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(54) **MODIFIED CELLS FOR IMMUNOTHERAPY**

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(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-L1. Also provided are related nucleic acids, vectors, compositions and method for enhancing the immune response towards cancers and pathogens.

Specification includes a Sequence Listing.

Figure 1

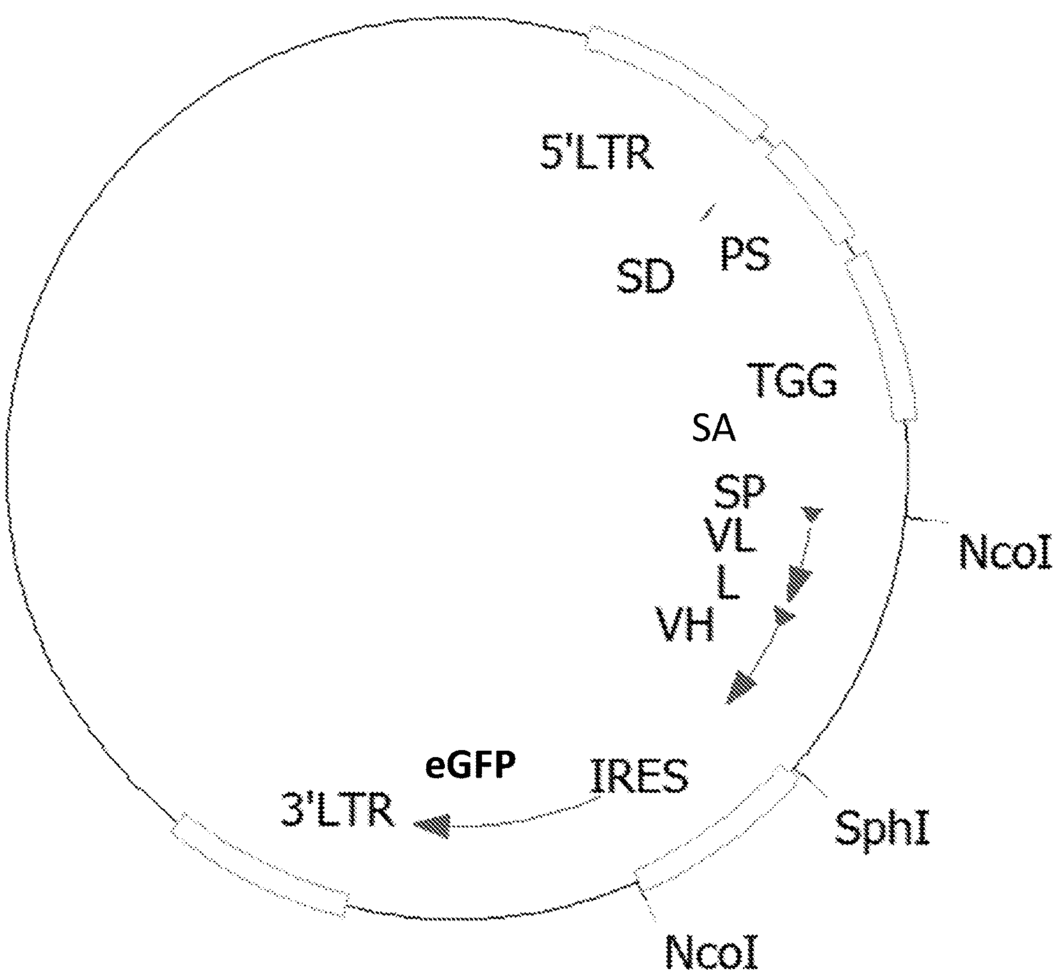


Figure 2

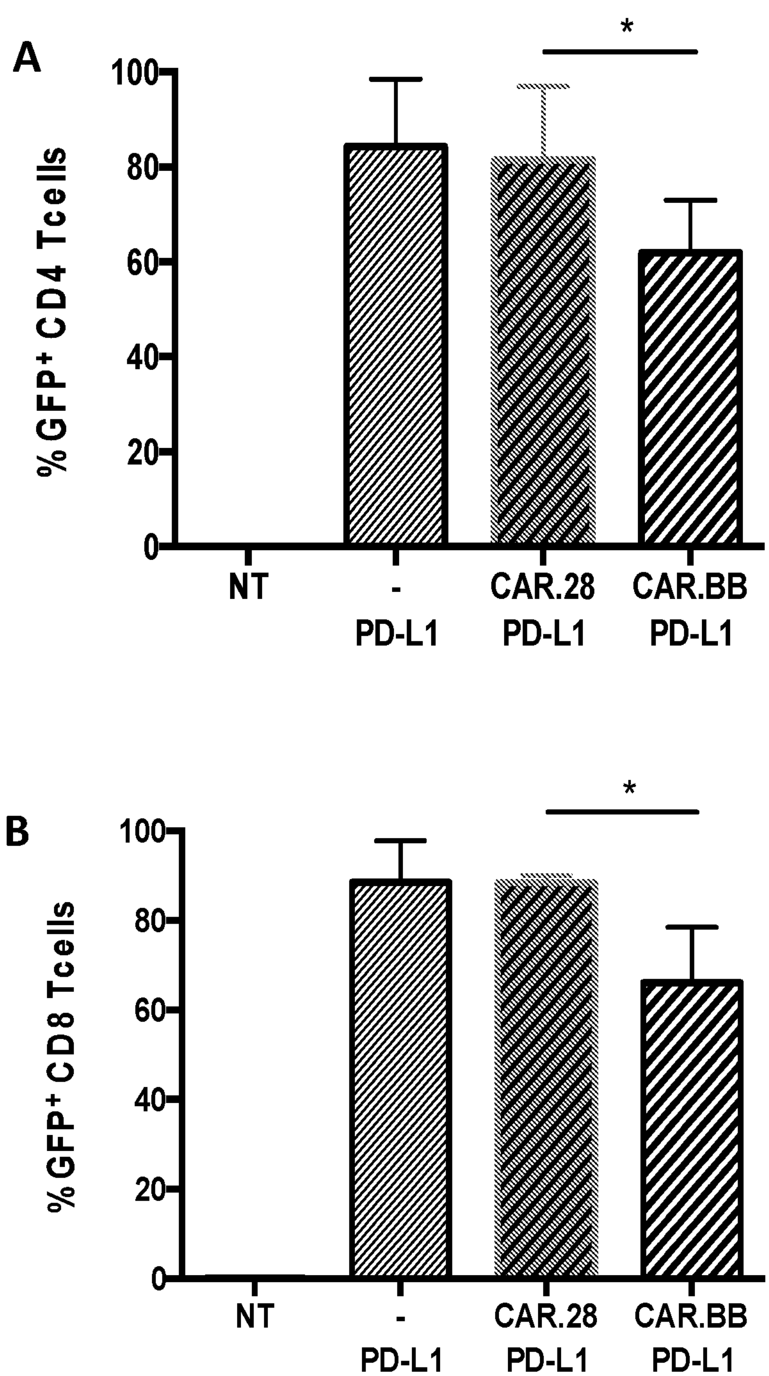

A
*
100
80
60
40
20
0
% GFP+ CD4 Tcells
NT
-
PD-L1
CAR.28
PD-L1
CAR.BB
PD-L1
B
*
100
80
60
40
20
0
% GFP+ CD8 Tcells
NT
-
PD-L1
CAR.28
PD-L1
CAR.BB
PD-L1

