases, but the encoded antibody retains the desired activity as described above. Variants may be naturally occurring, such as allelic or splice variants, or may be artificially constructed.

The term “identity” as used herein refers to the sequence match between two proteins or nucleic acids. The protein or nucleic acid sequences to be compared are aligned for maximum correspondence over a comparison window, for example using bioinformatics

tools such as EMBOSS Needle (pair wise alignment; available at www.ebi.ac.uk) or by manual alignment and visual inspection. When the same position in the sequences to be compared is occupied by the same nucleobase or amino acid residue, then the respective molecules are identical at that very position. Accordingly, the "percent identity" is a function of the number of matching positions divided by the number of positions compared and multiplied by 100%. For instance, if 6 out of 10 sequence positions are identical, then the identity is 60%. Aligning sequences for maximum correspondence may require introducing gaps. The percent identity between two protein sequences can, e.g., be determined using the Needleman and Wunsch algorithm (Needlemann S.B. and Wunsch C.D. A general method applicable to the search for similarities in the amino acid sequence of two proteins. J. Mol. Biol. 1970, vol. 48, p.443) which has been incorporated into EMBOSS Needle, using a BLOSUM62 matrix, a "gap open penalty" of 10, a "gap extend penalty" of 0.5, a false "end gap penalty", an "end gap open penalty" of 10 and an "end gap extend penalty" of 0.5, or a method of aligning sequences manually introducing gaps in a manner which maximises identity can be used. Two molecules having the same primary amino acid or nucleic acid sequence are identical irrespective of any chemical and/or biological modification. For example, two antibodies having the same primary amino acid sequence but different glycosylation patterns are identical by this definition. In case of nucleic acids, for example, two molecules having the same sequence but different linkage components such as thiophosphate instead of phosphate are identical by this definition. Similarly, nucleobases that differ only because of exocyclic modifications, for example cytosine and 5-methyl-cytosine, are identical by this definition.

A sequence being longer than any of the sequences provided herein, for example because it comprises several variable domains or one or more constant domains, shall nevertheless be identical to the reference sequence disclosed herein if sequence identity over a comparison window is given. A comparison window as used herein includes the entire sequence as claimed.

The term "CDR" refers to the hypervariable regions of the antibody which mainly contribute to antigen binding. Typically, an antigen binding site includes six CDRs, embedded into a framework scaffold. Herein, the CDRs of the VL are referred to as CDR-L1, CDR-L2 and CDR-L3 whereas the CDRs of the VH are referred to as CDR-H1, CDR-H2 and CDR-H3. These can be identified as described in KABAT, E.A., et al. Sequences of Proteins of Immunological Interest. 5th edition. Edited by U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES. NIH Publications, 1991. p. 91-3242. CDR-H1 as

used herein, however, differs from the Kabat definition in that it starts with position 27 and ends prior to position 36 (AHo positions 28 to 42, inclusive).

As used herein, the numbering system to identify amino acid residue positions in the VH and VL of the antibody corresponds to the “AHo”-system described by Honegger A. and Plückthun A. Yet another numbering scheme for immunoglobulin variable domains: An automatic modelling and analysis tool. J. Mol. Biol. 2001, vol. 309, p.657. The publication further provides conversion tables between the AHo and the Kabat system (Kabat E.A. et al., Sequences of Proteins of Immunological Interest. 5th edition. Edited by U.S. Department of Health and Human Services. NIH Publications, 1991. No. 91-3242).

The term “framework” (FR) refers to the scaffold of the variable antibody domain, either the variable light chain (VL) or variable heavy chain (VH), embedding the respective CDRs. A VL and/or VH framework typically includes four framework sections, FR1, FR2, FR3 and FR4, flanking the CDR regions. Thus, as known in the art, a VL has the general structure: (FR-L1) – (CDR-L1) – (FR-L2) – (CDR-L2) – (FR-L3) – (CDR-L3) – (FR-L4), whereas a VH has the general structure: (FR-H1) – (CDR-H1) – (FR-H2) – (CDR-H2) – (FR-H3) – (CDR-H3) – (FR-H4).Various aspects of the disclosure are described in further detail in the following subsections. It is understood that the various embodiments, preferences and ranges may be combined at will. Further, depending of the specific embodiment, selected definitions, embodiments or ranges may not apply.

In a first aspect, the invention provides an engineered immune cell expressing:

i) an antigen receptor, and

ii) an antibody that blocks PD-L1.

Such immune cell may e.g. be a T cell, a Natural Killer T (NKT) cell, a natural killer (NK) cell, a human embryonic stem cell, a hematopoietic stem cell (HSC) or a induced pluripotent stem cell (iPS).

Said T cell may be a cytotoxic T lymphocyte (CTL), a regulatory T lymphocyte, an inflammatory T- lymphocytes, or a helper T-lymphocyte or a gamma-delta T cell. Additionally or alternatively, said T cell is a CD4+ or CD8+ or a mixed population of CD4+ and CD8+ cells.

In some embodiments, the antigen receptor is a chimeric antigen receptor (CAR). As explained above, CARs comprise a cytoplasmic domain acting as intracellular signaling domain, a transmembrane domain and an extracellular domain serving antigen recognition. The extracellular domain may be connected to a signal peptide, to direct the transport of the
5 domain to the cell surface. Said signal peptide may be cleavable.
Typically, a spacer or hinge region is present between the transmembrane domain and the extracellular domain. Such hinge region may e.g. be selected from the group consisting of a CD8a hinge, an IgG1 hinge or a FcyRll hinge.
In some embodiments, the CAR comprises a CD3 zeta, a CD4, a CD28, a CD8 alpha or a 4-
10 1BB transmembrane domain.
Additionally, the CAR may comprise one or more costimulatory domains, e.g., selected from the group consisting of CD28, 4-1BB (CD137), ICOS, or OX40 (CD134) costimulatory domains, or functional fragments thereof, respectively. In preferred embodiments, the CAR comprises 4-1BB costimulatory domain or a functional fragment thereof. Exemplary
15 sequences of CD28 and 4-1BB co-stimulatory domains are provided in SEQ ID NOs: 49 and 47, respectively.
Typically, the cytoplasmic domain comprises a CD3 zeta signaling domain. An exemplary sequence of a CD3 zeta signaling domain is given in SEQ ID NO: 48.
Corresponding sequences are well-known and available in the art. Exemplary sequences of
20 signal peptides, hinge regions, transmembrane domains are provided in Figure 1 of WO2016/034666, which is herein incorporated by reference. Variants of said sequences may also be used. Other signal peptides, hinge regions, transmembrane domains, costimulatory domains and/or signaling domains could also be used within the scope of the invention.

25 In some embodiments, the CAR architecture is as shown in Figure 1 of Heczey A. et al, Blood. 2014 Oct 30; 124(18):2824-33, incorporated herein by reference.
In some embodiments, the CAR comprises an extracellular domain targeting GD2, such as the 14g2a scFv or an antibody comprising the 14g2a variable domains or an antibody comprising the CDRs of the 14g2a scFv, or variants thereof respectively, a CD3 zeta
30 signaling domain and a CD28 costimulatory domain. In some embodiments, the CAR comprises an extracellular domain targeting GD2, such as the 14g2a scFv, an antibody comprising the 14g2a variable domains or an antibody comprising the CDRs of the 14g2a scFv, or variants thereof, respectively, a CD3 zeta signaling domain and a 4-1BB costimulatory domain. The VH and VL sequences of the 14g2a scFv can e.g. be found in the

PDB database under accession number 4TUO_A and 4TUO_B, respectively (see also SEQ ID NOs: 13 and 14). Also contemplated is the use of variants of the 14g2a derived sequences, in particular variants having framework mutations, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mutations in the variable light and/or heavy chain. Preferred is an anti-GD2-CAR comprising at least one, preferably all of the CDR sequences as shown in SEQ ID NOs: 13 and 14, i.e. at least one, preferably all CDR sequences of SEQ ID NOs. 16 to 21. Exemplary GD2-specific CAR constructs are described in Heczey A. et al, Blood. 2014 Oct 30; 124(18):2824-33, in Pule, M.A. et al, Mol Ther, 12(5), November 2005, 933–941 (see Figure 1 for the amino acid sequence of transmembrane and endodomains of different receptors), as well as in WO2012033885, all three incorporated herein by reference.

In some embodiments, the CAR comprises an extracellular domain targeting CSPG4. Preferably, such CAR comprises at least one, preferably all CDRs of SEQ ID NOs.: 22 to 27. In some embodiments, such CAR comprises a VL sequence of SEQ ID NO.: 28 and/or a VH sequence of SEQ ID NO.: 29. Exemplary CSPG4-specific CAR constructs are described in WO2015/080981, incorporated herein by reference.

In some embodiments, the CAR comprises an extracellular domain targeting GPC3. Preferably, such CAR comprises at least one, preferably all CDRs of SEQ ID NOs.: 30 to 35. In some embodiments, such CAR comprises a VL sequence of SEQ ID NO.: 36 and/or a VH sequence selected from the group consisting of SEQ ID NO.: 37 and SEQ ID NO.: 38. Exemplary GPC3-specific CAR constructs are described in WO2016/049459, incorporated herein by reference.

In some embodiments, the CAR comprises an extracellular domain targeting 5T4. Preferably, such CAR comprises at least one, preferably all CDRs of SEQ ID NOs.: 39 to 44. In some embodiments, such CAR comprises a VL sequence of SEQ ID NO.: 45 and/or a VH sequence of SEQ ID NO.: 46. Exemplary 5T4-specific CAR constructs are described in WO2016/034666, incorporated herein by reference.

Additionally or alternatively, the antigen receptor is a T cell receptor (TCR). The TCR may be an endogenous (or native) TCR or an engineered TCR. The endogenous TCR may for example be selected for its specificity to antigen.

In one embodiment, the engineered TCR is a native TCR the sequence of which is recombinantly expressed in the immune cell. In some embodiments, the engineered TCR is derived from a native TCR, but comprises point mutations. In one embodiment, the engineered TCR comprises a disulfide bond in the constant region, for example as disclosed

in WO2006/000830, incorporated herein by reference. In particular the specific locations of the disulfide bonds in the constant regions are incorporated.

In one embodiment, the engineered TCR is modified to increase the surface expression as described in WO2016/170320, incorporated herein by reference. For example, the TCR comprises at least one of the following amino acid residues:
L96 of the alpha chain; R9 of the beta chain; Y10 of the beta chain; T24 of the alpha chain; V19 of the alpha chain; T20 of the alpha chain; M50 of the alpha chain; T5 of the alpha chain; Q8 of the alpha chain; S86 of the alpha chain; F39 of the alpha chain; D55 of the alpha chain; R43 of the beta chain; A66 of the alpha chain; V19 of the beta chain; L21 of the beta chain; L103 of the beta chain; T3 of the alpha chain; S7 of the alpha chain; P9 of the alpha chain; M11 of the alpha chain; A16 of the alpha chain; T18 of the alpha chain; L21 of the alpha chain; S22 of the alpha chain; D26 of the alpha chain; F40 of the alpha chain; S47 of the alpha chain; R48 of the alpha chain; Q49 of the alpha chain; I51 of the alpha chain; L52 of the alpha chain; V53 of the alpha chain; T67 of the alpha chain; E68 of the alpha chain; N74 of the alpha chain; F76 of the alpha chain; N79 of the alpha chain; Q81 of the alpha chain; A83 of the alpha chain; K90 of the alpha chain; S92 of the alpha chain; D93 of the alpha chain; and M101 of the alpha chain. Preferably, said at least one amino acid residue is not present in the corresponding germline framework TCR amino acid sequence.

In one embodiment, the engineered TCR comprises non-human constant regions, for example murine constant regions.

In one embodiment, the TCR, such as an endogenous TCR of a NKT cell, is capable of binding tumor associated macrophages.

In one embodiment, the TCR is specific for survivin. Exemplary TCRs that are specific for the survivin tumor antigen but do not have "on-target off tumor" toxicity are disclosed in WO2016/070119. Such survivin specific TCR preferably comprises the CDRs of SEQ ID NOs: 50 and 51. Preferably, the TCR comprises a beta chain of SEQ ID NOs: 50 and/or an alpha chain of 51.

In one embodiment, the TCR is specific for WT-1. Exemplary TCRs specific for WT-1 are disclosed in WO2005056595. WT-1 specific TCRs preferably comprise at least one CDR

from the group consisting of SEQ ID NOs: 52, 53, 54, 55, 56, 57, 58, and 59. In one embodiment, the alpha chain comprises SEQ ID NOs: 52, 53 and 54. In one embodiment, the alpha chain comprises SEQ ID NOs: 52, 53 and 55. In one embodiment, the beta chain comprises SEQ ID NOs: 56, 57 and 58. In one embodiment, the beta chain comprises SEQ
5 ID NOs: 56, 57 and 59. Such TCR may comprise the alpha chain of SEQ ID NOs: 60 or 62. Additionally or alternatively, such TCR may comprise the beta chain of SEQ ID NOs: 61 or 63. Thus, in one embodiment, the TCR comprises SEQ ID NOs: 60 and 61. In one embodiment, the TCR comprises SEQ ID NOs: 60 and 63. In one embodiment, the TCR comprises SEQ ID NOs: 62 and 61. In one embodiment, the TCR comprises SEQ ID NOs:
10 62 and 63.

In a preferred embodiment, said antigen receptor is recombinantly expressed. Accordingly, the immune cell is transduced or transfected with a vector encoding said antigen receptor.

15 The antigen to which the antigen receptor binds, is preferably expressed by or derived from a tumor or a pathogen. In some embodiments, in particular when using a CAR, the antigen receptor may bind more than one target. Also contemplated are immune cells expressing two or more, such as three, four or five, different recombinant antigen receptors. Exemplary antigens to which the antigen receptor binds may include, without being limited to, GD2,
20 WT-1, 5T4, GPC3, CSPG4, MUC16, MUC1, CA1X, CEA, CDS, CD7, CD 10, CD19, CD20, CD22, CD23, CD30, CD33, CD34, CD38, CD41, CD44, CD49f, CD56, CD70, CD74, CD133, CD138, CD123, cytomegalovirus (CMV) proteins such as pp65 or IE-1, human papillomavirus (HPV) proteins such as E6 or E7, Epstein-Barr virus (EBV) proteins such as EBNA-1, LMP-1, LMP-2, or BARF-1, ADV proteins such as hexon, EGP-2, EGP-
25 40, EpCAM, erb-B2, erb-B3, erb-B4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD3, Her-1, HER-2, HER2-HER3 in combination or HER1-HER2 in combination, hTERT, IL- 13R~a2, K-light chain, DR, LeY, LI cell adhesion molecule, MAGE-A1, MAGE-A4, MAGE-A10, Mesothelin, NKG2D ligands, NY-ESO-1, PSCA, PSMA, ROR1, TAG-72, VEGF-R2, EGFR, EGFRvIII, mutated p53, mutated ras, mutated raf, mutated RAC1, bcr/abl
30 fusions, c-Met, alphafetoprotein, CA-125, MUC-1, epithelial tumor antigen, prostate-specific antigen, melanoma-associated antigen, folate binding protein, HIV-1 envelope glycoprotein gpl20, HIV-1 envelope glycoprotein gp41, meothelin, HERV-K, or ERBB2 .

The antibody may be a full-length immunoglobulin or an antibody derivative. Over the past decades, full-length immunoglobulins have been dissected and the modules have been used to create monovalent, bivalent or multivalent derivatives as well as monospecific, bispecific or multispecific derivatives. Initially, smaller antigen binding fragments were produced by 5 proteolysis and later, artificial constructs have been generated by genetic engineering. Antibody derivatives are thus recombinant molecules including functional parts or the entirety of a full-length immunoglobulin, possibly in multiple copies. Exemplary antibody derivatives include, without being limited to, Fab, Fab', scFab, scFv, Fv fragment, nanobody, VHH, minimal recognition unit, diabody, single-chain diabody (scDb), tandem 10 scDb (Tandab), a linear dimeric scDb (LD-scDb), circular dimeric scDb (CD-scDb), BiTE (or tandem di-scFv or tandem scFv), DART, tandem tri-scFv, tri(a)body, bispecific Fab2, di-miniantibody, tetrabody, scFv-Fc-scFv fusion, scFv-Fc fusion, di-diabody, DVD-Ig, CrossMab, Duobody, scFab-Fc, scFab-Fc-scFab, IgG-scFab, scFab-dsscFv, Fv2-Fc, IgG-scFv fusion (such as e.g., bsAb, Bs1Ab, Bs2Ab, Bs3Ab, Ts1Ab, Ts2A)b, Knob-into-Holes 15 (KiHs), DuoBody, (see e.g., Holliger P and Hudson J. Engineered antibody fragments and the rise of single domains. Nature Biotechnol. 2005, vol. 23, 9, p.1126; Dimasi N. et al (2009), JMB 393, 672-692)).