Figure 2

C

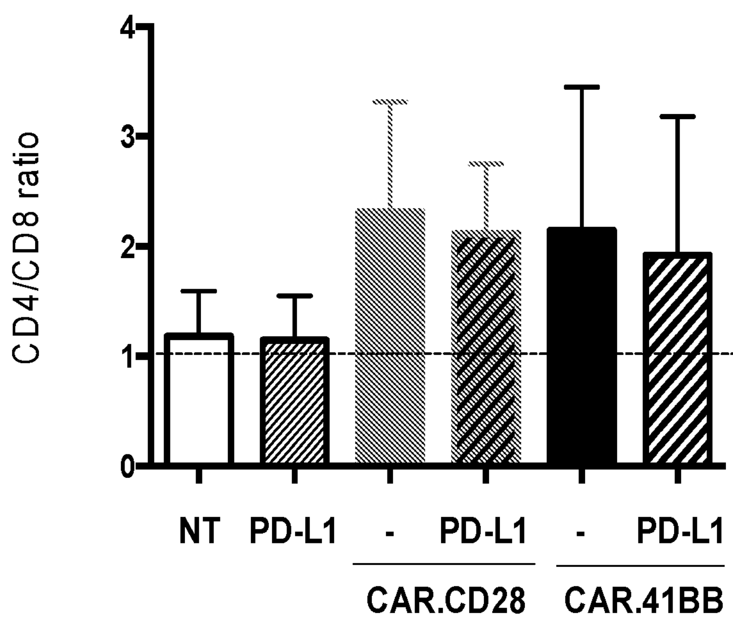


Figure 3

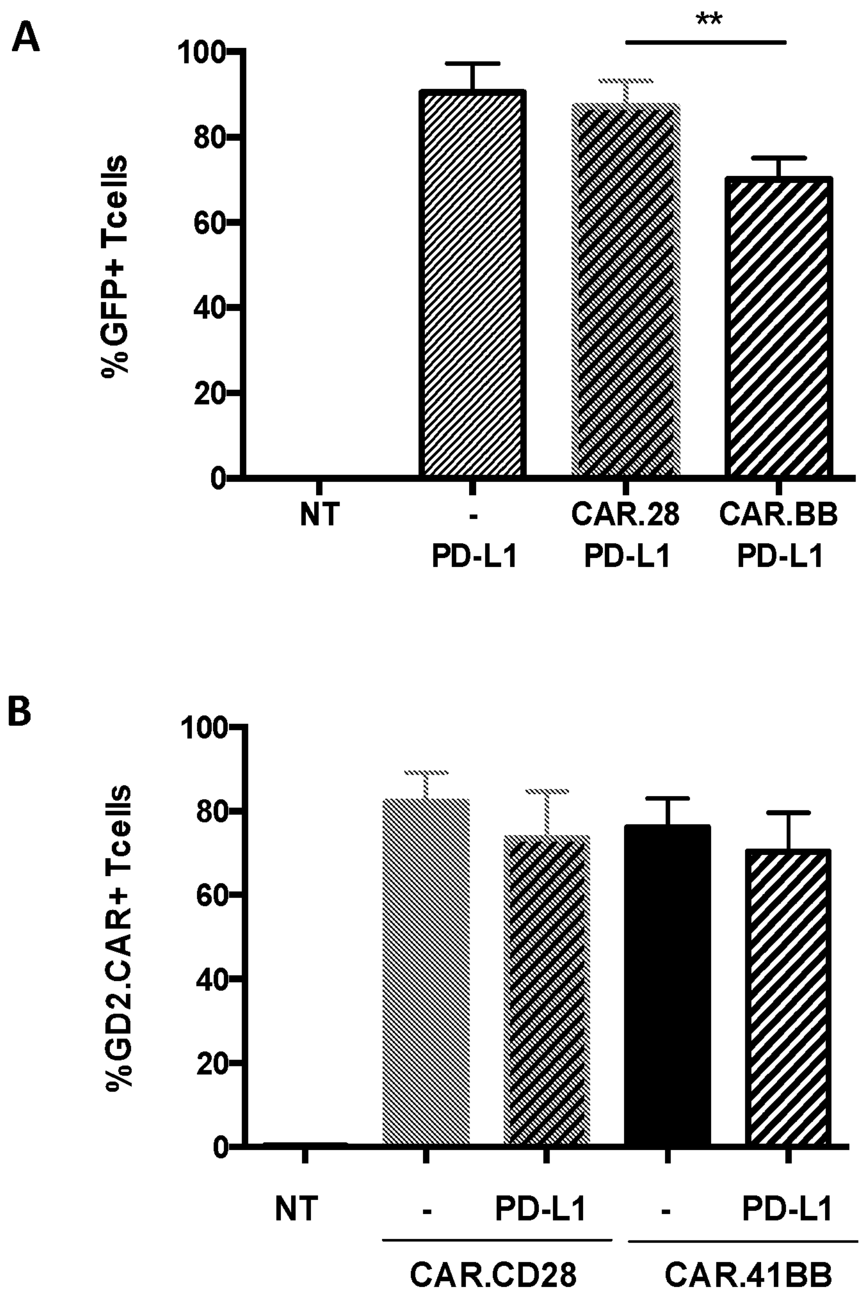
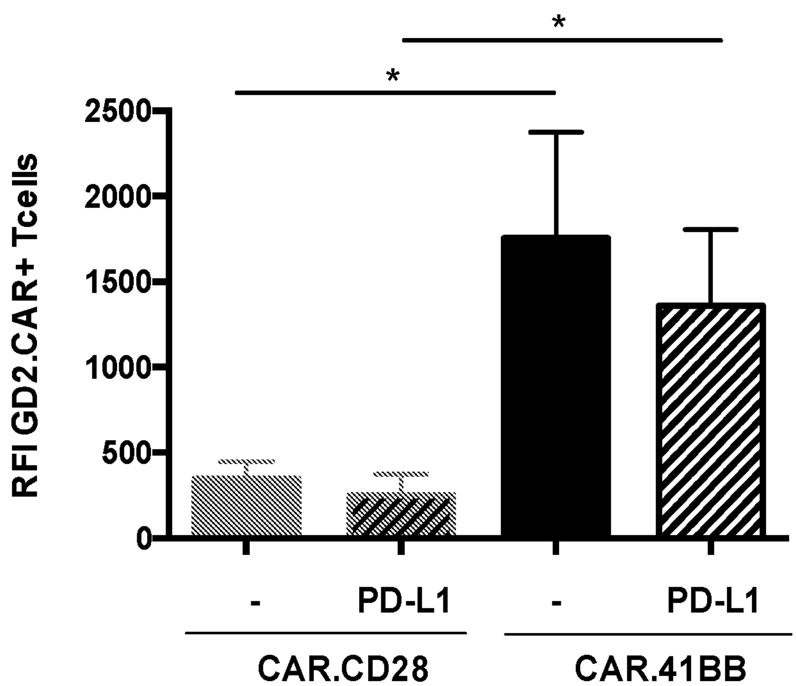

A
**
100
80
60
40
20
0
%GFP+ Tcells
NT
-
PD-L1
CAR.28
PD-L1
CAR.BB
PD-L1
B
100
80
60
40
20
0
%GD2.CAR+ Tcells
NT
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB

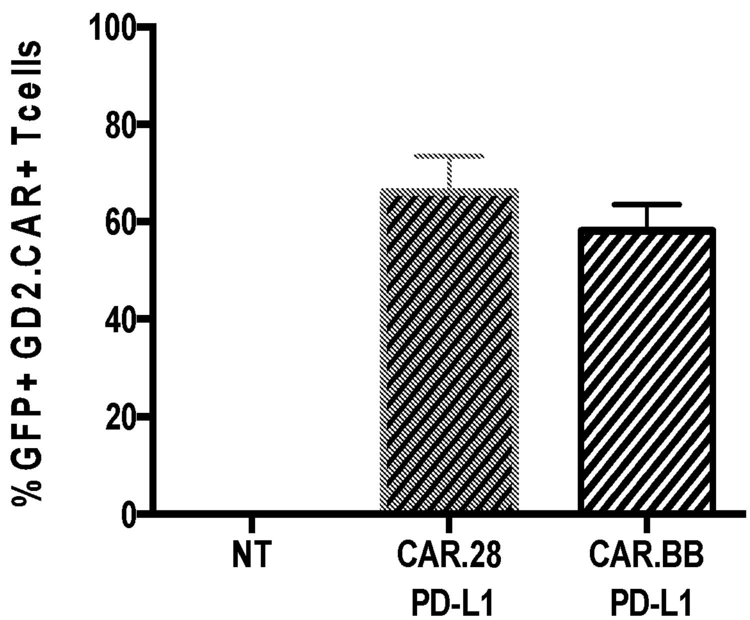
Figure 3

C



*
*
2500
2000
1500
1000
500
0
RFI GD2.CAR+ Tcells
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB

D



100
80
60
40
20
0
% GFP+ GD2.CAR+ Tcells
NT
CAR.28
PD-L1
CAR.BB
PD-L1

Figure 3

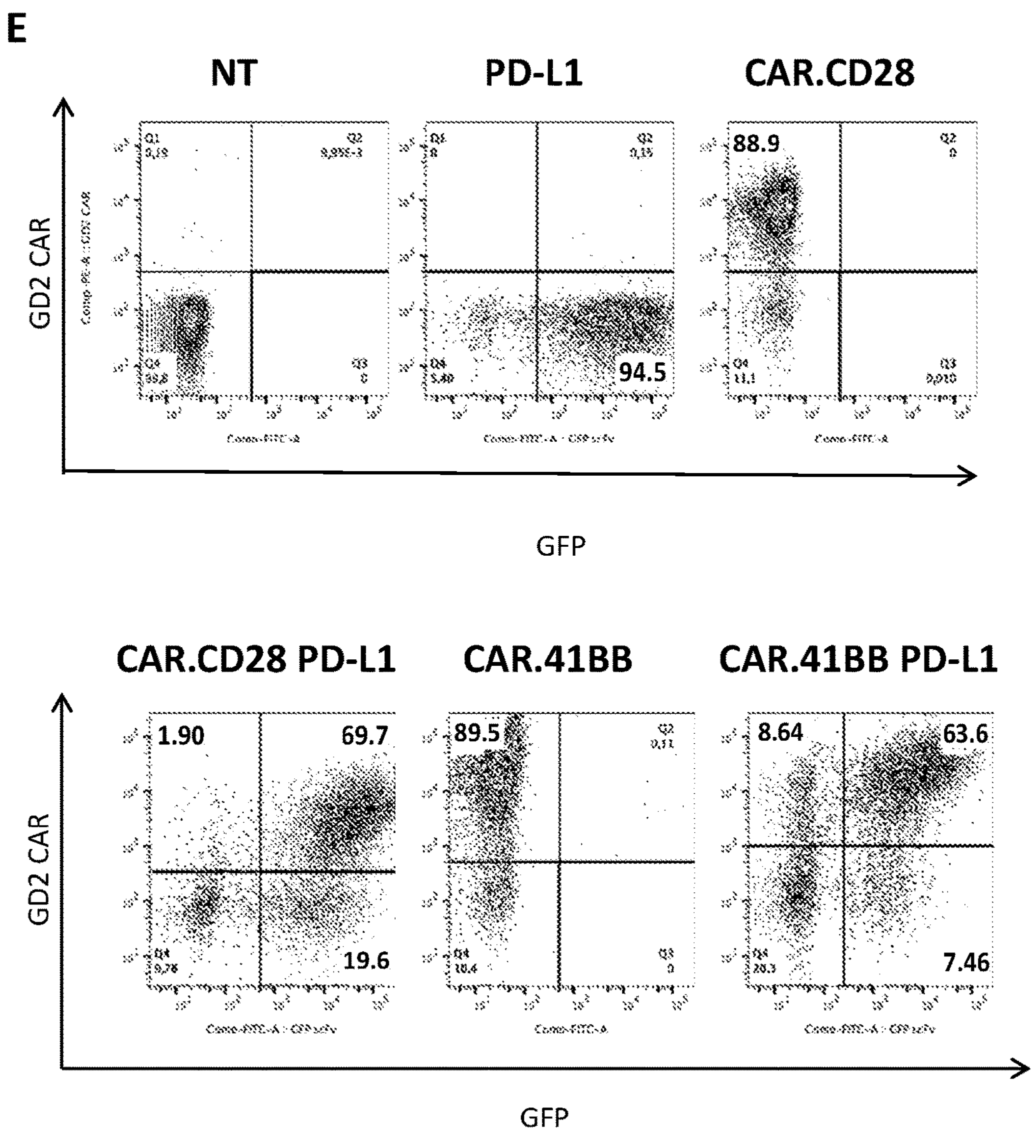

E
NT
PD-L1
CAR.CD28
88.9
94.5
GD2 CAR
GFP
CAR.CD28 PD-L1
CAR.41BB
CAR.41BB PD-L1
1.90
69.7
19.6
89.5
8.64
63.6
7.46
GD2 CAR
GFP