A subgroup of antibody derivatives are antibody fragments. As used herein, the term "antibody fragments" refers to (i) monovalent and monospecific antibody derivatives which 20 comprise the variable heavy and/or light chains or functional fragments of an antibody and lack an Fc part; and (ii) BiTE (tandem scFv), DARTs, diabodies and single-chain diabodies (scDB). Thus, an antibody fragment is e.g. selected from the group consisting of: Fab, Fab', scFab, scFv, Fv fragment, nanobody, VHH, dAb, minimal recognition unit, single-chain diabody (scDb), BiTE and DART. The recited antibody fragments have a molecular weight 25 below 60 kDa. In one embodiment, the antibody derivative is an antibody fragment, preferably a humanized antibody fragment.

In one embodiment, the antibody comprises an Fc domain which is capable of mediating cytotoxic immune responses. Non-limiting examples of antibodies including an Fc domain 30 are full-length immunoglobulins, DVD-Ig, scFv-Fc and scFv-Fc.scFv fusions, IgG-scFab, scFab-Fc, scFab-Fc-scFab,Fv2-Fc, IgG-scFv fusions (such as e.g., bsAb, Bs1Ab, Bs2Ab, Bs3Ab, Ts1Ab, Ts2Ab), DuoBody and CrossMab.

In one embodiment, the antibody comprises an Fc domain which is modified such that it does not induce cytotoxic immune responses and/or or does not activate complement. In one embodiment, the antibody derivative lacks an Fc domain. Exemplary antibody derivatives lacking an Fc domain are Fab, Fab', scFab, scFv, Fv fragment, nanobody, VHH, minimal recognition unit, diabody, single-chain diabody (scDb), tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), circular dimeric scDb (CD-scDb), BiTE (also called tandem di-scFv or tandem scFv), tandem tri-scFv, tri(a)body, bispecific Fab2, di-miniantibody, di-diabody, scFab-dsscFv or DART. In one embodiment, the antibody comprises a Fc domain engineered using Knob into Holes (KiHs) technology.

The Fc part mediates cytotoxic immune responses such as ADCC, ADCP and/or CDC; however, such Fc mediated effects are not required or are even undesired when targeting the PD-1:PD-L1 axis as both proteins are expressed on the surface of antitumor cytotoxic T cells. Hence, administering full-length monoclonal antibodies with functional Fc parts may result in the depletion of the very lymphocytes they are intended to activate. Treatment with anti–PD-1 antibodies was found to correlate with lower circulating T-cell numbers in patients. PD-L1 is expressed on non-tumor cells as well and it is not desirable to target these cells and mediate ADCC, ADCP and/or CDC.

In some embodiments, the antibody derivative has a molecular weight of about 60 kDa or lower, such as about 55 kDa, 50 kDa, 45 kDa, 40 kDa, 35 kDa, 30 kDa or 27 kDa or lower. Solid tumors have substantial physical barriers that often prevent full-length immunoglobulins to penetrate to the center which results in reduced therapeutic effects (Christiansen, J., and Rajasekaran, A.K. (2004), Mol. Cancer Ther. 3, 1493–1501). Smaller antibody derivatives may in contrast penetrate deeper into the tumor. Exemplary antibody derivates having a molecular weight of about 60 kDa or lower are antibody fragments, including, without being limited to, Fab, Fab', scFab, scFv, Fv fragment, nanobody, VHH, dAb, minimal recognition unit, single-chain diabody (scDb), or DART.

The size and/or architecture of the antibody has implications on its half-life. To decrease side-effects in a therapeutic setting, it may be advantageous to use antibodies with a short half-life. This may e.g. be achieved by using an antibody derivative lacking an Fc part, more preferably an antibody derivative having a low molecular weight, such as about 60 kDa or lower, such as about 55 kDa, 50 kDa, 45 kDa, 40 kDa, 35 kDa, 30 kDa or 27 kDa or lower.

Preferred antibody derivatives having these characteristics are e.g. Fab, Fab', scFab, scFv, Fv fragment, nanobody, VHH, dAb, minimal recognition unit, single-chain diabody (scDb), BiTE or DART.

5 The antibody can thus be monovalent or multivalent, i.e. having one or more antigen binding sites. Non-limiting examples of monovalent antibodies, in particular antibody derivatives, include, without being limited to, scFv, Fv fragments, Fab, scFab, dAb, VHH, nanobody or minimal recognition unit. A multivalent antibody can have two, three, four or more antigen binding sites. Full-length immunoglobulins, F(ab')2 fragments, single-chain 10 diabody (scDb), tandem scDb (Tandab), a linear dimeric scDb (LD-scDb), circular dimeric scDb (CD-scDb), BiTE (or tandem di-scFv or tandem scFv), DART, tandem tri-scFv, tri(a)body, bispecific Fab2, di-miniantibody, tetrabody, scFv-Fc-scFv fusion, scFv-Fc fusion, di-diabody, DVD-Ig, CrossMab, Duobody, scFab-Fc, scFab-Fc-scFab, IgG-scFab, scFab-dsscFv, Fv2-Fc, IgG-scFv fusion, diabodies, triabodies and tetrabodies are non-15 limiting examples of multivalent antibodies; an exemplary multivalent antibody comprises two binding sites, i.e. the antibody is bivalent.
In some embodiments, the antibody, in particular the antibody derivative, is bispecific, i.e. the antibody derivative is directed against two different targets or two different epitopes on one target molecule. In some embodiments, the antibody derivative is multivalent and 20 comprises more than two, e.g., three or four different binding sites for three or four, respectively, different antigens. Such antibody is multivalent and multispecific, in particular tri- or tetra-specific, respectively.

Preferably, the antibody derivative above is an scFv (a "single chain variable fragment" or 25 a "single chain antibody"). An scFv is a fusion protein that includes the VH and VL domains of an antibody connected by a linker. It thus lacks the constant Fc region which is present in a full-length antibody. The VH and VL domains can be connected in either orientation, VL-linker-VH or VH-linker-VL, by a flexible linker. In a preferred embodiment, the orientation is VL-linker-VH, i.e. the light chain variable region being at the N-terminal end 30 and the heavy chain variable region being at the C-terminal end of the polypeptide. The linker may have the sequence of SEQ ID NO: 10, however, shorter or longer linkers or variants of SEQ ID NO: 10 may also be used.

Thus, in one embodiment, the cell provided herein is a T cell expressing a scFv blocking PD-L1 and at least one CAR according to the present disclosure. In one embodiment, the cell provided herein is a T cell expressing a scFv blocking PD-L1 and at least one TCR according to the present disclosure. In one embodiment, the cell provided herein is a T cell expressing a scFv blocking PD-L1 and at least one CAR as well as at least one TCR according to the present disclosure.

Thus, in one embodiment, the cell provided herein is a Natural Killer T (NKT) cell, expressing a scFv blocking PD-L1 and at least one CAR according to the present disclosure. In one embodiment, the cell provided herein is a NKT cell expressing a scFv blocking PD-L1 and at least one TCR according to the present disclosure. In one embodiment, the cell provided herein is a NKT cell expressing a scFv blocking PD-L1 and at least one CAR as well as at least one TCR according to the present disclosure.

Thus, in one embodiment, the cell provided herein is a Natural Killer (NK) cell expressing a scFv blocking PD-L1 and at least one CAR according to the present disclosure. In one embodiment, the cell provided herein is a NK cell expressing a scFv blocking PD-L1 and at least one TCR according to the present disclosure. In one embodiment, the cell provided herein is a NK cell expressing a scFv blocking PD-L1 and at least one CAR as well as at least one TCR according to the present disclosure.

Thus, in one embodiment, the cell provided herein is a human embryonic stem cell, expressing a scFv blocking PD-L1 and at least one CAR according to the present disclosure. In one embodiment, the cell provided herein is a human embryonic stem cell expressing a scFv blocking PD-L1 and at least one TCR according to the present disclosure. In one embodiment, the cell provided herein is a human embryonic stem cell expressing a scFv blocking PD-L1 and at least one CAR as well as at least one TCR according to the present disclosure.

Thus, in one embodiment, the cell provided herein is a hematopoietic stem cell (HSC) expressing a scFv blocking PD-L1 and at least one CAR according to the present disclosure. In one embodiment, the cell provided herein is a hematopoietic stem cell expressing a scFv blocking PD-L1 and at least one TCR according to the present disclosure. In one embodiment, the cell provided herein is a hematopoietic stem cell expressing a scFv blocking PD-L1 and at least one CAR as well as at least one TCR according to the present disclosure.

Thus, in one embodiment, the cell provided herein is an induced pluripotent stem cell (iPS) expressing a scFv blocking PD-L1 and at least one CAR according to the present disclosure.

In one embodiment, the cell provided herein is an induced pluripotent stem cell expressing a scFv blocking PD-L1 and at least one TCR according to the present disclosure. In one embodiment, the cell provided herein is an induced pluripotent stem cell expressing a scFv blocking PD-L1 and at least one CAR as well as at least one TCR according to the present disclosure.

Antibodies having the frameworks as used herein have been described as being surprisingly stable in the scFv format (see, e.g., WO/2009/155726 or Borras et al., JBC, Vol. 285, no. 12, 9 March 2010, pages 9054-9066). Thus, the antibody preferably comprises the framework sequences as comprised in SEQ ID Nos: 1 and/or 2 or variants thereof. Variants may e.g. include modifications as described in WO2014/206561, in particular including VL framework sequences SEQ ID NOs. 15 to 22 of WO2014/206561.

The antibody is preferably humanized, to avoid an immune response against the protein. "Humanized" antibodies refer to antibodies that include one or more, typically all six CDR regions of a non-human parent antibody or variants thereof or synthetic CDRs, and of which the framework is, e.g., (i) a human framework, potentially including one or more framework residues of the non-human parent antibody, or (ii) a framework from a non-human antibody modified to increase similarity to naturally produced human frameworks. Methods of humanizing antibodies are known in the art, see e.g. Leger O., and Saldanha J. Antibody Drug Discovery. Edited by Wood C. London: Imperial College Press, 2011. ISBN 1848166281. p.1-23.

In some embodiments, the antibody is fully human.

In preferred embodiments, the antibody binds to an epitope on PD-L1 such that PD-L1 interaction with both CD80 and PD-1 is blocked. As PD-L1 binding to PD-1 drives T cell exhaustion and PD-L1 binding to CD80 drives T cell anergy, simultaneously blocking the binding of PD-L1 to CD80 and PD-1 prevents anergy and reverts exhaustion.

In preferred embodiments, the antibody comprises
i) at least one of the variable heavy chain (VH) CDR sequences CDR-H1 , CDR-H2 or CDR-H3 as set forth in SEQ ID NOs.: 6, 7 and 8, respectively, or variants thereof,

ii) at least one of the variable light chain (VL) CDR sequences CDR-L1 , CDR-L2 or CDR-L3 as set forth in SEQ ID NOs.: 3, 4, and 5, respectively, or variants thereof.

Preferably, the antibody comprises
5 i) at least one of VH sequence of SEQ ID NO: 2, and/or
ii) at least one VL sequence of SEQ ID NO: 1,
or variants thereof, respectively.

In some embodiments, said antibody is a scFv comprising SEQ ID NO.: 9 or a variant
10 thereof.

A variant may in some embodiments be an antibody that differs from a given antibody, in one, two, three, four, five or more positions of its amino acid sequence. Such difference may e.g., be a substitution, addition, modification or deletion. In one embodiment, the variant has
15 at least 85%, more preferably 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and most preferably 100% sequence identity to the sequences disclosed herein, in particular SEQ ID NO: 1, 2 or 9.

Variants of the antibodies provided herein may be prepared by introducing appropriate
20 modifications into the nucleic acid sequence encoding the antibody. Any combination(s) of deletions, substitutions, additions, modifications and insertions can be made to the framework or to the CDRs, provided that the generated antibody possesses the desired characteristics for which it can be screened using appropriate methods. Of particular interest are substitutions, preferably conservative substitutions.
25

As used herein, "conservative substitution" refers to a modification and a substitution, that maintains physically, biologically, chemically and/or functionally the properties with regard to the corresponding reference. A molecule that includes a sequence with conservative substitution for instance may have a similar size, shape, electric charge, chemical properties,
30 including a comparable ability to form covalent or hydrogen bonds, and/or comparable polarity. Such conservative modifications include, but are not limited to, one or more nucleobases and amino acid substitutions, additions and deletions.
For example, conservative amino acid substitutions include those in which the amino acid residue is replaced with an amino acid residue having a similar side chain. For example,

amino acid residues being non-essential with regard to binding to an antigen can be replaced with another amino acid residue from the same side chain family, e.g. serine may be substituted for threonine. Amino acid residues are usually divided into families based on common, similar side-chain properties, such as:

5 1. nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, methionine),
2. uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, proline, cysteine, tryptophan),
3. basic side chains (e.g., lysine, arginine, histidine, proline),
4. acidic side chains (e.g., aspartic acid, glutamic acid),
10 5. beta-branched side chains (e.g., threonine, valine, isoleucine) and
6. aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

A conservative substitution can be taken to be a substitution of a first amino acid within one of the six groups above by a further amino acid within the same group of the six groups. Preferred conservative substitutions include:

15 1. Substituting alanine (A) by valine (V);
2. Substituting arginine (R) by lysine (K);
3. Substituting asparagine (N) by glutamine (Q);
4. Substituting aspartic acid (D) by glutamic acid (E);
5. Substituting cysteine (C) by serine (S);
20 6. Substituting glutamic acid (E) by aspartic acid (D);
7. Substituting glycine (G) by alanine (A);
8. Substituting histidine (H) by arginine (R) or lysine (K);
9. Substituting isoleucine (I) by leucine (L);
10. Substituting methionine (M) by leucine (L);
25 11. Substituting phenylalanine (F) by tyrosine (Y);
12. Substituting serine (S) by threonine (T);
13. Substituting tryptophan (W) by tyrosine (Y);
14. Substituting phenylalanine (F) by tryptophan (W);

and/or

30 15. Substituting valine (V) by leucine (L)

and vice versa. Other substitutions such as substituting proline (P) by alanine (A) are also permissible and can be determined empirically or in accord with other known conservative or non-conservative substitutions. A conservative substitution may also involve the use of a non-natural amino acid.

The antibody described herein may comprise one or more, such as two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more of such conservative substitutions.

In another embodiment, non-conservative substitutions are introduced into any sequence disclosed herein to produce a variant. In one embodiment, the antibody comprises one or more, such as two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more of such non-conservative substitutions.

A particularly preferred type of variant is one where one or more entire CDRs are replaced. Typically, the CDR-H3 and CDR-L3 contribute most significantly to antigen binding. For example, the entire CDR-L1, CDR-L2, CDR-H1 and/or CDR-H2 may be replaced by a different CDR of natural or artificial origin. In some embodiments, one or more CDRs are replaced by an alanine-cassette.

In some embodiments, the variant does not show any improvement over the parent antibody. In some embodiments, a variant antibody as described herein

(i) retains specific binding to PD-L1, in particular to hPD-L1, preferably blocking the interaction between PD-L1 and PD-1; and/or

(ii) has a KD to human PD-L1 of lower than 500 pM, preferably lower than 250 pM, 100 pM, 75 pM, 50 pM, 40 pM, 30 pM, 20 pM, more preferably of lower than 10 pM as measured by KinExA®; and/or

(iv) competes with the antibody disclosed herein for binding to PD-L1; and/or

(v) has at least 80%, preferably at least 85%, 90%, 95% or 97% sequence identity to the sequences disclosed herein.