Figure 4

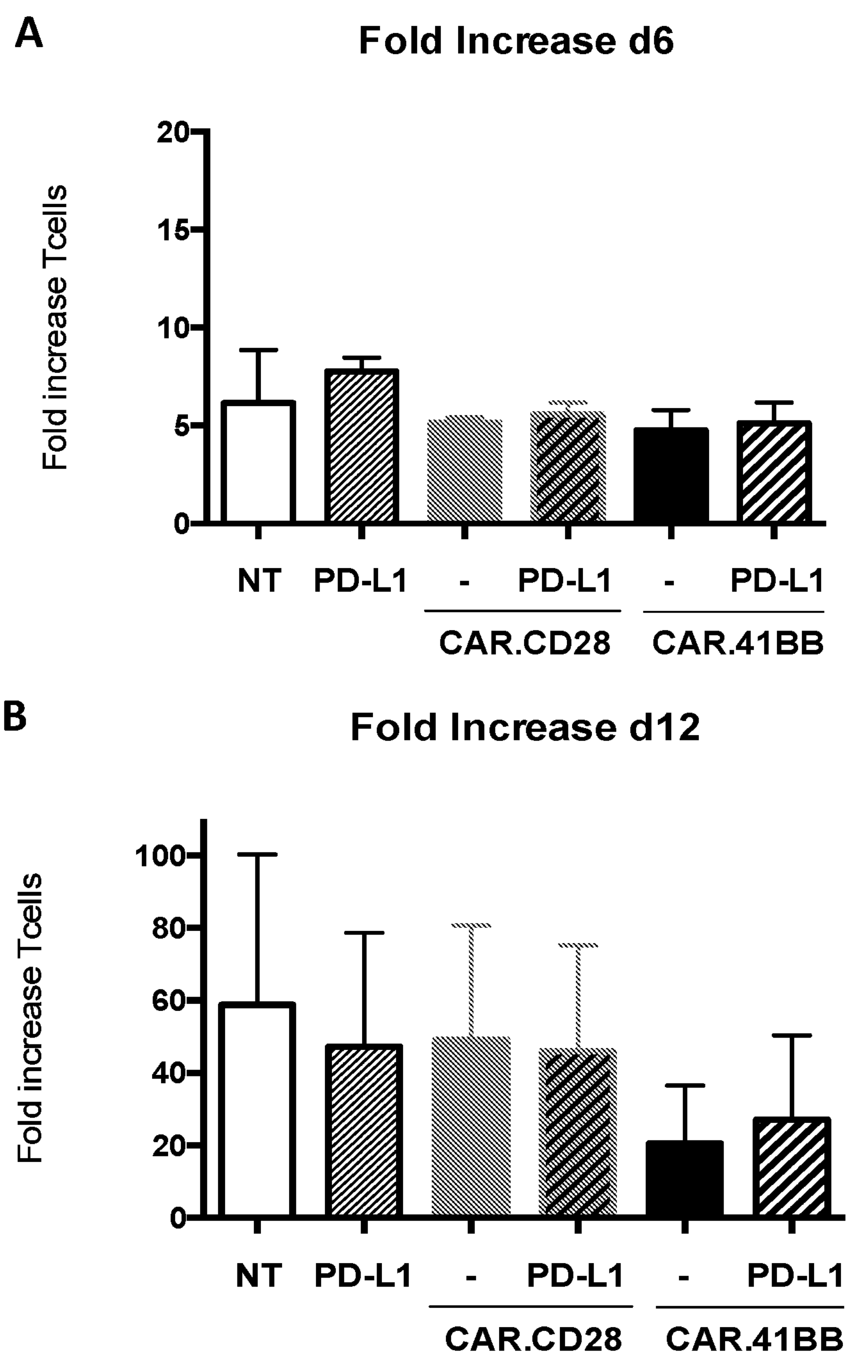

A
Fold Increase d6
Fold increase Tcells
20
15
10
5
0
NT
PD-L1
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB
B
Fold Increase d12
Fold increase Tcells
100
80
60
40
20
0
NT
PD-L1
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB

Figure 5

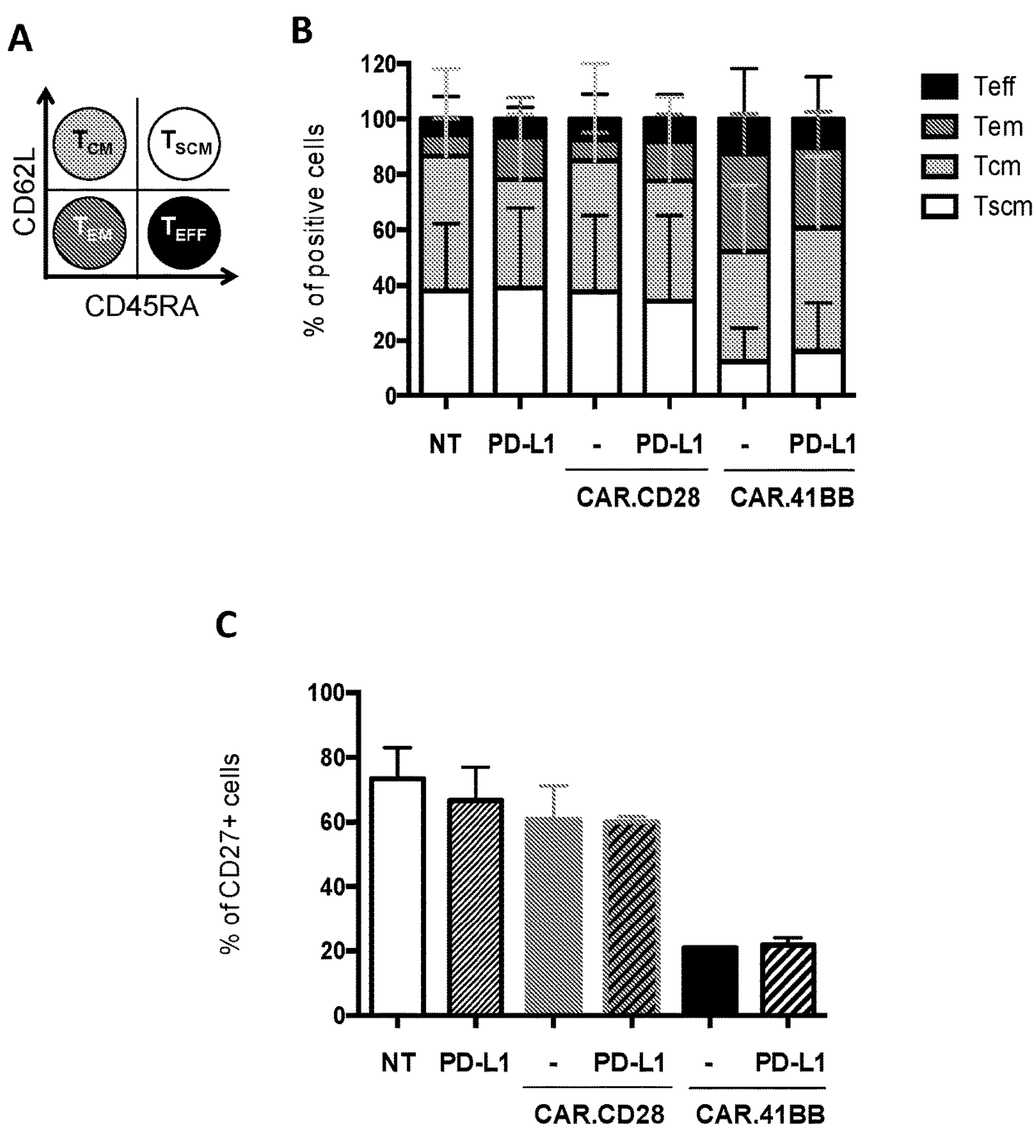
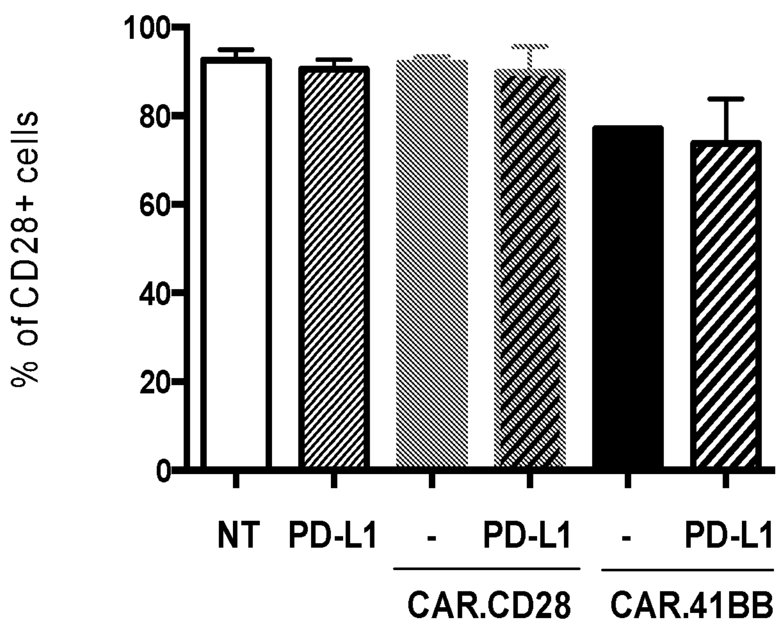