In some embodiments, the antibody has high affinity for PD-L1 and binds hPD-L1 with a KD of lower than 100 pM, such as lower than about 75 pM, 50 pM, 25 pM or 10 pM . For example, the antibody is bivalent and binds PD-L1 with a KD of lower than 10 pM as measured by KinExA®, preferably lower than 5 pM, more preferably about 3 pM, e.g., 2.9 pM, 2.8 pM or 2.7 pM. In some embodiments, such bivalent antibody is a full-length immunoglobulin.

In some embodiments, the antibody provided herein is monovalent and binds human PD-L1 with a KD of lower than 50 pM as measured by KinExA®. Said KD is preferably lower than

about 10 pM, such as about 9 pM, e.g., 9.0 pM, 8.9 pM, 8.8 pM or 8.7 pM. In some embodiments, said monovalent antibody is a scFv.

In some embodiments, the antibody provided herein is monovalent and binds monkey PD-L1 with a KD of lower than 50 pM as measured by KinExA®. Said KD is preferably lower than about 10 pM, more preferably lower than about 5 pM, such as e.g., about 3.4 pM, 3.3 pM or 3.2 pM as measured by KinExA®.

Such KinExA®.measurements are preferably done at room temperature, more preferably under the conditions as described in example 1.

High affinity antibodies may be advantageous to provide a protective effect even if a small amount of engineered immune cells is present at its target site, accordingly expressing a limited amount of antibody.

Also provided are engineered immune cells as described above expressing antibodies that bind to human PD-L1 as well as to monkey PD-L1. Preferably, the affinity to monkey PD-L1 is at least twice as tight as the affinity to human PD-L1. In some embodiments, the affinity KD of a monovalent antibody, preferably a scFv, to monkey PD-L1 is about 3.3 pM as measured by KinExA®, for example as measured at room temperature, preferably under the conditions indicated in Example 1.

Further provided are antibodies that have no cross-reactivity to other member of the B7 family, such as PD-L2 and B7-H3.

Further contemplated are engineered immune cells as described above expressing antibodies that compete with the antibodies disclosed herein for binding to PD-L1.

The engineered immune cell described herein may secreted the antibody and/or express the antibody on its surface. In preferred embodiments, the antibody is secreted.

In some embodiments, the cell may additionally recombinantly express at least one further protein compound such as a second antibody or a cytokine. Cytokines are e.g. selected from the group consisting of IL-2, IL-4, IL-7, IL-12, IL-15, IL-21 or MIP-1alpha and are preferably of human origin, i.e. hIL-2, hIL-4, hIL-7, hIL-15, hIL-21, or hMIP-1alpha. In some embodiments, such cell expresses hIL-15. IL-15 has been described to improve in vivo persistence and anti-tumor activity of CAR NKT cells (see WO2013/040371). Such second

antibody may e.g., target an immune inhibitory molecule, such as transforming growth factor-beta (TGF-β), IL-10, Fas, CD47, CTLA-4, Tim-3, LAG-3, or ligands thereof.

In some embodiments, the engineered immune cell expresses an antigen receptor being a CAR comprising a 4-1BB costimulatory domain, an antibody as described herein and further IL-15. In some embodiments, the engineered immune cell expresses an antigen receptor being a CAR comprising a CD28 costimulatory domain, an antibody as described herein and further IL-15.

Thus there is provided an NKT cell with a native TCR, a chimeric antigen receptor (in particular specific for a tumor antigen), and an antibody to PD-L1 according to the present disclosure, in particular a scFv. The NKT cell further encodes a cytokine, such as IL-15.

Further contemplated is a nucleic acid encoding the antigen receptor and/or the antibody described herein. The proteins may be encoded by a plurality of nucleic acid sequences. In some embodiments, the proteins are encoded by a single nucleic acid sequence. Typically, the nucleic acid is an isolated nucleic acid.

Knowing the sequence of the antibody and/or the antigen receptor, cDNAs encoding the respective polypeptide sequence can be generated by methods well known in the art, e.g. by gene synthesis. These cDNAs can be cloned by standard cloning and mutagenesis techniques into a suitable vector such as an expression vector or a cloning vector. Thus, further contemplated is cDNA encoding the antigen receptor and/or the antibody as described herein.

Based on the cloning strategy chosen, genetic constructs may generate an antibody and/or an antigen receptor having one or more additional residues at the N-terminal or C-terminal end. It is therefore to be understood that the antibodies disclosed herein include the disclosed sequences rather than consist of them.

Basic protocols of standard cloning, mutagenesis and molecular biology techniques are described in, e.g., Molecular Cloning, A Laboratory Manual (Green M. and Sambrook, J. Molecular Cloning: a Laboratory Manual. 4th edition. Cold Spring Harbor Laboratory, 2012. ISBN 1936113422.).

Further contemplated are isolated nucleic acids hybridizing with the nucleic acids described herein under stringent conditions.

Also provided are vectors comprising the nucleic acid provided herein, such as an expression vector or a cloning vector. One, two or more nucleic acids encoding the antigen receptor and the antibody described herein may be comprised in a vector, which may be the same vector (bicistronic or multicistronic) or separate vectors. The expression vector may be a multicistronic vector, such as a bicistronic vector, e.g., using internal ribosome entry sites (IRES), 2A-like sequences or dual promoters.

The expression vector may e.g. be a lentiviral, a retroviral, an adenoviral or an Adeno-Associated Virus (AAV) vector. The expression vector may also be a non-viral vector, including a plasmid, a transposon, an inserting sequence, or an artificial chromosome.

A nucleic acid molecule may in some embodiments define an expression cassette. An expression cassette is a nucleic acid molecule capable of directing expression of a particular nucleotide sequence in an appropriate host cell. An expression cassette includes a promoter operatively linked to the nucleotide sequence of interest, which is operatively linked to one or more termination signals. It may also include sequences required for proper translation of the nucleotide sequence. The coding region can encode a polypeptide of interest. The expression of the nucleotide sequence in the expression cassette can be under the control of a constitutive promoter or of an inducible promoter that initiates transcription only when the host cell is exposed to some particular external stimulus. In some embodiments, the antibody is under the control of the 5" end LTR of the retrovirus. The nucleic acid encoding the antigen receptor and/or the nucleic acid encoding the antibody may each be operably linked to a promoter which may be the same or different promoters.

The nucleic acid and/or vectors may further comprise a signal peptide. Typically, a signal peptide is a 5-30 amino acid peptide attached to the N-terminus of the protein to be secreted and is attached to increase protein secretion. In preferred embodiments, the signal peptide is a human signal peptide. In some embodiments, the signal peptide is hIgG1. In some embodiments, the signal peptide comprises SEQ ID NO: 15.

Additionally or alternatively, the antibody is membrane anchored. Such membrane anchored antibody may comprise a transmembrane domain. In some embodiments, the membrane anchored antibody does not comprise no signaling domain. In one embodiment, the antibody is secreted and in also provided as membrane anchored form.

A genetically engineered immune cell may comprise a safety switch, such as a suicide switch. Such switches suppress the cell's activity if serious side effects emerge, or make the cells self-destruct if they attack healthy tissue. Typically, such switches are controllable and

therefore require an additional receptor or other target on the cell. Such safety switches are controlled by administering a second medication to the subject. Thus, in some embodiments, the vector comprises a nucleic acid sequence encoding a safety switch, preferably a suicide switch.

5

The invention also provides a method of generating an immune cell as described herein, comprising the steps of:

(a) Providing an immune cell,

(b) Introducing into said cell at least one nucleic acid encoding said antigen receptor and at

10 least one nucleic acid encoding said antibody; and

(c) Expressing said nucleic acids by said cell.

In some embodiments, step (b) comprises introducing the expression vector as described above into said cell.

The method may comprise the additional step of:

15 (i) Introducing into said cell at least one other antigen receptor having a different antigen specificity than the antigen receptor of step (b); and/or introducing into said cell at least one other antibody having a different antigen specificity than the antibody of step (b).

The invention also relates to a pharmaceutical composition comprising

20 i) an effective amount of the engineered immune cell described herein or of the expression vector described herein, and

ii) a pharmaceutically acceptable excipient.

Suitable “excipients” include, without being limited to: (i) buffers such as phosphate, citrate, or other, organic acids; (ii) antioxidants such as ascorbic acid and tocopherol; (iii)

25 preservatives such as 3-pentanol, hexamethonium chloride, benzalkonium chloride, benzyl alcohol, alkyl paraben, catechol, or cyclohexanol; (iv) amino acids, such as e.g. histidine, arginine; (v) peptides, preferably up to 10 residues such as polylysine; (vi) proteins, such as bovine or human serum albumin; (vii) hydrophilic polymers such as polyvinylpyrrolidone; (viii) monosaccharides, disaccharides, polysaccharides and/or other carbohydrates including

30 glucose, mannose, sucrose, mannitol, trehalose, sorbitol, aminodextran or polyamidoamines; (ix) chelating agents, e.g. EDTA; (x) salt-forming ions such as sodium, potassium, and/or chloride; (xi) metal complexes (e.g. Zn-protein complexes); (xii) ionic and non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG), (xiii) cryopreservatives such as dimethyl sulfoxide (DMSO).

The engineered immune cell described herein, the expression vector described herein and/or the pharmaceutical composition described herein are useful for therapy. Thus, further provided is the engineered immune cell described herein, the expression vector described herein and/or the pharmaceutical composition described herein for use in therapy.

Also provided is a method of treating a subject in need thereof comprising:
(a) Providing the engineered immune cell described herein; and
(b) Administrating said immune cells to said subject.
Additionally or alternatively, the method of treatment involves the provision and administration of the expression vector described herein and/or the pharmaceutical composition described herein.

The terms “treatment” and “treating” as used herein, include a prophylactic or preventative measure having a therapeutic effect and/or preventing, slowing down (lessening), or at least partially alleviating or abrogating an abnormal, including pathologic, condition in the organism of a subject. Treatment according to the present disclosure involves the administration of a pharmaceutically effective amount of a molecule, nucleic acid, vector, pharmaceutical composition, and/or an engineered immune cell as described herein, i.e. inter alia, the cell, the vector or the composition disclosed herein, to a subject in need thereof to prevent, cure, delay the onset and/or progression, reduce the severity of, stabilize, modulate, cure or ameliorate one or more symptoms of the condition to be treated. Those in need of treatment include those already with the disorder as well as those prone to having the disorder or those in whom the disorder is to be prevented (prophylaxis). Generally, a treatment reduces, stabilizes, or inhibits progression of a symptom that is associated with the presence and/or progression of a disease or pathological condition.

The subject in need of a treatment can be a human or a non-human animal. Typically, the subject is a mammal, e.g., a mouse, a rat, rabbit, a hamster, a dog, a cat, a monkey, an ape, a goat, a sheep, a horse, a chicken, a guinea pig or a pig. In typical embodiments, the subject is diagnosed with a cancer and/or a PD-L1-related disorder or may acquire such a disorder. In case of an animal model, the animal might be genetically engineered to develop such disorder.

Typically, an effective amount of the cell, the vector or the composition disclosed herein is administered to the subject. An "effective amount" is an amount – either as a single dose or as part of a series of doses – which at the dosage regimen applied yields the desired therapeutic effect, i.e., to reach a certain treatment goal. A therapeutically effective amount is generally an amount sufficient to provide a therapeutic benefit in the treatment or management of the relevant pathological condition, or to delay or minimize one or more symptoms associated with the presence of the condition. The dosage will depend on various factors including patient and clinical factors (e.g., age, weight, gender, clinical history of the patient, severity of the disorder and/or response to the treatment), the nature of the disorder being treated, the particular composition to be administered, the route of administration, and other factors.

The term "administering", as used herein, refers to any mode of transferring, delivering, introducing, or transporting matter such as the cell, the vector or the composition described herein, to a subject. Administration may be administered locally or systemically. Preferred modes of administration include, without being limited to, parenteral, e.g., intravenous, or systemic, administration. Administration "in combination with" further matter such as one or more therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.
The cells, the vector or the composition are administered one or more times to said subject.

The cells, the vector or the composition may be administered in combination with one or more therapies selected from the group of antibody therapy, chemotherapy, cytokine therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy. The therapies of the present invention may precede or follow the other agent treatment by intervals ranging from minutes to weeks.

The cells may have originated from the subject or from another individual of the same species, i.e. they are autologous or allogeneic. Autologous adoptive transfer requires extraction of the patient's cells, their genetic modification, e.g. as described above and culturing said cells in vitro before returning them to the same patient. Such individual preparation for each new patient limits application of cellular immunotherapies in treating cancer. As an off-the-shelf product, however, allogeneic T cells derived from healthy donors carry the risk of recognizing the patient's body as foreign, which can cause a serious side

effect called graft versus host disease (GvHD). Off-the-shelf therapies based on CAR-modified NKT cells generated in large volumes from healthy donors offer great promise. While endowed with powerful cancer-killing properties like conventional T cells, invariant NKT cells express special T cell receptors that are not associated with GvHD. Hence
5 allogeneic NKT cells can be used to treat multiple cancer patients with minimal risk of GvHD.

The subject in need of treatment may have a condition, without being limited to, a pre-malignant or malignant cancer condition, such as NSCLC (non small cell lung carcinoma),
10 urothelial cancer, melanoma, renal cell carcinoma, Hodgkin's lymphoma, head and neck squamous cell carcinoma, ovarian cancer, gastrointestinal cancer, hepatocellular cancer, glioma, breast cancer, lymphoma, small cell lung carcinoma, myelodysplastic syndromes, prostate cancer, bladder cancer, cervical cancer, non clear cell kidney cancer, colorectal cancer, sarcomas, colon cancer, kidney cancer, lung cancer, pancreatic cancer or gastric
15 cancer, skin cancer, uterine cancer, glioblastoma, neuroblastoma, sarcoma, head and neck cancer, leukemia, carcinoma, Merkel cell carcinoma or renal cell carcinoma (RCC), multiple myeloma, lymphoblastic leukemia (ALL), B cell leukemia, chronic lymphocytic leukemia, non-Hodgkin's lymphoma; pathogen infection, an autoimmune disorder.

20 The invention further provides a kit for treatment of cancer, pathogen infection, an autoimmune disorder comprising the engineered immune cell described herein, the expression vector described herein or the pharmaceutical composition described herein, and written instructions for use.
In some embodiments, the kit may further comprise an inducer of a safety switch.
25

SEQUENCES
The sequences disclosed herein are
SEQ ID NO: 1 - VL of scFv
EIVMTQSPSTLSASVGDRVIITCQASEDIYSLLAWYQQKPGKAPKLLIYDASDLASG
30 VPSRFSGSGSGAEFTLTISSLQPDDFATYYCQGNYGSSSSSSYGAVFGQGTKLTVLG

SEQ ID NO: 2 – VH of scFv

EVQLVESGGGLVQPGGSLRLSCTVSGIDLSSYTMGWVRQAPGKGLEWVGIISSGG
RTYYASWAKGRFTISRDTSKNTVYLQMNSLRAEDTAVYYCARGRYTGYPYYFAL
WGQGTLVTVSS

5 SEQ ID NO: 3 – CDR-L1 of scFv
QASEDIYSLLA

SEQ ID NO: 4 – CDR-L2 of scFv
DASDLAS

10

SEQ ID NO: 5 – CDR-L3 of scFv
QGNYGSSSSSSYGAV

SEQ ID NO: 6 – CDR-H1 of scFv
15 IDLSSYTMG

SEQ ID NO: 7 –CDR-H2 of scFv
IISSGGRTYYASWAKG

20 SEQ ID NO: 8 –CDR-H3 of scFv
GRYTGYPYYFAL

SEQ ID NO: 9 – scFv
EIVMTQSPSTLSASVGDRVIITCQASEDIYSLLAWYQQKPGKAPKLLIYDASDLASG
25 VPSRFSGSGSGAEFTLTISSLQPDDFATYYCQGNYGSSSSSSYGAVFGQGTKLTVLG
GGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTVSGIDLSSYTMG
WVRQAPGKGLEWVGIISSGGRTYYASWAKGRFTISRDTSKNTVYLQMNSLRAED
TAVYYCARGRYTGYPYYFALWGQGTLVTVSS

30 SEQ ID NO: 10 – linker
GGGGSGGGGSGGGGSGGGGS

SEQ ID NO: 11 – forward primer
TAACCATGGAGTTTGGGCTGAG

SEQ ID NO: 12 – reverse primer
GACGCATGCTCAGCTCGACACGGTGACC

5 SEQ ID NO: 13 – VL sequence of 14g2a scFv
DVVMTQTPLSLPVSLGDQASISC**RSSQSLVHRNGNTYLH**WYLQKPGQSPKLLIH**K
VSNRFS**GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC**SQSTHVPPLT**FGAGTKLE
LKR
(CDR sequences highlighted in bold)
10

SEQ ID NO: 14 – VH sequence of 14g2a scFv
EVQLLQSGPELEKPGASVMISCKASG**SSFTGYNMN**WVRQNIGKSLEWIG**AIDPYY
GGTSYNQKFKG**RATLTVDKSSSTAYMHLKSLTSEDSAVYYCVS**GMEY**WGQGTS
VTVSS
15 (CDR sequences highlighted in bold)

SEQ ID NO: 15 – hIgG1 signal peptide
MEFGLSWLFLVAILKGVQ

20 SEQ ID NO: 16 – CDR-L1 of anti-GD2-CAR
RSSQSLVHRNGNTYLH

SEQ ID NO: 17 – CDR-L2 of anti-GD2-CAR
KVSNRFS
25

SEQ ID NO: 18 – CDR-L3 of anti-GD2-CAR
SQSTHVPPLT

SEQ ID NO: 19 – CDR-H1 of anti-GD2-CAR
30 SSFTGYNMN

SEQ ID NO: 20 – CDR-H2 of anti-GD2-CAR
AIDPYYGGTSYNQKFKG

SEQ ID NO: 21 – CDR-H3 of anti-GD2-CAR
GMEY

SEQ ID NO: 22 – CDR-L1 of anti-CSPG4 CAR
5 RASQTIYKNLH

SEQ ID NO: 23 – CDR-L2 of anti-CSPG4 CAR
YGSDSIS

10 SEQ ID NO: 24 – CDR-L3 of anti-CSPG4 CAR
LQGYSTPWT

SEQ ID NO: 25 – CDR-H1 of anti-CSPG4 CAR
YTFTDYSMH

15

SEQ ID NO: 26 – CDR-H2 of anti-CSPG4 CAR
WINTATGEPTYADDFKG

SEQ ID NO: 27 – CDR-H3 of anti-CSPG4 CAR
20 YYDY

SEQ ID NO: 28 - VL sequence of anti-CSPG4 CAR
LDIKLTQSPSILSVTPGETVSLSCRASQTIYKNLHWYQQKSHRSPRLLIKYGSDSISG
IPSRFTGSGSGTDYTLNINSVKPEDEGIYYCLQGYSTPWTFGGGTKLEIKR

25

SEQ ID NO: 29 - VH sequence of anti-CSPG4 CAR
QVKLKESGPELKKPGETVKISCKASGYTFTDYSMHWVKKTPGKGLKWLGWINTA
TGEPTYADDFKGRFAISLETSARTVYLQINNLRNEDTATYFCFSYYDYWGQGTTV
TVSS

30

SEQ ID NO: 30 - CDR-L1 of anti-GPC3 CAR
RSSQSLVHSNRNTYLH

SEQ ID NO: 31 - CDR-L2 of anti-GPC3 CAR

KVSNRFS

SEQ ID NO: 32 - CDR-L3 of anti-GPC3 CAR
SQNTHVPPT

5

SEQ ID NO: 33 - CDR-H1 of anti-GPC3 CAR
YTFTDYEMH

SEQ ID NO: 34 - CDR-H2 of anti-GPC3 CAR
10 ALDPKTGDTAYSQKFKG

SEQ ID NO: 35 - CDR-H3 of anti-GPC3 CAR
FYSYTY

15 SEQ ID NO: 36 - VL of anti-GPC3 CAR
DVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNRNTYLHWYLQKPGQSPQLLIYKV
SNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQNTHVPPTFGQGTKLEIK
R

20 SEQ ID NO: 37 – VH of anti-GPC3 CAR
QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYEMHWVRQAPGQGLEWMGALD
PKTGDTAYSQKFKGRVTLTADKSTSTAYMELSSLTSEDTAVYYCTRFYSYTYWG
QGTLVTVSS

25 SEQ ID NO.: 38 - VH of anti-GPC3 CAR
QVQLQQSGAELVRPGASVKLSCKASGYTFTDYEMHWVKQTPVHGLKWIGALDP
KTGDTAYSQKFKGKATLTADKSSSTAYMELRSLTSEDSAVYYCTRFYSYTYWGQ
GTLVTVSA

30 SEQ ID NO: 39 - CDR-L1 of anti-5T4 CAR
YSFTGYYMH

SEQ ID NO: 40 - CDR-L2 of anti-5T4 CAR
RINPNNGVTLYNQKFKD

SEQ ID NO: 41 - CDR-L3 of anti-5T4 CAR
STMITNYVMDY

5 SEQ ID NO: 42 - CDR-H1 of anti-5T4 CAR
KASQSVSNDVA

SEQ ID NO: 43 - CDR-H2 of anti-5T4 CAR
YTSSRYA

10

SEQ ID NO: 44 - CDR-H3 of anti-5T4 CAR
QQDYNSPPT

SEQ ID NO: 45 - VL of anti-5T4 CAR
15 SIVMTQTPTFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYA
GVPDRFIGSGYGTDFTFTISTLQAEDLAVYFCQQDYNSPPTFGGGTKLEIKR

SEQ ID NO: 46 - VH of anti-5T4 CAR
EVQLQQSGPDLVKPGASVKISCKASGYSFTGYYMHWVKQSHGKSLEWIGRINPNN
20 GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDY
WGQVTSVTVSS

SEQ ID NO: 47 – 41BB costimulatory domain
KRGRKKLLYI FKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

25

SEQ ID NO: 48 – CD3 zeta intracellular domain
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM
QALPPR

30

SEQ ID NO: 49 – CD28 costimulatory domain
RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

SEQ ID NO: 50 – Survivin specific TCR, beta chain

DAMVIQNPRYQVTQFGKPVTLSCSQTLNHNVMYWYQQKSSQAPKLLFHYYDKD
FNNEADTPDNFQSRRPNTSFCFLDIRSPGLGDAAMYLCATSRGDSTAEPQHFGDGT
RLSIL

5 SEQ ID NO: 51 – Survivin specific TCR, alpha chain
GESVGLHLPTLSVQEGDNSIINCAYSNSASDYFIWYKQESGKGPQFIIDIRSNMDKR
QGQRVTVLLNKTVKHLSLQIAATQPGDSAVYFCAETVTDSWGKLQFGAGTQVVV
TPD

10 SEQ ID NO: 52 – WT-1 specific TCR, CDR1 alpha
SSYSPS

SEQ ID NO: 53 – WT-1 specific TCR, CDR2 alpha
YTSAATL

15

SEQ ID NO: 54 – WT-1 specific TCR, CDR3 alpha
WSPFSGGGADGLT

SEQ ID NO: 55 – WT-1 specific TCR, CDR3 alpha
20 SPFSGGGADGLT

SEQ ID NO: 56 – WT-1 specific TCR, CDR1 beta
DFQATT

25 SEQ ID NO: 57 – WT-1 specific TCR, CDR2 beta
SNEGSKA

SEQ ID NO: 58 – WT-1 specific TCR, CDR3 beta
SARDGGEG

30

SEQ ID NO: 59 – WT-1 specific TCR, CDR3 beta
RDGGEGSETQY

SEQ ID NO: 60 –WT-1 specific TCR, alpha chain

MLLLLVPVLEVIFTLGGTRAQSVTQLDSHVSVSEGTPVLLRCNYSSSYSPSLFWYV
QHPNKGLQLLLKYTSAATLVKGINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCV
VSPFSGGGADGLT

5 SEQ ID NO: 61 – WT-1 specific TCR, beta chain
MLLLLLLLGPGSGLGAVVSQHPSWVICKSGTXVKIECRSLDFQATTMFWYRQFPK
QSLMLMATSNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYIC
SARDGGEG

10 SEQ ID NO: 62 – WT-1 specific TCR, alpha chain
MLLLLVPVLEVIFTLGGTRAQSVTQLDSHVSVSEGTPVLLRCNYSSSYSPSLFWYV
QHPNKGLQLLLKYTSAATLVKGINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCV
V SPFSGGGADGLT

15 SEQ ID NO: 63 – WT-1 specific TCR, beta chain
MLLLLLLLGPGSGLGAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQFPK
QSLMLMATSNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYICSAR
DGGEGSETQY

20 The following are examples, illustrating the methods and compositions disclosed herein. It is understood that various other embodiments may be practiced, given the general description provided above.

EXAMPLES

25 Cell lines

The 293T cells were obtained from ATCC while neuroblastoma tumor cell line CHLA-255 was kindly provided by Dr Leonid Metelitsa Baylor College of Medicine (Houston, TX). Cells were maintained in culture with IMDM (Gibco) for 293T or RPMI 1640 (Gibco) for CHLA-255, containing 10% FBS (Corning), 1% GlutaMAX and 1% penicillin/streptavidin
30 (Gibco) in a humidified atmosphere containing 5% CO_2 at 37°C.

Example 1 – Characterization of the scFv

Neutralization of Human PD-L1

The anti-PD-L1 scFv (see SEQ ID NO: 9) is a humanized protein comprising rabbit CDRs. Its ability to inhibit the binding of PD-L1 to PD-1 was tested by competition ELISA. Briefly, rhPD-L1 Fc fusion was coated onto 96-well microplates. After blocking, serial dilutions of scFv were added to plates and incubated for one hour at room temperature. Half of the scFv dilution was replaced with biotinylated PD-1 Fc fusion and bound PD-1 was detected with streptavidin-HRP.

Similarly, the ability to inhibit the binding of PD-L1 to CD80 was tested by competition ELISA using rhCD80-His. After blocking, serial dilution of scFv was prepared with a constant concentration of 50 nM rhPD-L1 Fc fusion. This mixture was incubated with the CD80 coated plates for 2 hours at room temperature. The background level corresponding to no binding of PD-L1 to CD80 was determined by including a dilution series of scFv1 in the absence of any PD-L1-Fc. Bound PD-L1 Fc fusion was detected with goat anti-human IgG Fc-HRP. In this assay, the ability of PD-L1 to interact with CD80 generates an absorbance signal, which is effectively neutralized to background level by the scFv. Taken together, these results indicate that scFv blocks the interaction of PD-L1 with both PD-1 and CD80.

Stability

Two different processes can be observed that may affect the stability of scFvs. Firstly, scFvs could be prone to dimerization, often followed by oligomerization and further aggregation and precipitation. Secondly, scFv degradation, leading to smaller fragments, can occur over time.

The stability of the scFv formulated in PBS pH 7.2 at 10 mg/mL concentration upon storage at different temperature conditions (4°C, 22°C, 37°C and -20°C) was investigated. Only a minor amount of dimerization of the scFv or formation of high molecule weight molecules was observed upon storage for 2 weeks at 4°C, 22°C and 37°C. The scFv formed up to 1.8% and 2.7% of dimers after 1 or 2 weeks of storage at 37°C, respectively.

The thermal stability was assessed by differential scanning fluorimetry (DSF) in a real-time PCR device (Corbett, Rotor-Gene). The midpoint melting temperatures (Tm) of the scFv calculated using Rotor-Gene 6000 Series Software 1.7. was 81.5°C.

Proteinaceous biologics may become exposed to freeze/thaw stress during manufacturing, storing and shipping which may cause aggregation and degradation. In order to assess stability of the anti-PD-L1 scFv during freeze/thaw cycles, it was formulated in PBS pH 7.2 at 10 mg/mL in 1.5 mL polypropylene tubes. The vials were submerged into liquid nitrogen,

followed by incubation in a water bath at room temperature. After centrifugation, supernatants were analyzed by SE-HPLC. Virtually 100% of the scFv remained monomeric after 10 freeze/thaw cycles and no protein loss or precipitation was observed.
The stability of the anti-PD-L1 scFv in human serum (Sigma-Aldrich, cat no H4522) was
5 assessed by ELISA. There was no loss of binding activity of the scFv after up to 20 hours of incubation with human serum at 37°C.

Kinetic Exclusion Assay of the scFv and the full-length antibody

The affinity of a monovalent and a bivalent antibody to PD-L1 was determined by Kinetic
10 Exclusion Assay (KinExA®) with a KinExA 3200 (Sapidyne Instruments, USA, cat. no. 5001). The KinExA® measures the equilibrium binding affinity and kinetics between unmodified molecules in solution. The measurement requires the immobilization of one interaction partner on a solid phase solely to act as a probe to determine the concentration of the corresponding binding partner in solution.
15 a) Monovalent antibody

The affinity of the scFv to PD-L1-Fc fusion was determined at room temperature in PBS with 0.02% sodium azide, pH7.4. Two curves were measured, one using 20 pM scFv1 with an incubation time of 5 hours and the other at 10 pM scFv1 with an incubation time of 9 hours. The KD value for the scFv was 8.8 pM, calculated using the "n-curve analysis" of the
20 KinExA® Pro software version 4.1.9 or 4.2.10.

b) Bivalent antibody

The scFv was reformatted into IgG format and expressed in suspension-adapted CHO K1 cells originally received from ATCC and adapted to serum-free growth in suspension culture. IgG antibodies were purified by Protein A chromatography followed by size
25 exclusion chromatography. The KD for binding to PD-L1-His was calculated at room temperature in PBS with 0.02% sodium azide, pH7.4, using two curves. One curve used 100 pM of IgG with an incubation time of 5 hours, and the other used 10 pM of IgG with an incubation time of 10 hours. The KD value calculated for the binding of the IgG to human PD-L1 was 2.77 pM. The results demonstrate that the IgG binds PD-L1 with an affinity
30 around three times tighter than the affinity of the scFv to PD-L1

Selectivity

Cross-reactivity of the scFv to PD-L1 from other species was determined by ELISA, using PD-L1 Fc fusions from human (RnD Systems, USA, cat. no. 156-B7), rat (Sino Biological,

China, cat. no. 80450-R02H) or monkey (Sino Biological, China, cat. no. 90251-C02H). The results indicated that the scFv specifically bound to human and monkey PD-L1, but not to rat PD-L1. Crossreactivity of the anti-PD-L1 scFv to monkey PD-L1 was further investigated using KinExA®. The KD value was calculated at room temperature in PBS with 0.02% sodium azide, pH7.4 using two curves. One curve used 50 pM of scFv with an incubation time of 6 hours, and the other used 10 pM of scFv with an incubation time of 16 hours. The KD value calculated for the scFv was 3.3 pM. The results demonstrate that the scFv binds to monkey PD-L1 with an affinity around 2.7 times tighter than binding to human PD-L1.

Cross-reactivity of the scFv to recombinant human proteins sharing sequence similarity to PD-L1 was determined by ELISA using rhPD-L1 Fc fusion (RnD Systems, USA, cat. no. 156-B7), rhPD-L2 Fc fusion (RnD Systems, USA, cat. no. 1224-PL) or rhB7-H3 Fc fusion (RnD Systems, USA, cat. no. 1027-B3). The results indicated that scFv specifically bound to human PD-L1, with no cross-reactivity to human PD-L2 or B7-H3.

Binding to Cell Surface PD-L1

The ability of the scFv to bind to PD-L1 on the surface of cells was determined by extracellular FACS staining of ES-2 cells (ATCC, USA, cat. no. CRL-1978). The results demonstrate that the scFv is able to specifically recognize the natural form of PD-L1 expressed on the surface of cells.

The binding of the scFv to cell surface PD-L1 was further investigated at room temperature in PBS with 0.02% sodium azide, pH7.4 using KinExA®. One curve was constructed, using 50 pM scFv and an incubation time of 5 hours. The calculated KD value for the scFv binding to cell surface expressed PD-L1 on ES-2 cells is 12.8 pM.