A
CD62L
CD45RA
T_{CM}
T_{SCM}
T_{EM}
T_{EFF}
B
% of positive cells
120
100
80
60
40
20
0
Teff
Tem
Tcm
Tscm
NT
PD-L1
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB
C
% of CD27+ cells
100
80
60
40
20
0
NT
PD-L1
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB

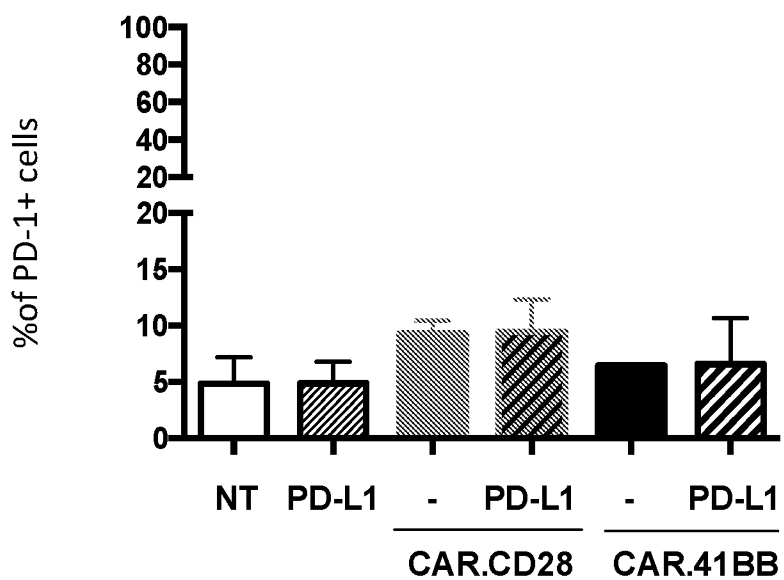
Figure 5

D



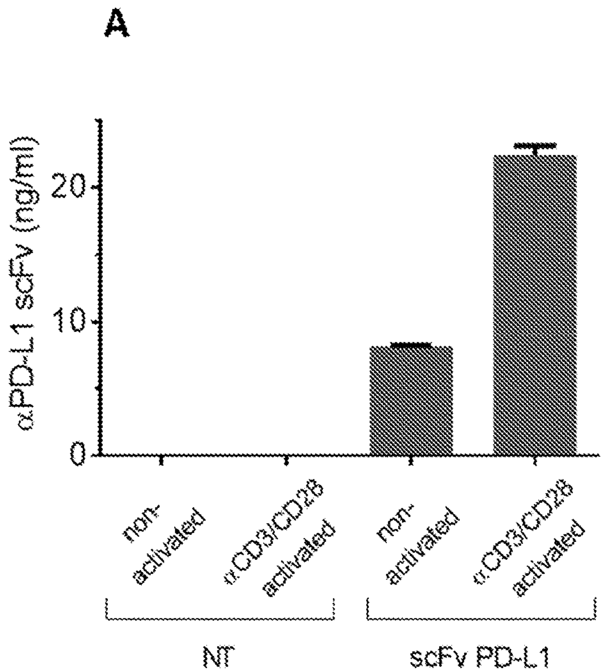
100
80
60
40
20
0
% of CD28+ cells
NT
PD-L1
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB

E



100
80
60
40
20
20
15
10
5
0
%of PD-1+ cells
NT
PD-L1
-
PD-L1
-
PD-L1
CAR.CD28
CAR.41BB

Figure 6


A
αPD-L1 scFv (ng/ml)
0
10
20
non-activated
αCD3/CD28 activated
non-activated
αCD3/CD28 activated
NT
scFv PD-L1