Example 2 - Generation of the retroviral construct encoding the anti-PD-L1 scFv and retrovirus production

DNA encoding the anti-PD-L1 scFv was amplified by PCR with the following primers: Forward: 5'- TAACCATGG AGTTTGGGCTGAG -3' (SEQ ID NO: 11) and Reverse: 5'-GACGCATGCTCAGCTCGACACGGTGACC -3' (SEQ ID NO: 12) in order to add the NcoI restriction site at 5' end (forward primer) and the stop codon TGA and the restriction site SphI at the 3' end (reverse primer). The PCR product and the retroviral backbone SFG(I)eGFP were digested with NcoI and SphI and ligated. The insert was sequenced to confirm that no mutations occurred during the cloning. The final vector was named

SFG.scFv.anti-PD-L1(I)eGFP (see Figure 1). The reporter gene GFP expressed upon IRES is used to assess the transduction efficiency. Transient retroviral supernatant was prepared by transfection of 293T cells with the retroviral vector and two plasmids encoding gag-pol and RDF envelop, respectively. The supernatant collected at 48 hrs was used to transduce 5 activated T cells isolated from healthy donors. The retroviral vectors encoding the CAR that targets the GD2 antigen (GD2.CAR) including either the CD28 (CAR.CD28 or CAR.28) or the 4-1BB (CAR.41BB or CAR.BB) endodomains were previously described (Heczey A. et al, Blood. 2014 Oct 30; 124(18):2824-33). Said CAR comprises the CDR sequences of SEQ ID NOs: 16 to 21.

10

Example 3 - Generation and expansion of CAR T cells producing the anti-PD-L1 scFv

Peripheral blood mononuclear cells (PBMCs) from healthy human donors were isolated by Lymphoprep (Fresenius) density gradient centrifugation. Primary T cells were cultured in complete T cell media containing 44% Click's medium (Irvine Scientific), 44% RPMI 1640 15 (Hyclone), 10% FBS (Hyclone), 1% Glutamax and 1% penicillin/streptavidin in the presence of IL-7 (10 ng/mL) and IL-15 (5 ng/mL) (from PeproTech). T cells were activated with immobilized anti-CD3 (1 mcg/mL) (Miltenyi, Catalog Number: 130-093-387) and anti-CD28 (1 mcg/mL) (BD Biociences, Catalog Number: 555725) in 24-well plate at the concentration of $0.5x10^6$ cell/mL in T cells media without cytokines. Twenty four hours after 20 the stimulation, IL-7 and IL-15 were added to the medium. By day 2, T cells were transduced with the retroviral supernatant SFG.scFv.anti-PD-L1(I)eGFP and/or for the GD2.CAR (1 mL/well of retroviral supernatant in a retronectin-coated 24-well plate). In order to generate T cells expressing the GD2.CAR and releasing the anti-PD-L1 scFv a co-transduction with both retroviral constructs has been performed (1 mL/well of GD2.CAR supernatant plus 1 25 mL/well of anti-PD-L1 scFv). Non-Transduced (NT) T cells were plated at the same concentration ($0.25x10^6$ cells/mL) in a non-tissue culture plate coated with retronectin. Seventy two hours after the transduction T cells were washed, counted and suspended in complete T cell media with IL-7/IL-15 at $1x10^6$ cells/mL. T cells were expanded *in vitro* for 5 days and then analyzed by flow cytometry to assess transduction efficiency and T cell 30 composition. Eleven to twelve days after initiation, T cells were tested in functional assays.

Example 4 – T cells co-transduced with the GD2.CAR and SFG.scFv.anti-PD-L1 vectors express both GD2.CAR and anti-PD-L1 scFvs

The phenotype of T cells was assessed using mAbs for CD3, CD4, CD8, CD60L, CD45RA, CD95, CD27, CD2, PD-L1 (BD Bioscience or Biolegend). GD2.CAR expression was detected using the anti-idiotype 1A7 mAb followed by staining with a secondary rat anti-mouse-IgG1-PE mAb (BD Bioscience). The transduction efficiency of the SFG.scFv.anti-PD-L1(I)eGFP vector was assessed by measuring GFP expression. GD2.CAR Relative Fluorescence Intensity (RFI) was calculated as the Mean Fluorescence Intensity (MFI) of CAR T cells divided by the MFI of non-transduced T cells. As shown in Figure 2, CD4 and CD8 T cells were successfully transduced with the anti-PD-L1 scFv retroviral construct of Example 2 without alteration of the CD4/CD8 ratio. T cells co-expressed GD2.CAR and eGFP anti-PD-L1 upon double retroviral transduction (Figure 3). Transduction with the anti-PD-L1 scFv did not affect T cell proliferation (Figure 4). Moreover, expression of the anti-PD-L1 scFv did not affect T cell subset compositions (Figure 5). This example demonstrates the generation of CAR T cells co-expressing anti-GD2 CARs and anti-PD-L1 scFvs.

Example 5 – T cells transduced with the SFG.scFv.anti-PD-L1 vector release functionally active anti-PD-L1 scFvs upon activation through the endogenous TCR/CD3 complexes

To test whether T cells are capable of secreting anti-PD-L1 scFvs the cells were stimulated through the endogenous TCR. Non-transduced T cells or anti-PD-L1 scFv transduced T cells were plated in tissue culture treated 24-well plates uncoated or coated with immobilized anti-CD3 (1μg/ml, Miltenyi) and anti-CD28 (1μg/ml, BD Bioscences) anti-CD3/CD28 anti-CD3/CD28 antibodies at the concentration of 0.5×10^6 cells/mL in 2 mL/well of T cells medium with 10% FBS. After 18 hours 1 mL of supernatant was collected for the quantification of the anti-PD-L1 scFv released by T cells using a specific sandwich ELISA assay. A matched pair of anti-scFv monoclonal antibodies of mouse origin were used for this sandwich ELISA. As shown in Figure 6 A, the anti-PD-L1 scFv was released by transduced T cells after stimulation with immobilized anti-CD3/anti-CD28 antibodies. These T cells express their natural endogenous TCR/CD3 complexes. The results suggest that activation of transduced T cells through the endogenous TCR/CD3 complexes is sufficient to induce synthesis and extracellular secretion of anti-PD-L1 scFvs. The quantitative ELISA showed large amounts of secreted anti-PD-L1 scFv (Figure 6 A). Non-transduced cells did not secrete scFvs. Since the anti-PD-L1 scFv is under the control of the constitutively active 5'LTR of a retroviral vector, transduced T cells plated on uncoated

wells released basal levels of anti-PD-L1 scFvs which however significantly increased upon T cell activation.

The anti-PD-L1 scFv produced by activated T cells is capable of binding to immobilized PD-L1 (Figure 6 B). Briefly, recombinant human PD-L1-Fc (R&D Systems) was immobilized at a concentration of 2 μg/mL onto microplates in PBS. After, blocking with 5% non-fat dry milk, increasing concentrations of scFv was added and detected by Protein L-HRP (Sigma-Aldrich). Figure 6 B shows that T cell-produced anti-PD-L1 scFv binds to PD-L1 equally well as a reference control, the anti-PD-L1 scFv produced in *E. coli*. These data demonstrate that the transduced T cells release functionally active anti-PD-L1 scFvs in large amounts upon activation through the endogenous TCR. These data also suggest that transduced T cells will readily secrete anti-PD-L1 scFvs when they are activated through genetically modified TCR and through CARs.

<u>Example 6 - T cells co-transduced with the GD2.CAR-4-1BB and SFG.scFv.anti-PD-L1 vectors have enhanced tumor-killing activity</u>

Transduced and non-transduced T cells ($0.5x10^5$ cells/well) were co-cultured with the tumor cell line CHLA-255 ($2.5x10^5$ cells/well) at an effector:target (E:T) ratio of 1:5 in 24-well plates, in the absence of exogenous cytokines. After 7 days of co-culture, T cells were harvested and counted. If the percentage of residual tumor cells was <5% (as assessed by flow cytometry), T cells were re-plated with fresh tumor cells at the same 1:5 E:T ratio for a second cycle of co-culture. After an additional 7 – 8 days, cells were then collected and analyzed by flow cytometry to enumerate T cells and residual tumor cells. Specifically, CD3 and GD2 antibodies were used to stain T cells and tumor cells, respectively. CountBright beads (Invitrogen) were used for cell counting by flow cytometry. Supernatant was also collected after 24 hours of culture to measure IFNgamma release by ELISA (R&D System) according to the manufacturer's instruction.

GD2.CAR T cells with the 4-1BB endodomain transduced with the anti-PD-L1 scFv showed better killing of tumor cells in the second cycle of co-culture (14 days of culture) (Figure 7) and produced higher levels of IFNgamma in the second cycle of co-culture (14 days of culture) with tumor cells (Figure 8) than the GD2.CAR T cells without the anti-PD-L1 svFv. Thus exhaustion of GD2.CAR T cells engaging repetitively tumor cells occurs with 4-1BB costimulation, but the presence of the anti PD-L1 scFv protect CAR T cells from exhaustion and may provide prolonged anti tumor activity in vivo and prevent tumor recurrence.

While there are shown and described presently preferred embodiments of the invention, it is to be understood that the invention is not limited thereto but may be otherwise variously embodied and practiced within the scope of the following claims. Since numerous modifications and alternative embodiments of the present invention will be readily apparent
5 to those skilled in the art, this description is to be construed as illustrative only and is for the purpose of teaching those skilled in the art the best mode for carrying out the present invention. Accordingly, all suitable modifications and equivalents may be considered to fall within the scope of the following claims.

CLAIMS

1. An engineered immune cell expressing:

i) an antigen receptor, and

5 ii) an antibody blocking PD-L1.

2. The engineered immune cell of claim 1, wherein the antibody inhibits PD-L1 interaction with both CD80 and PD-1.

10 3. The engineered immune cell of claim 1 or 2, wherein the antibody is humanized.

4. The engineered immune cell of any one of the preceding claims, wherein said immune cell is

a T cell,

15 a Natural Killer T (NKT) cell,

a Natural Killer (NK) cell

a human embryonic stem cell,

a hematopoietic stem cell (HSC) or

an induced pluripotent stem cell (iPS).

20

5. The engineered immune cell of any one of the preceding claims, wherein said T cell is a cytotoxic T lymphocyte (CTL), a regulatory T lymphocyte, an inflammatory T-lymphocytes, a helper T-lymphocyte or a gamma-delta T cell.

25 6. The engineered immune cell of claims 4 or 5, wherein said T cell is a CD4+ or CD8+ or a mixed population of CD4+ and CD8+ cells.

7. The engineered immune cell of any one of the preceding claims, wherein said antigen receptor is a CAR, said CAR comprising a cytoplasmic domain, a transmembrane domain

30 and an extracellular domain.

8. The engineered immune cell of claim 7, wherein said transmembrane domain is a CD3 zeta, CD4, a CD28, a CD8 alpha or a 4-1BB transmembrane domain.

9. The engineered immune cell of any one of the preceding claims, wherein the CAR further comprises one or more costimulatory domains,

10. The engineered immune cell of any of claims 1-6, wherein said antigen receptor is a
5 TCR, such as an endogenous TCR or an engineered TCR.

11. The engineered immune cell of any one of the preceding claims, wherein said antigen receptor is recombinantly expressed.

10 12. The engineered immune cell of any one of the preceding claims, wherein said antibody is a full-length immunoglobulin or an antibody derivative.

13. The engineered immune cell of any one of the preceding claims, wherein said antibody comprises a functional Fc domain.

15

14. The engineered immune cell of any one of the preceding claims, wherein said antibody comprises a Fc domain which is modified such that it does not induce cytotoxic immune responses or complement activation.

20 15. The engineered immune cell of any one of the preceding claims, wherein said antibody does not comprise a Fc domain.

16. The engineered immune cell of claim 15, wherein said antibody is an antibody fragment selected from the group consisting of Fab, Fab', scFab, scFv, Fv fragment,
25 nanobody, VHH, dAb, minimal recognition unit, diabody, single-chain diabody (scDb), BiTE or DART.

17. The engineered immune cell of any one of the preceding claims, wherein said antibody binds human PD-L1 with a KD of lower than 100 pM,

30

18. The engineered immune cell of any one of the preceding claims, wherein said antibody comprises

i) at least one of the variable heavy chain (VH) CDR sequences CDR-H1, CDR-H2 or CDR-H3 as set forth in SEQ ID NOs.: 6, 7, and 8, respectively, or variants thereof,

ii) at least one of the variable light chain (VL) CDR sequences CDR-L1 , CDR-L2 or CDR-L3 as set forth in SEQ ID NOs.: 3, 4, and 5, respectively, or variants thereof.

19. The engineered immune cell of any one of the preceding claims, wherein said
5 antibody comprises

i) at least one VH sequence of SEQ ID NO.: 2, and/or

ii) at least one VL sequence of SEQ ID NO.: 1.

20. The engineered immune cell of any one of the preceding claims, wherein said
10 antibody comprises SEQ ID NO.: 9.

21. The engineered immune cell of any one of the preceding claims, comprising

i) at least one VH framework sequence of SEQ ID NO.: 2, and/or

ii) at least one VL framework sequence of SEQ ID NO.: 1.

15

22. The engineered immune cell of any one of claims 1 to 11, wherein the antibody competes with the antibody of claims 17 to 19 for binding to PD-L1.

23. The engineered immune cell of any one of the preceding claims, wherein said
20 antibody is secreted by the cell and/or expressed on its surface, for example is secreted.

24. The engineered immune cell of any one of the preceding claims, wherein the cell further expresses one or more cytokine, preferably human cytokine, such as IL-2, IL-4, IL-7, IL-12, IL-15, IL-21 and/or MIP-1alpha, and/or
25 further expresses one or more antibodies targeting an immune inhibitory molecule, such as transforming growth factor-beta (TGF-β), IL-10, Fas, CD47, CTLA-4, Tim-3, LAG-3, and ligands thereof

25. The engineered immune cell of any one of the preceding claims, wherein the antigen
30 receptor binds to an antigen that is expressed by or derived from a tumor or a pathogen.

26. The engineered immune cell of any one of the preceding claims, wherein the antigen is selected from the group consisting of GD2, WT-1, 5T4, GPC3, CSPG4, MUC16, MUC1, CA1X, CEA, CDS, CD7, CD 10, CD19, CD20, CD22, CD23, CD30, CD33, CD34, CD38,

CD41, CD44, CD49f, CD56, CD70, CD74, CD133, CD138, CD123, cytomegalovirus (CMV) proteins such as pp65 or IE-1, human papillomavirus (HPV) proteins such as E6 or E7, Epstein-Barr virus (EBV) proteins such as EBNA-1, LMP-1, LMP-2, or BARF-1, ADV proteins such as hexon, EGP-2, EGP-40, EpCAM, erb-B2, erb-B3, erb-B4, FBP, Fetal acetylcholine receptor, folate receptor-a, GD3, Her-1, HER-2, HER2-HER3 in combination or HER1-HER2 in combination, hTERT, IL- 13R~a2, K-light chain, DR, LeY, LI cell adhesion molecule, MAGE-A1, MAGE-A4, MAGE-A10, Mesothelin, NKG2D ligands, NY-ESO-1, PSCA, PSMA, ROR1, TAG-72, VEGF-R2, EGFR, EGFRvIII, mutated p53, mutated ras, mutated raf, mutated RAC1, bcr/abl fusions, c-Met, alphafetoprotein, CA-125, MUC-1, epithelial tumor antigen, prostate-specific antigen, melanoma-associated antigen, folate binding protein, HIV-1 envelope glycoprotein gpl20, HIV-1 envelope glycoprotein gp41, meothelin, HERV-K, or ERBB2 ..

27. A nucleic acid encoding the antigen receptor and the antibody according to any one of the preceding claims.

28. An expression vector comprising a nucleic acid encoding the antigen receptor and/or the antibody of any one of claims 1 to 26.

29. The expression vector of claim 28, being a lentiviral, a retroviral, an adenoviral, an Adeno-Associated Virus (AAV), a plasmid, a transposon, and insertion sequence, or an artificial chromosomal vector.

30. The expression vector of claim 28 or 29, being a multicistronic vector, such as a bicistronic vector.

31. The expression vector of any one of claims 28 to 30, comprising at least one IRES sequence and/or at least one self-cleaving sequence, such as a 2A sequence.

32. The expression vector of any one of claims 28 to 31, further comprising a safety switch, for example an inducible suicide switch.