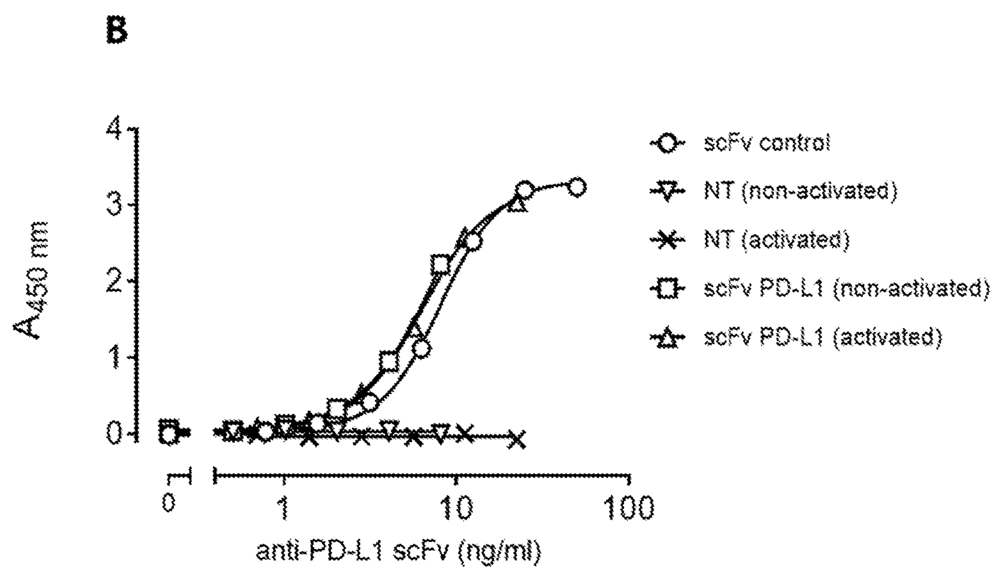
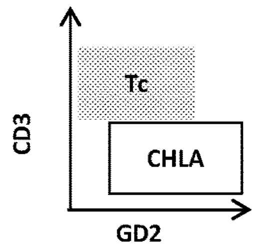

B
A450 nm
0
1
2
3
4
0
1
10
100
anti-PD-L1 scFv (ng/ml)
scFv control
NT (non-activated)
NT (activated)
scFv PD-L1 (non-activated)
scFv PD-L1 (activated)

Figure 7 A



E:T = 5:1

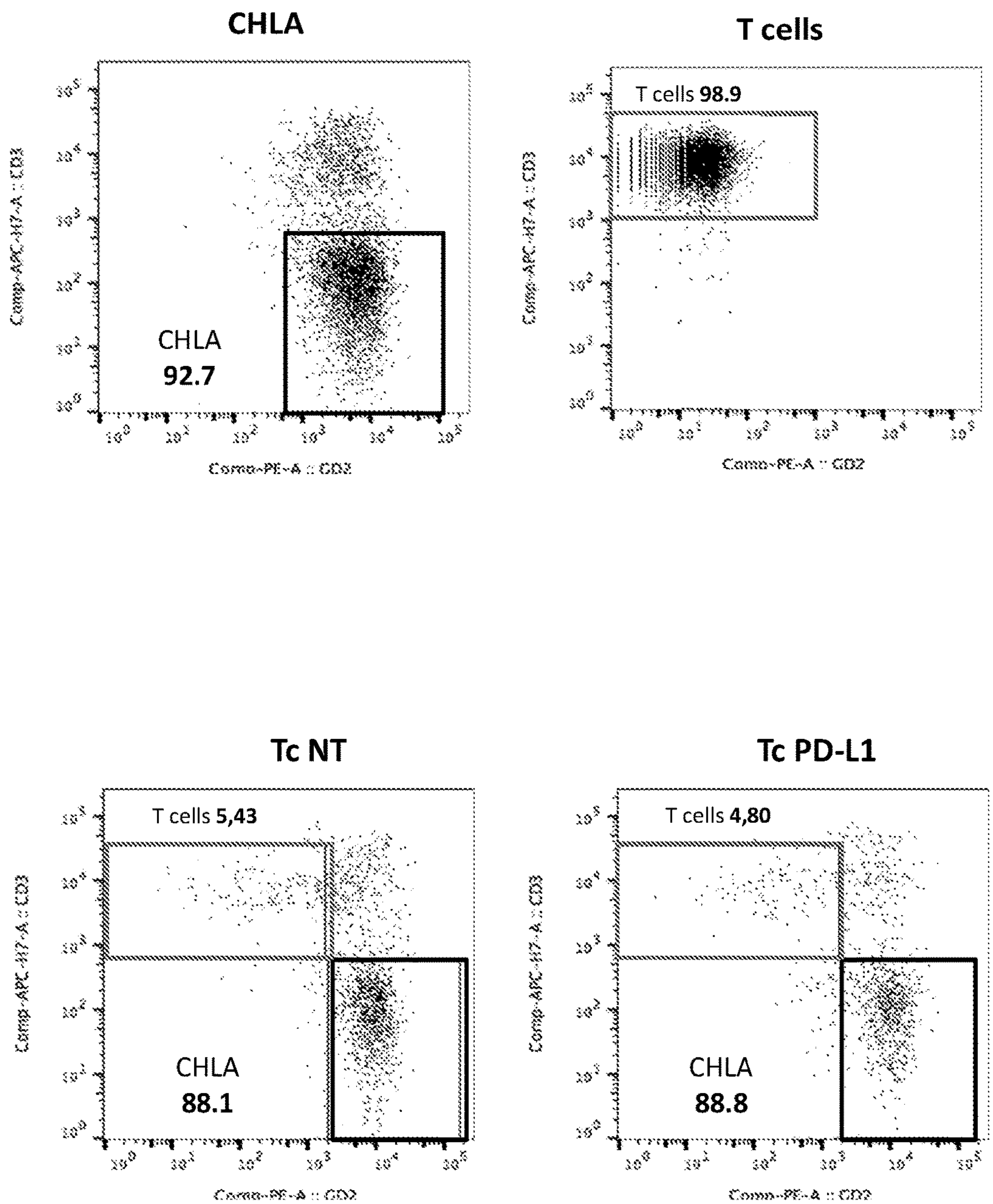
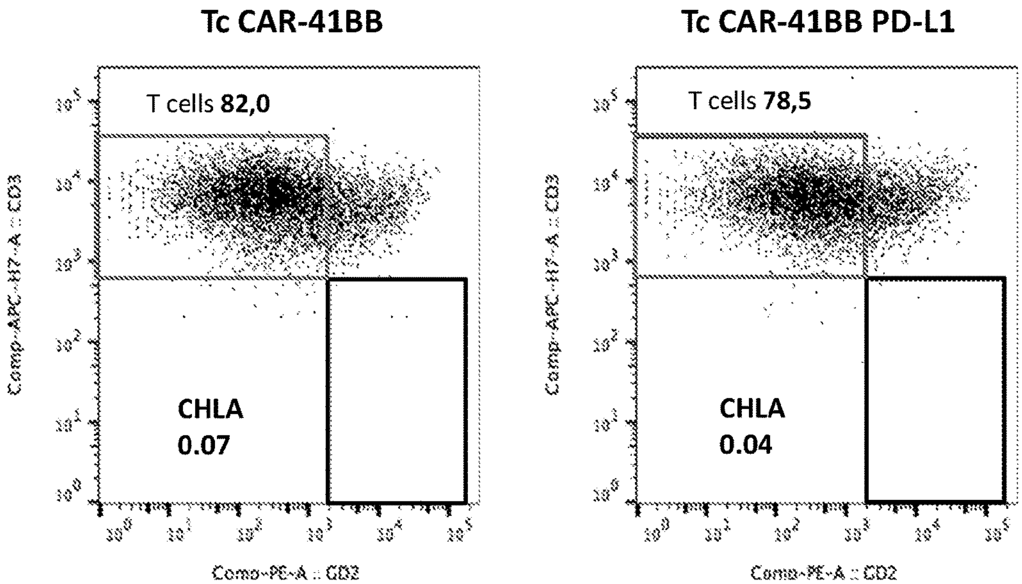


Figure 7 B


Tc CAR-41BB
T cells 82,0
CHLA
0.07
Comp-APC-H7-A :: CD3
Comp-PE-A :: CD2
Tc CAR-41BB PD-L1
T cells 78,5
CHLA
0.04
Comp-APC-H7-A :: CD3
Comp-PE-A :: CD2

C

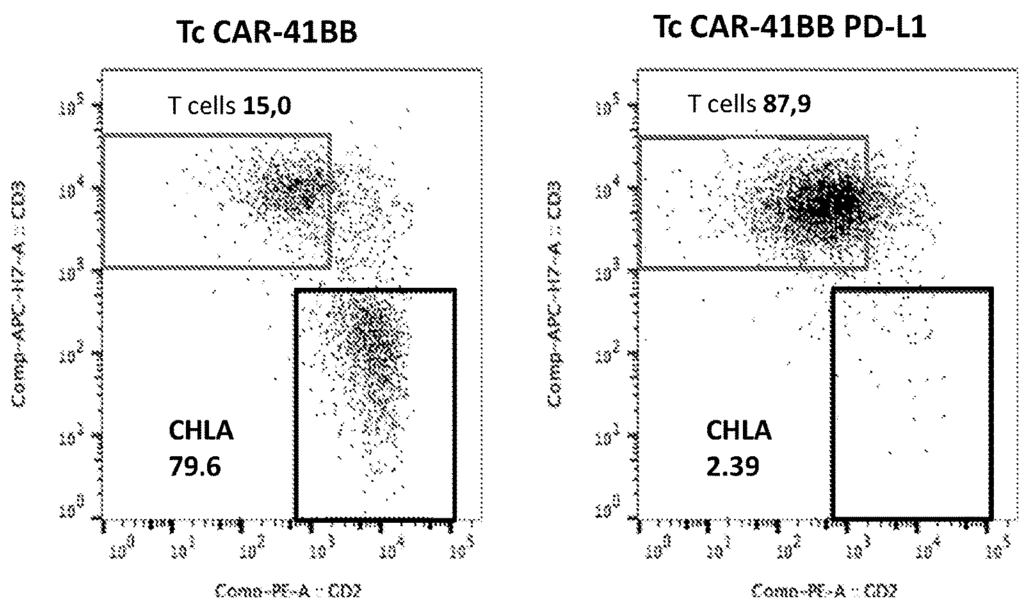
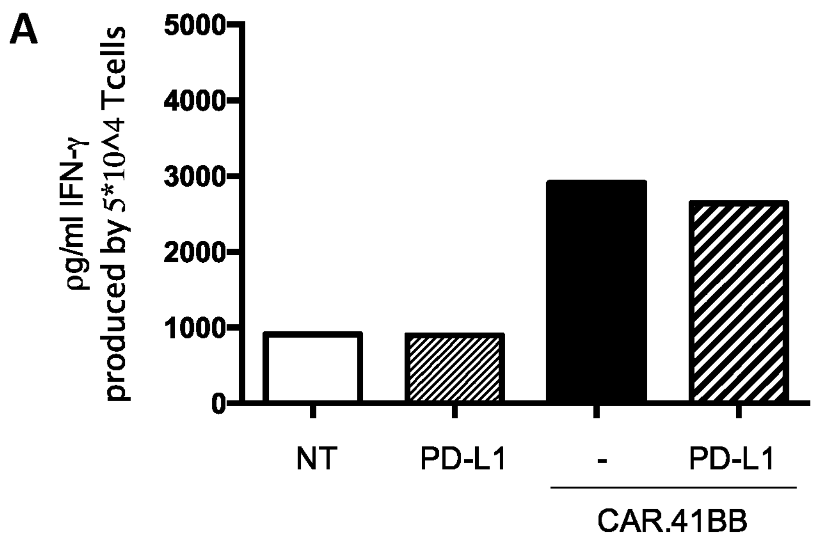
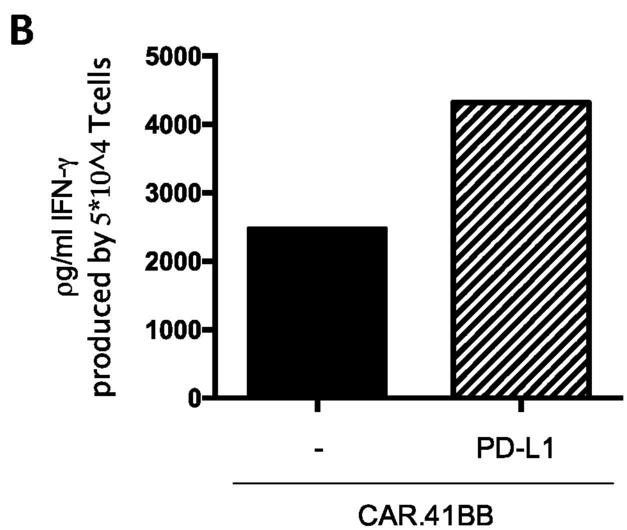

Tc CAR-41BB
T cells 15,0
CHLA
79.6
Comp-APC-H7-A :: CD3
Comp-PE-A :: CD2
Tc CAR-41BB PD-L1
T cells 87,9
CHLA
2.39
Comp-APC-H7-A :: CD3
Comp-PE-A :: CD2

Figure 8


A
ρg/ml IFN-γ
produced by 5*10^4 Tcells
5000
4000
3000
2000
1000
0
NT
PD-L1
-
PD-L1
CAR.41BB


B
ρg/ml IFN-γ
produced by 5*10^4 Tcells
5000
4000
3000
2000
1000
0
-
PD-L1
CAR.41BB

MODIFIED CELLS FOR IMMUNOTHERAPY

RELATED APPLICATIONS

[0001] This application is a US National stage entry of International Application No. PCT/US2017/019301, which designated the United States and was filed on Feb. 24, 2017, published in English. This application claims priority under 35 U.S.C. § 119 or 365 to EP Application No. 16020057.2, filed Feb. 25, 2016. The entire teachings of the above applications are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] 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

[0003] 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.

[0004] 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.

[0005] Still another approach is the transfer of antigen-specific T cell receptors (TCRs) with defined specificity into immune cells.

[0006] 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.

[0007] 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.

[0008] 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).

[0009] 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.

[0010] WO2014134165 describes the co-expression of a chimeric antigen receptor (CAR) and an 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 U.S. Pat. No. 7,858,746. 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.