33. A cloning vector comprising the nucleic acid of claim 27.

34. A method of generating an immune cell according to any one of claims 1 to 26, comprising the steps of:

(a) Providing an immune cell,

(b) Introducing into said cell at least one nucleic acid encoding said antigen receptor and at least one nucleic acid encoding said antibody; and

(c) Expressing said nucleic acids by said cell.

35. The method of claim 34, wherein step (b) comprises introducing the expression vector of any one of claims 28 to 32 into said cell.

36. The method of claim 34 or 35, further comprising the step of:

(i) Introducing into said cell at least one other antigen receptor having different antigen specificity than the antigen receptor of claim 34; and/or introducing into said cell at least one other antibody having a different antigen specificity than the antibody of claim 34.

37. A pharmaceutical composition comprising

i) an effective amount of the engineered immune cell of any one of claims 1 to 26 or of the expression vector of any one of claims 28 to 32, and

ii) a pharmaceutically acceptable excipient.

38. The engineered immune cell of any one of claims 1 to 26, the expression vector of any one of claims 28 to 32 or the pharmaceutical composition of claim 37 for use in therapy.

39. The engineered immune cell, the expression vector or the pharmaceutical composition of claim 38, wherein therapy is in combination with one or more therapies selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

40. A method of treating a subject in need thereof comprising:

(a) Providing the engineered immune cell according to any one of claims 1 to 26;

(b) Administrating said immune cells to said subject.

41. The method of claim 40, wherein said immune cell are autologous or allogeneic.

42. The method of any one of claims 40 or 41, wherein cells are administered one or more times to said subject.

43. A method of treating a subject in need thereof comprising:
5 (a) Providing the expression vector according to any one of claims 28 to 32 or the pharmaceutical composition of claim 37;
(b) Administrating said expression vector or said pharmaceutical composition to said subject.

10 44. The method of any one of claims 40 to 43, in combination with one or more therapies selected from the group of antibody therapy, chemotherapy, cytokine therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

45. The cell or the vector of claim 38 or 39, or the method of any one of claims 40 to 44,
15 wherein the condition to be treated is a pre-malignant or malignant cancer condition, such as NSCLC (non small cell lung carcinoma), urothelial cancer, melanoma, renal cell carcinoma, Hodgkin's lymphoma, head and neck squamous cell carcinoma, ovarian cancer, gastrointestinal cancer, hepatocellular cancer, glioma, breast cancer, lymphoma, small cell lung carcinoma, myelodysplastic syndromes, prostate cancer, bladder cancer, cervical
20 cancer, non clear cell kidney cancer, colorectal cancer, sarcomas, colon cancer, kidney cancer, lung cancer, pancreatic cancer or gastric cancer, skin cancer, uterine cancer, glioblastoma, neuroblastoma, sarcoma, head and neck cancer, leukemia, carcinoma, Merkel cell carcinoma or renal cell carcinoma (RCC), blood cancer, multiple myeloma, lymphoblastic leukemia (ALL), B cell leukemia, chronic lymphocytic leukemia, non-
25 Hodgkin's lymphoma, and ovarian cancer; pathogen infection, an autoimmune disorder.

46. A kit for treatment of cancer, pathogen infection, an autoimmune disorder comprising
the engineered immune cell of any one of claims 1 to 26 or the expression vector of
30 any one of claims 28 to 32, and
written instructions for use.

47. The kit of claim 46, further comprising an inducer of a safety switch, such as an inducible suicide switch.

48. The engineered immune cell of claim 9, wherein the CAR further comprises one or more costimulatory domains, for example CD28, 4-1BB (CD137), ICOS, or OX40 (CD134), or functional fragments thereof, respectively.

5

Figure 1

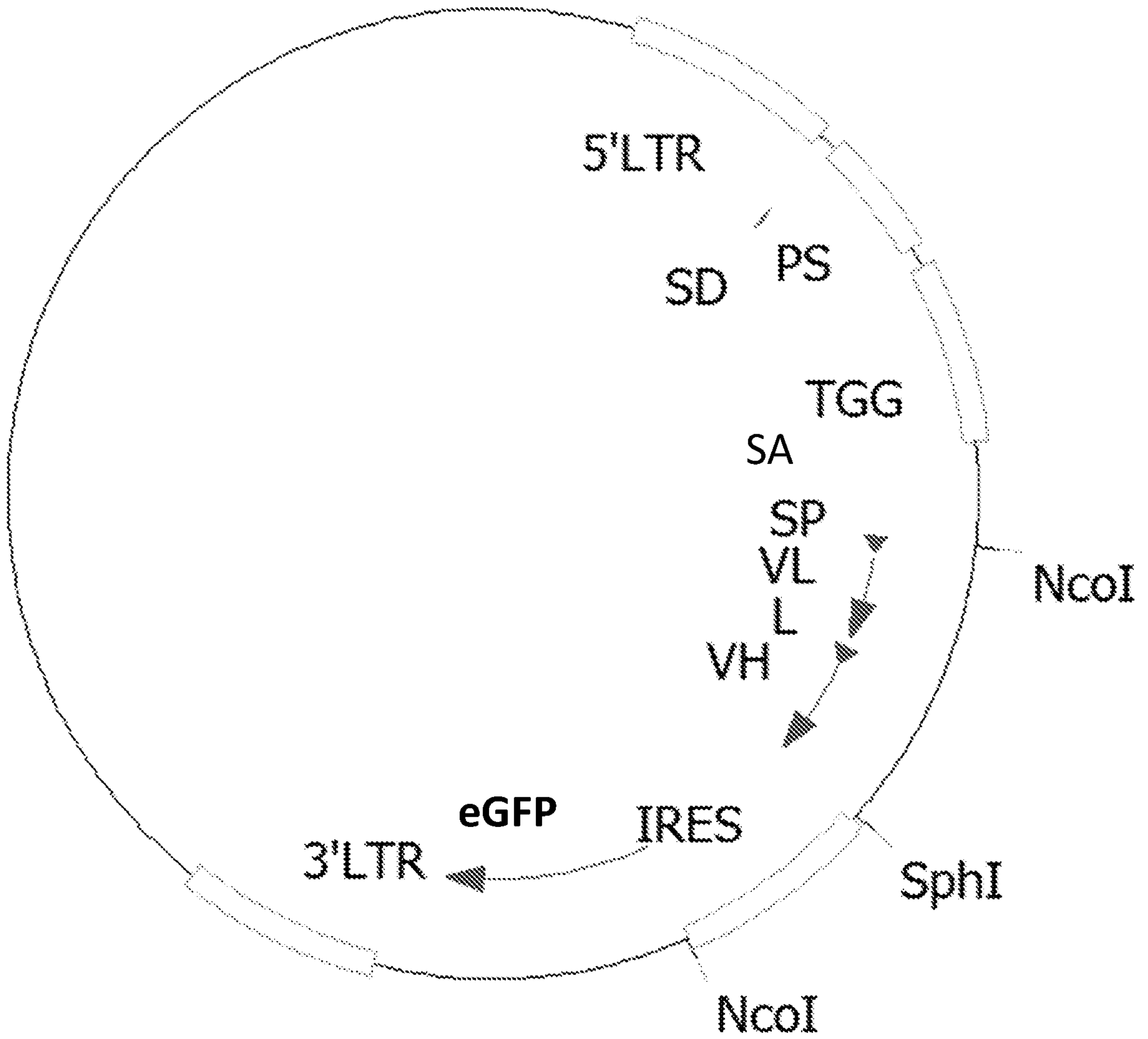
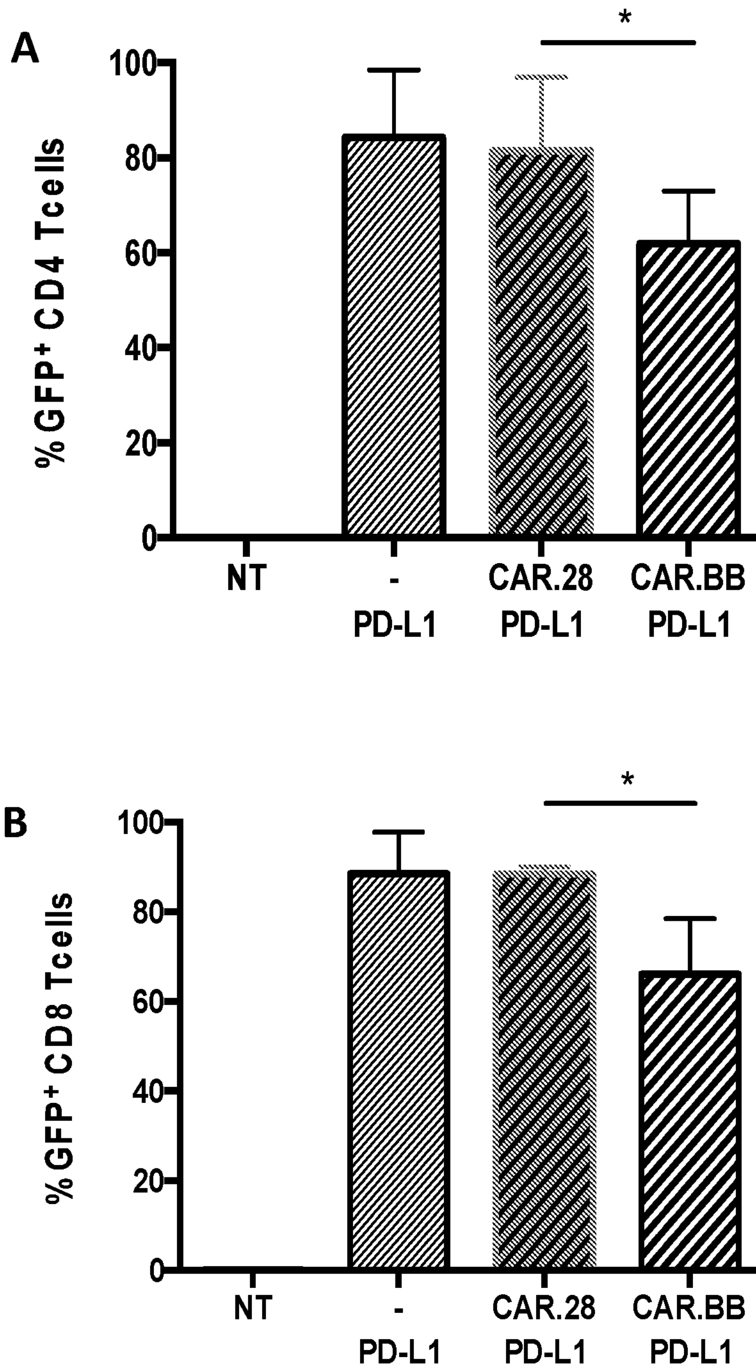


Figure 2



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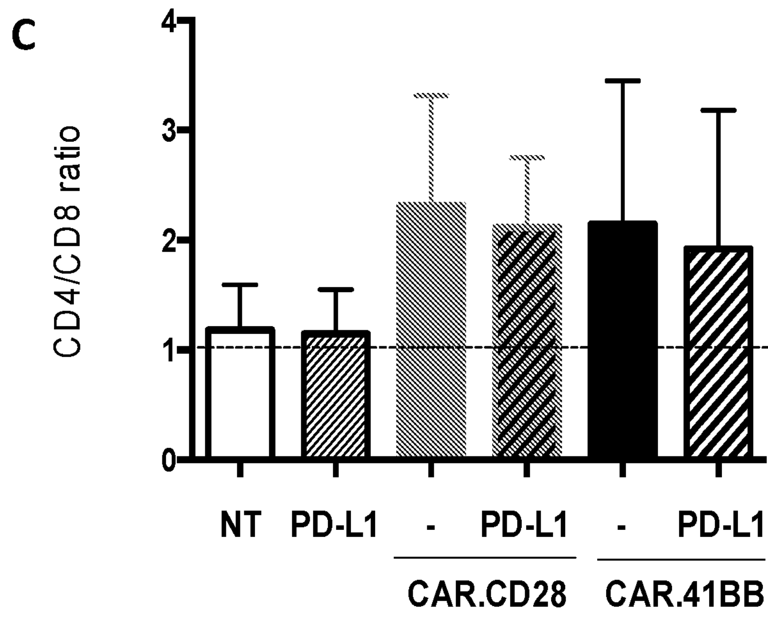

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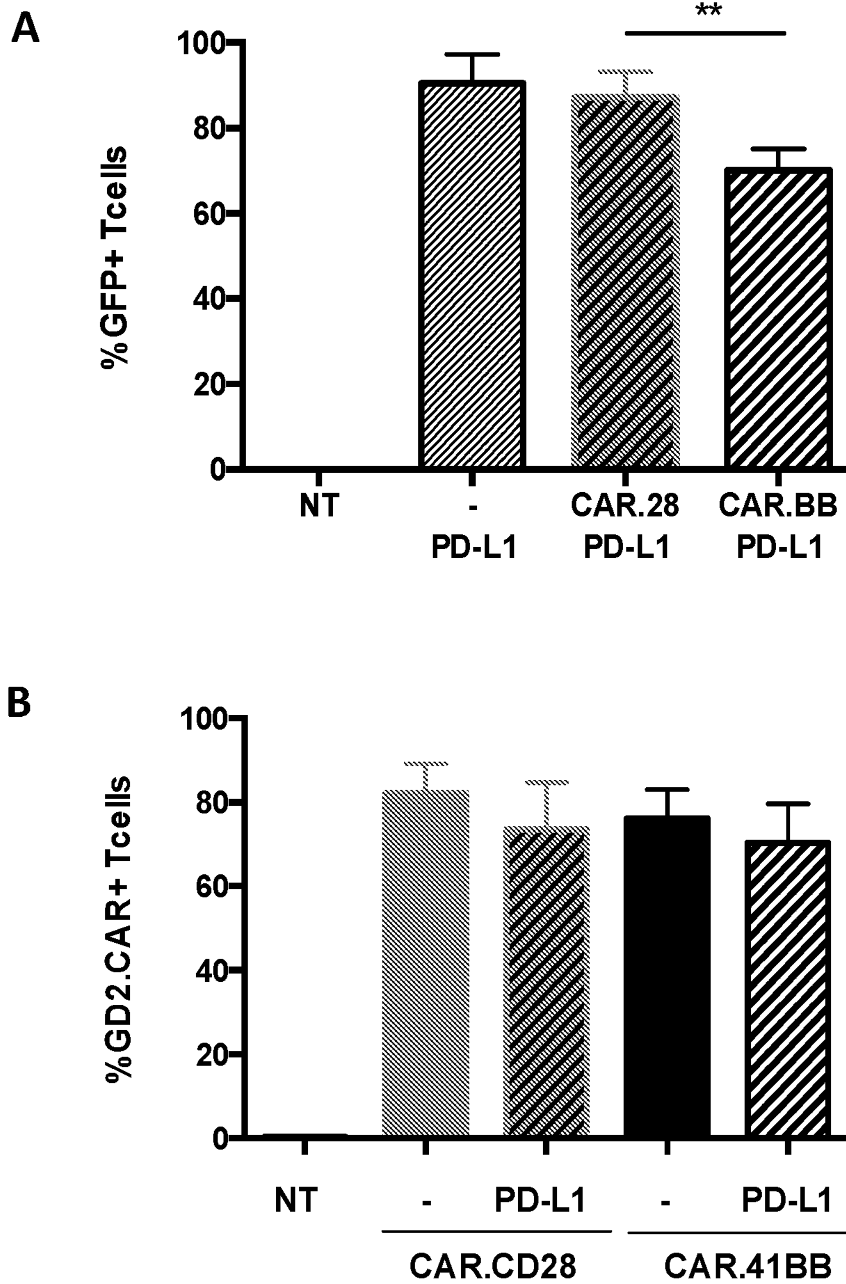

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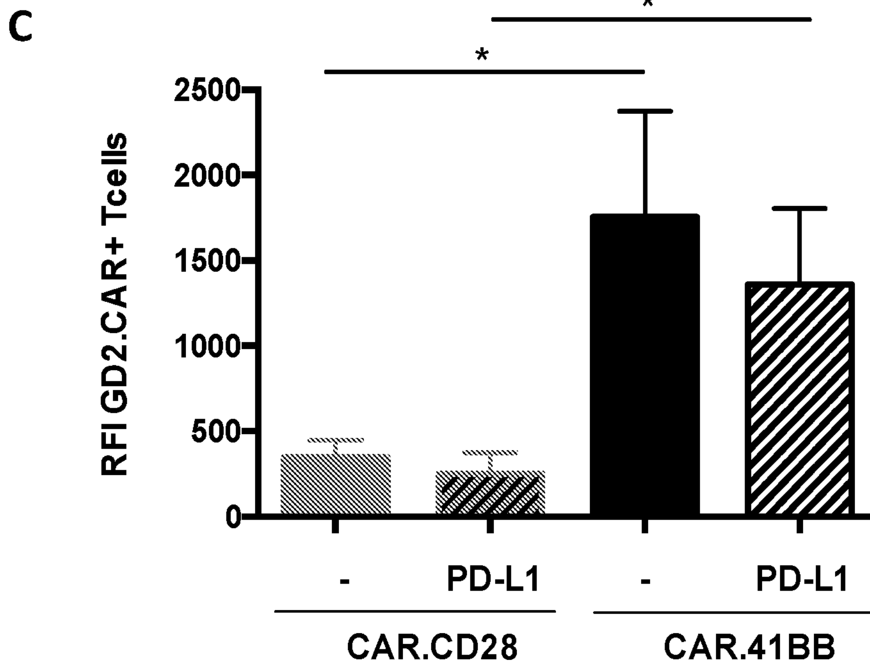

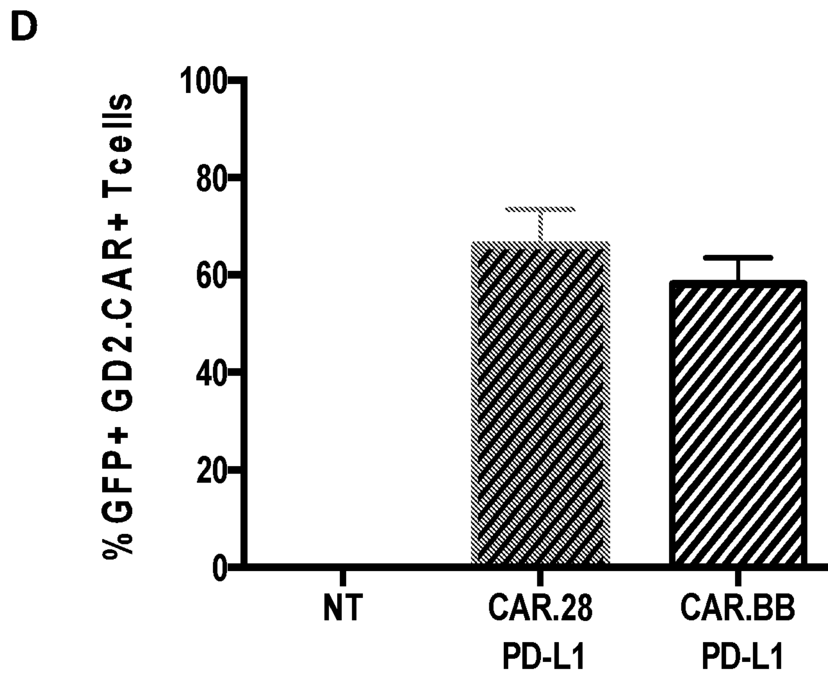

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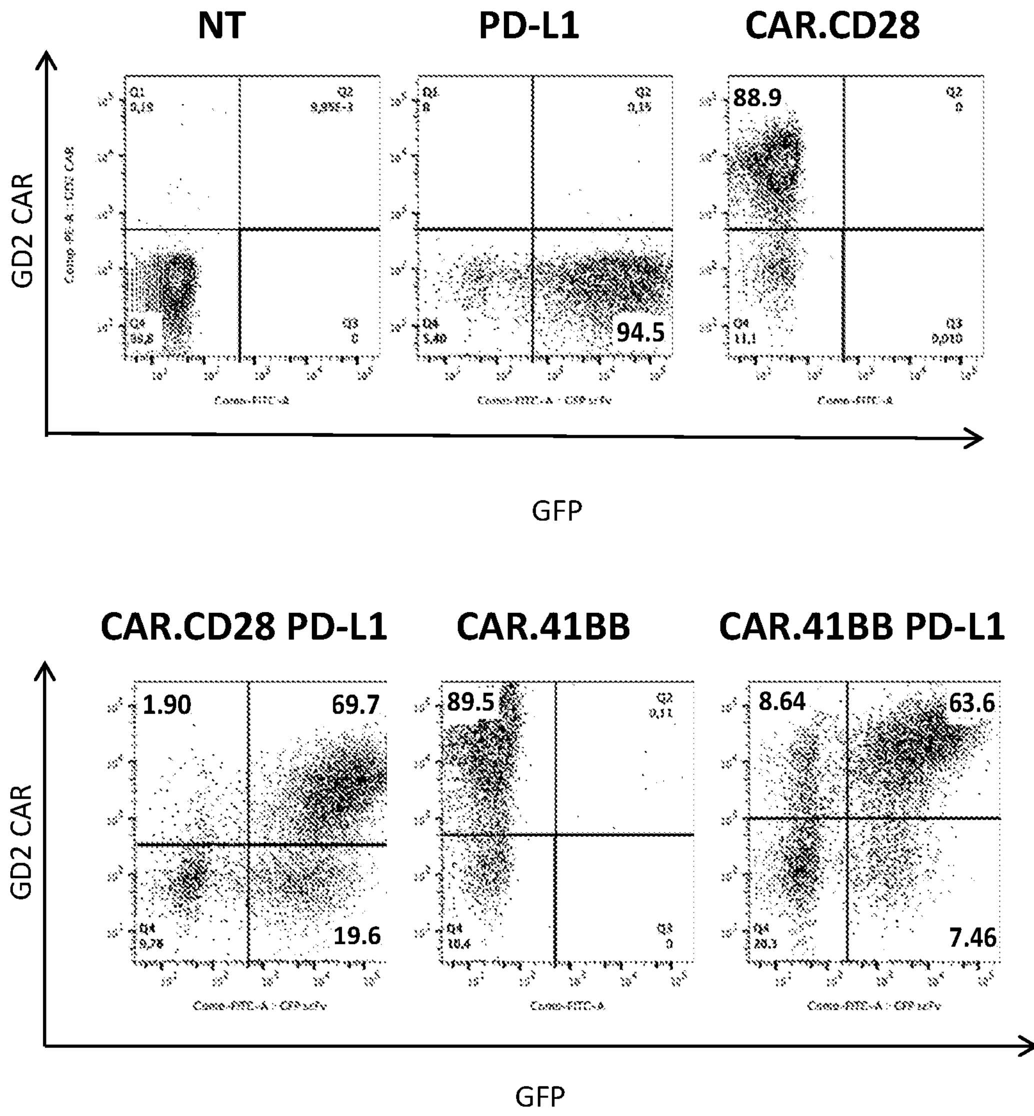

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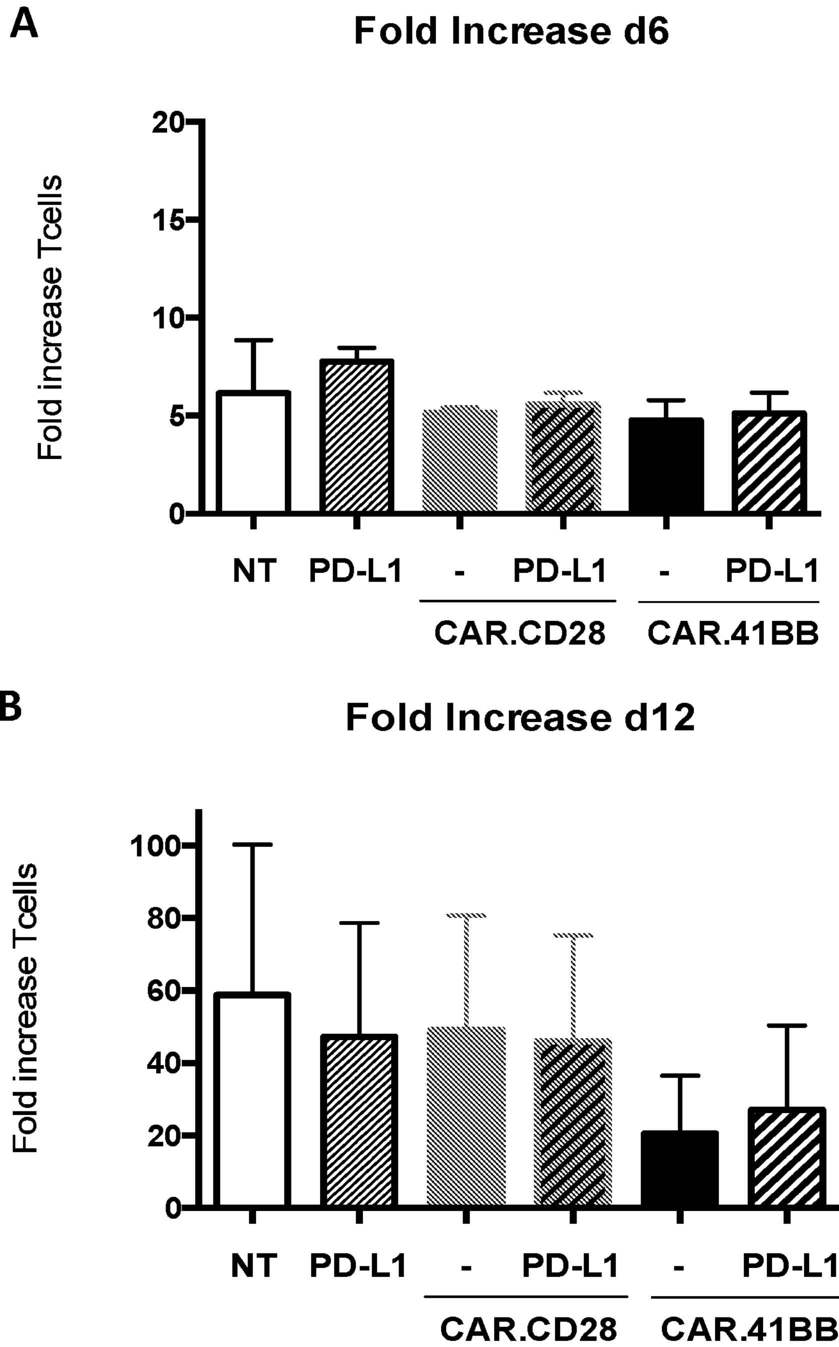

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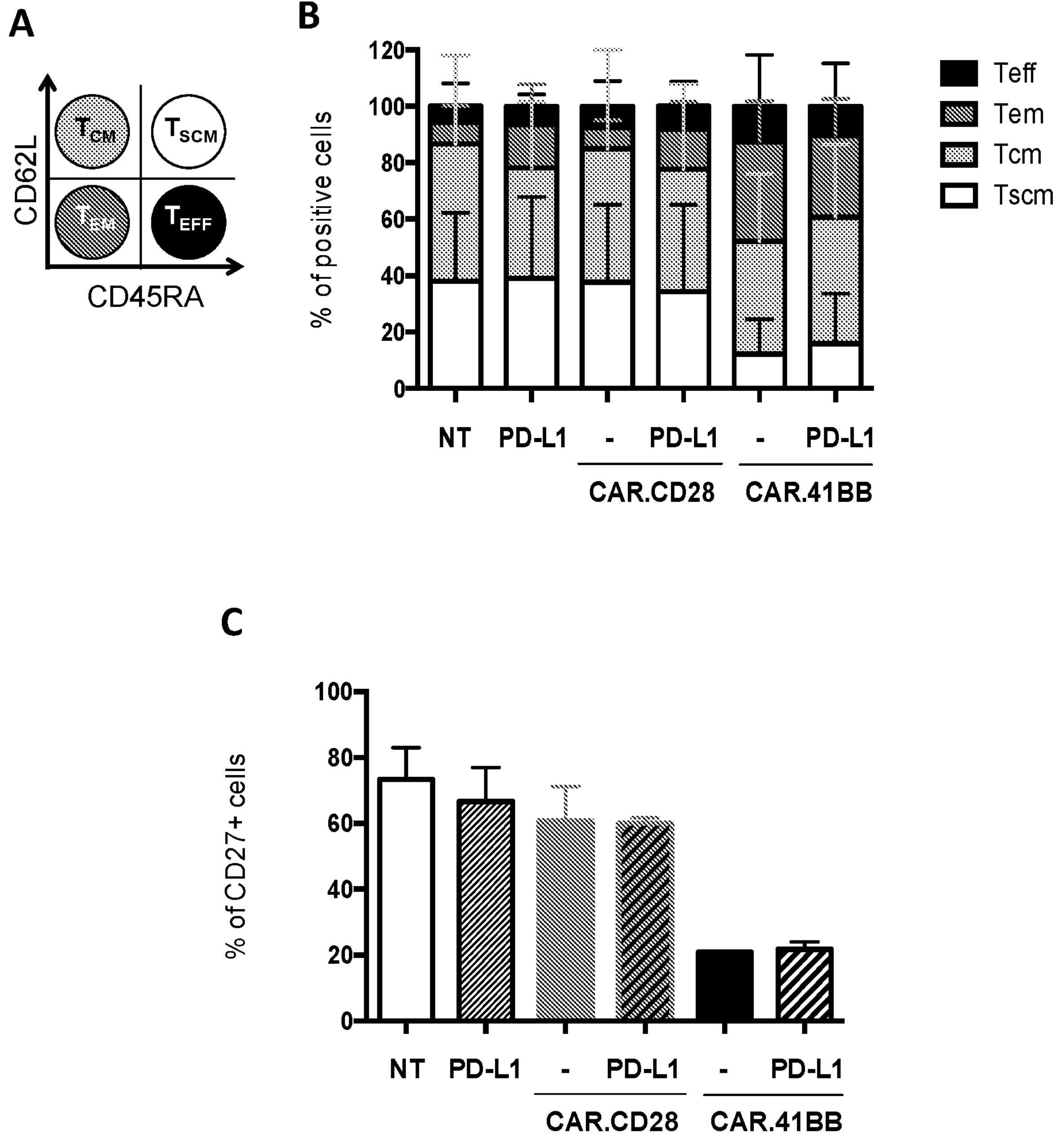

Figure 5

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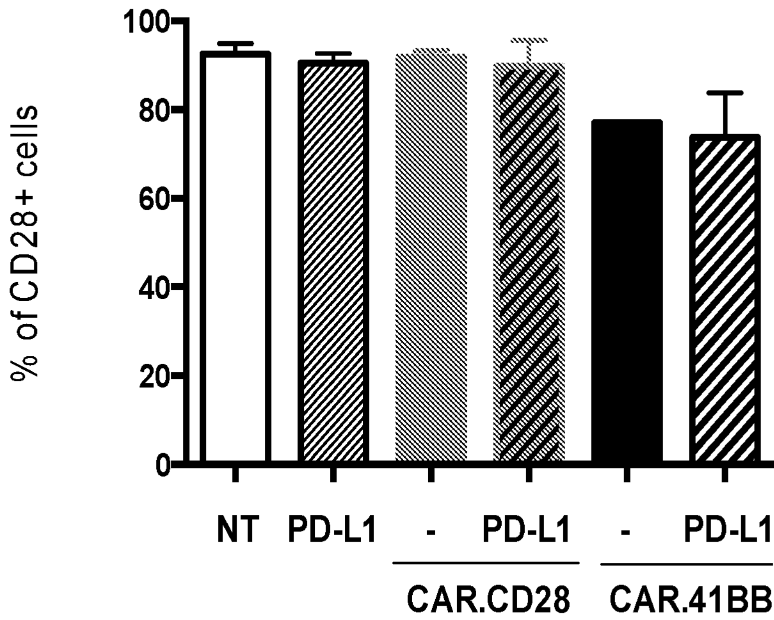

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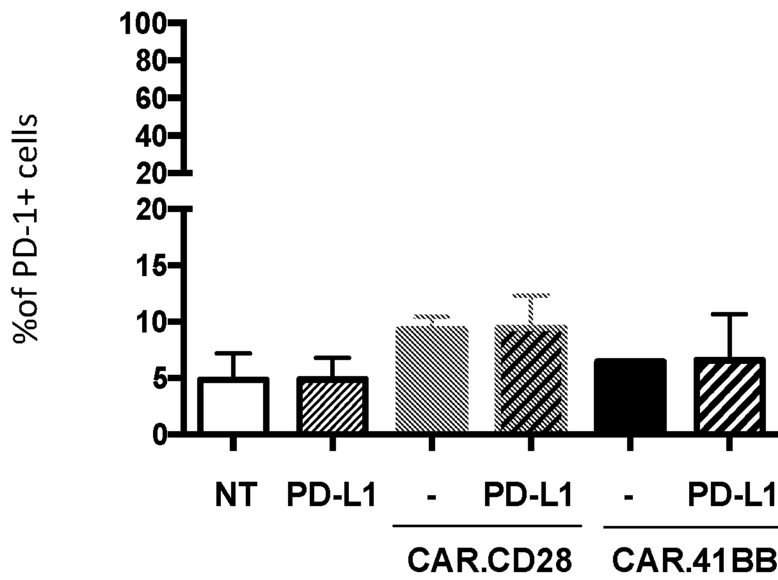

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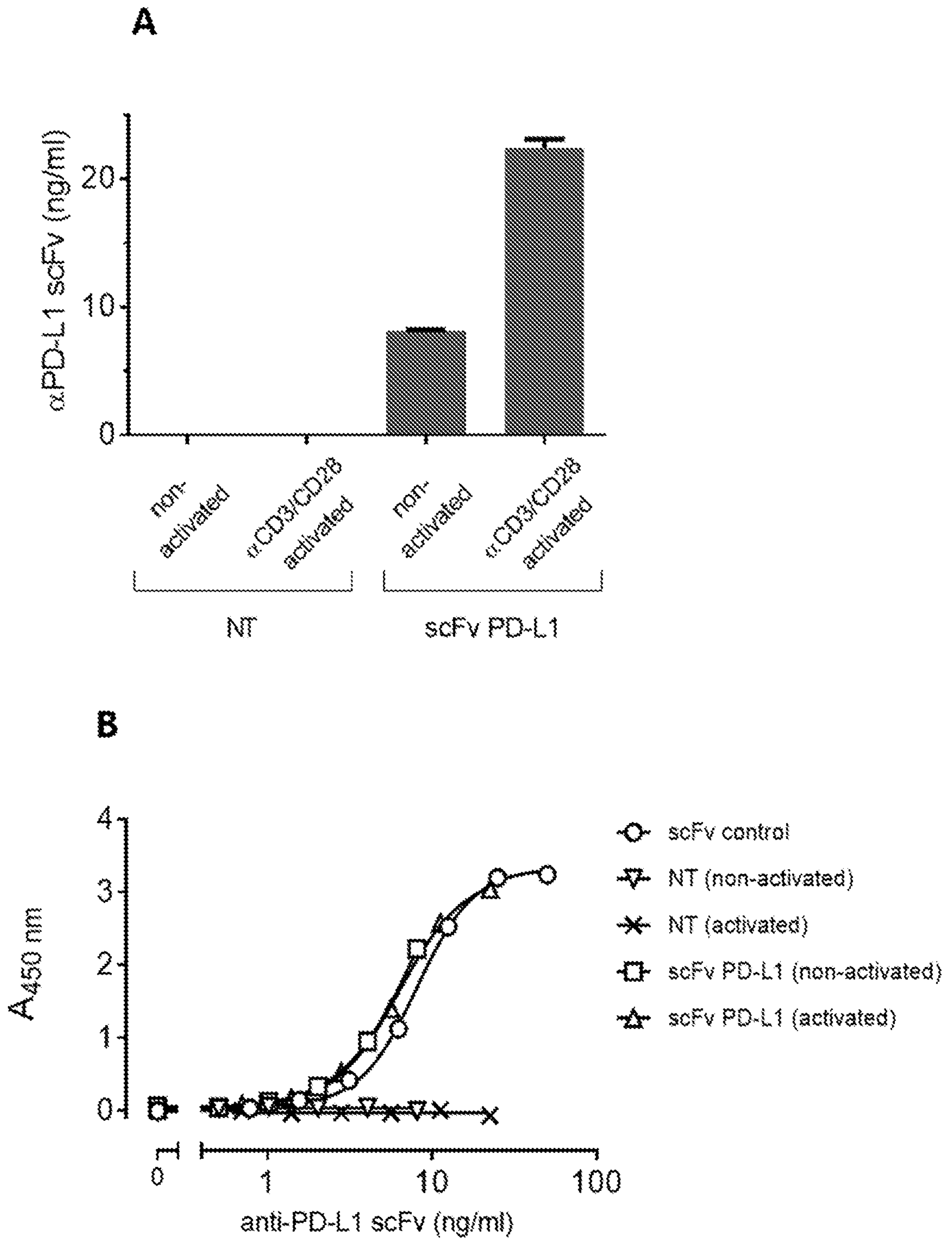
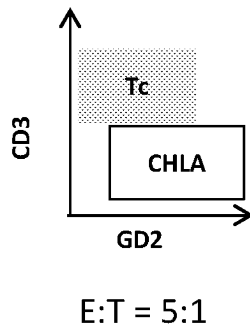


Figure 7 A



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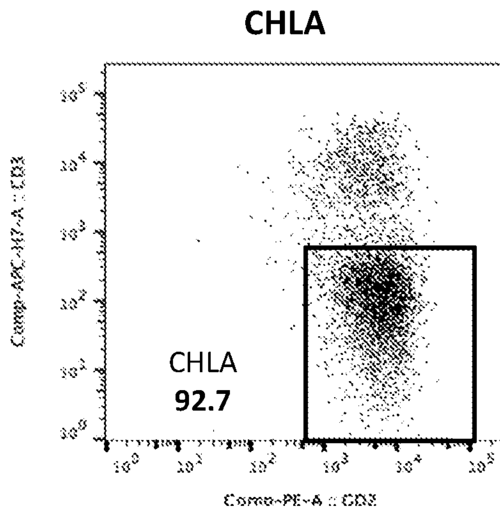

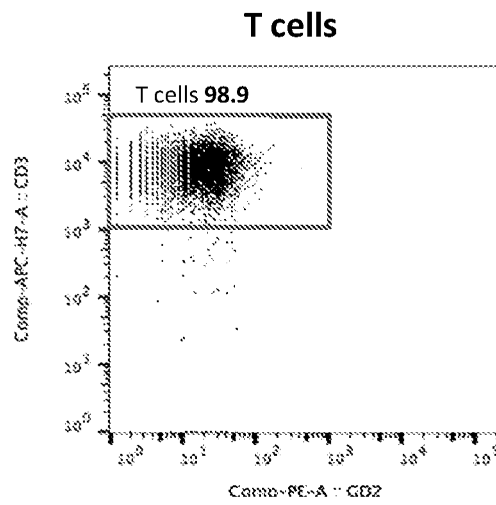

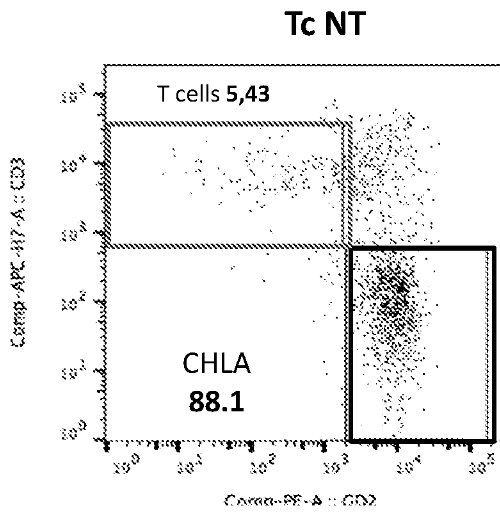

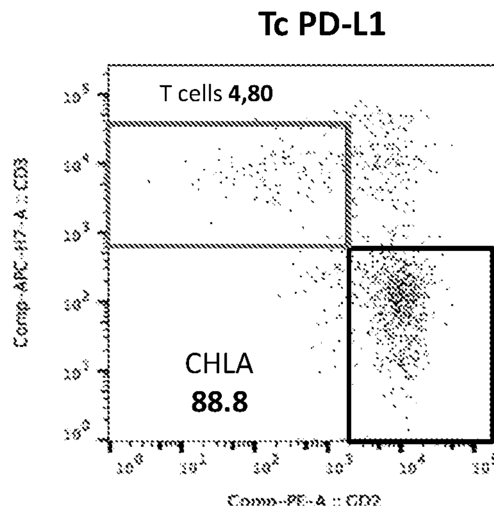

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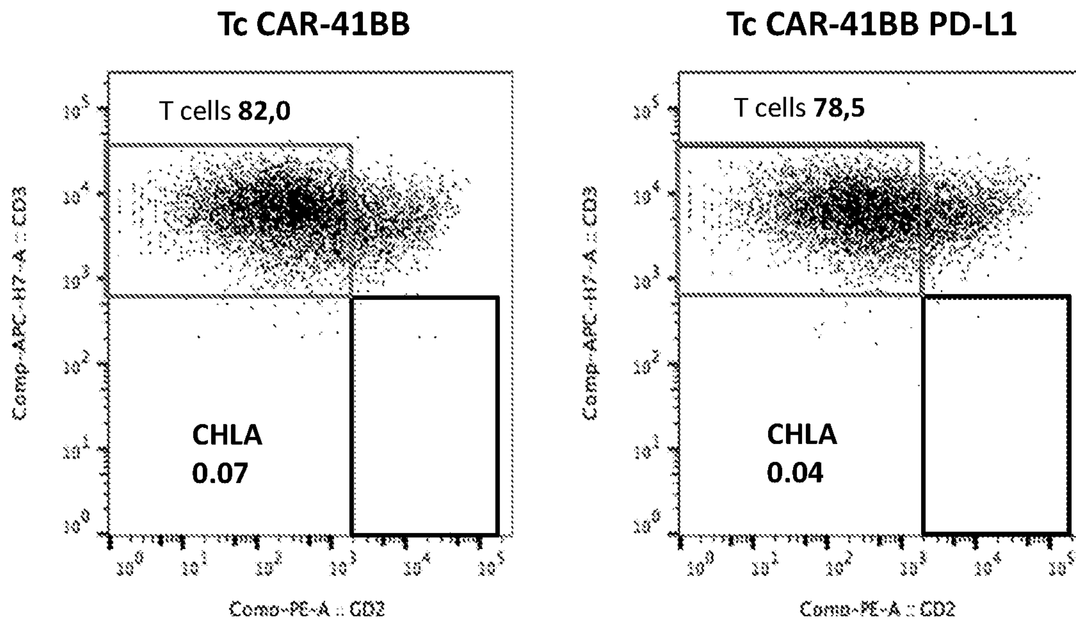

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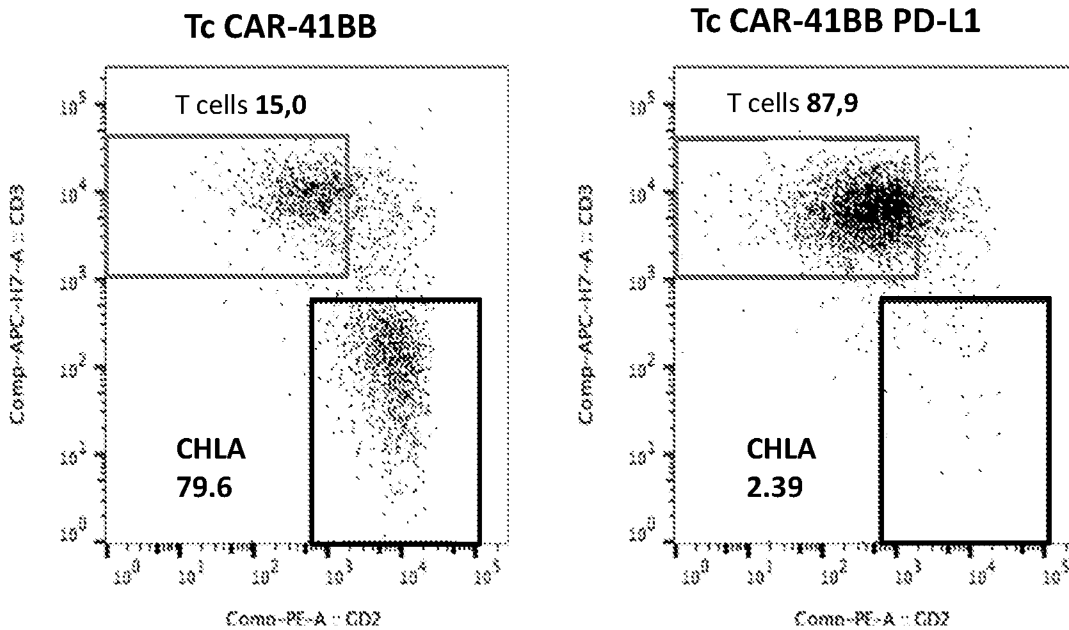

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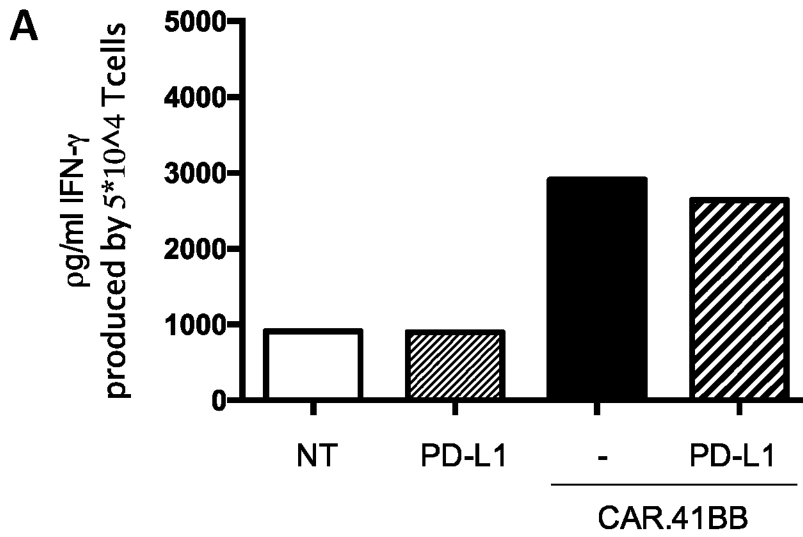

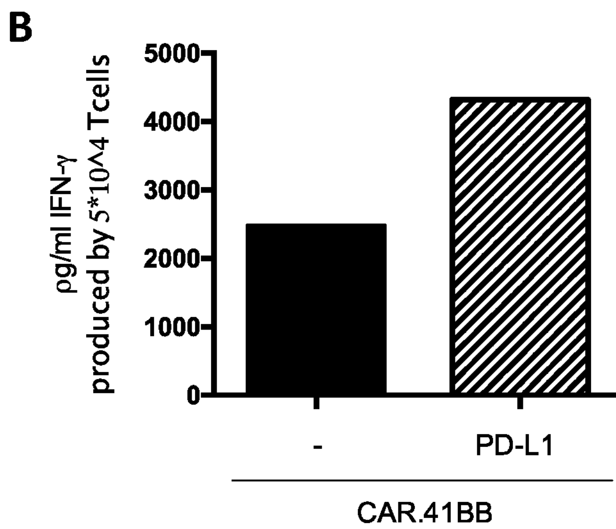

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/019301

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61K35/17 C12N5/10
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 A61K C12N

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

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

EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AUSTIN W BOESCH ET AL: "Highly parallel characterization of IgG Fc binding interactions", MABS, vol. 6, no. 4, 16 April 2014 (2014-04-16), pages 915-927, XP55370473, US ISSN: 1942-0870, DOI: 10.4161/mabs.28808 the whole document -----	1-48
X	WO 2014/134165 A1 (SLOAN KETTERING INST CANCER [US]) 4 September 2014 (2014-09-04) page 35 -----	1-46

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"E" earlier application or patent but published on or after the international filing date

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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	Date of mailing of the international search report
18 May 2017	26/05/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Scheffzyk, Irmgard

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2017/019301

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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