[0011] Both Suarez E. et al, Oncotarget. 2016 Jun 7; 7(23): 34341-34355 and WO2016100985 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 ADCC, which led to human NK cells recruitment to the tumor site in the vivo model.

[0012] 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.

SUMMARY OF THE INVENTION

[0013] Thus, in one aspect, the invention provides for an engineered cell expressing:

[0014] i) an antigen receptor, and

[0015] ii) an antibody that binds and blocks PD-L1. Such antibody may be a humanized antibody or a fully human antibody. In some 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.

[0016] 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 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.

[0017] 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.

[0018] 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.

[0019] In one embodiment, the cell is a NKT cell.

[0020] In one embodiment, the cell is of human in origin. In one embodiment, the cell is autologous; in one embodiment, the cell is allogenic.

[0021] 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.

[0022] 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.

[0023] In one embodiment, the antigen receptor is a chimeric antigen receptor (CAR).

[0024] In one embodiment, the antigen receptor is membrane anchored.

[0025] 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.

[0026] 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.

[0027] Further provided are a nucleic acid encoding the antigen receptor and the antibody as described herein, as well as vectors comprising such nucleic acid.

[0028] Also provided are methods of generating an immune cell as described herein, comprising the steps of:

[0029] (a) Providing an immune cell,

[0030] (b) Introducing into said cell at least one nucleic acid encoding said antigen receptor and at least one nucleic acid encoding said antibody; and

[0031] (c) Expressing said nucleic acids by said cell.

[0032] Further provided are pharmaceutical compositions comprising

[0033] i) an effective amount of the engineered immune cell or of the expression vector described herein, and

[0034] ii) a pharmaceutically acceptable excipient.

[0035] 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:

[0036] (a) Providing the recombinant immune cell as described herein;

[0037] (b) Administrating said immune cells to said subject.

[0038] 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.

[0039] 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.

[0040] 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

[0041] FIG. **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.

[0042] FIG. **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 (FIG. **2**A) or CD8 (FIG. **2**B) T cells expressing eGFP. FIG. **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-IBB endodomain.

[0043] FIG. **3** shows that T cells co-expressed GD2.CAR and eGFP anti-PD-L1 upon double retroviral transduction. FIG. **3**A: Transduction efficiency of the SFG.scFv.anti-PD-L1(I)eGFP vector. Shown is the percentage of eGFP positive T cells. FIG. **3**B: Transduction efficiency of GD2.CAR encoding either the CD28 or the 4-1BB endodomains.

[0044] FIG. **3**C: Expression of GD2.CAR shown as RFI (Relative Fluorescence Intensity). FIG. **3**D: Percentage of T cells co-transduced with the GD2.CAR and the scFv anti-PD-L1 retroviral vectors. FIG. **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.

[0045] FIG. **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 (FIG. **4**A) and at day 12 (FIG. **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;

[0046] FIG. **5** shows that expression of the anti-PD-L1 scFv did not affect T cell subset compositions. FIG. **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 (TEFF) CD62L-CD45RA+]. T cell subset compositions (FIG. **5**B), percentage of CD27 (FIG. **5**C), CD28 (FIG. **5**D) and PD-1 (FIG. **5**E) positive cells 10 days after stimulation with immobilized anti-CD3/CD28. Mean and s.d. are shown (n=4 independent experiments).

[0047] FIG. **6** shows that the anti-PD-L1 scFv is released by transduced T cells. FIG. **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. FIG. **6**B: The anti-PD-L1 scFv secreted by transduced T cells binds PD-L. The cell culture supernatants of non-transduced (NT) and anti-PD-L1 transduced T cells (scFv PD-L1) were tested for their binding to recombinant human PD-L1. Anti-PD-L1 scFv produced in *E. coli* was used as a reference control (scFv control).

[0048] FIG. **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. FIG. **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 the anti-PD-L1 scFv. Representative plots of T cells at the end of the first (7 days of culture; FIG. **7**B) and second cycle of co-culture (14 days of culture; FIG. **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 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.

[0049] FIG. **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 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; FIG. **8**A) and second (14 days of culture; FIG. **8**B) tumor specific stimulation.

[0050] As used throughout the Figures "CAR.BB" refers to "CAR.41BB" as well as "CAR.4-1BB".

DETAILED DESCRIPTION

[0051] 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.

[0052] The term "engineered immune cell" as used herein refers to an immune cell which was genetically modified to express the proteins described herein.

[0053] 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).

[0054] 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.

[0055] In some embodiments, binding of the antigen to the antigen receptor activates the immune cell through induction of signal transduction or changes in protein expression in the immune cell which results in initiation of an immune response.

[0056] The term "endogenous" refers to a nucleic acid or a polypeptide that is normally expressed in a cell or tissue, absent recombinant engineering.

[0057] As used herein, "PD-L1" refers to the protein also known as "programmed cell death ligand 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 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 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, 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-CO2H. "Rat PD-L1" refers to PD-L1 of Rattus norvegicus (Norway rat). The amino acid sequence of exemplary rat PD-L1 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-RO2H. "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-MO3H 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 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 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.

[0058] "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 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, 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.

[0059] "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 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.

[0060] 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.

[0061] 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.

[0062] 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.

[0063] 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.

[0064] 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.

[0065] 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.

[0066] 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).

[0067] 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.

[0068] In a first aspect, the invention provides an engineered immune cell expressing:

[0069] i) an antigen receptor, and

[0070] ii) an antibody that blocks PD-L1.

[0071] 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).

[0072] 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.

[0073] 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 domain to the cell surface. Said signal peptide may be cleavable.

[0074] 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.

[0075] In some embodiments, the CAR comprises a CD3 zeta, a CD4, a CD28, a CD8 alpha or a 4-1BB transmembrane domain.

[0076] 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 sequences of CD28 and 4-1BB costimulatory domains are provided in SEQ ID NOs: 49 and 47, respectively.

[0077] 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.

[0078] Corresponding sequences are well-known and available in the art. Exemplary sequences of signal peptides, hinge regions, transmembrane domains are provided in FIG. **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.

[0079] In some embodiments, the CAR architecture is as shown in FIG. **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 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 FIG. **1** for the amino acid sequence of transmembrane and endodomains of different receptors), as well as in WO2012033885, all three incorporated herein by reference.

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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:

[0085] 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; 151 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.

[0086] In one embodiment, the engineered TCR comprises non-human constant regions, for example murine constant regions.

[0087] In one embodiment, the TCR, such as an endogenous TCR of a NKT cell, is capable of binding tumor associated macrophages.

[0088] 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.

[0089] 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 ID NOs: 56, 57 and 59. Such TCR may comprise the alpha chain of SEQ ID NOs: 60 or 62.

[0090] 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: 62 and 63.

[0091] 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.

[0092] 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, 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-AL 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 gp120, HIV-1 envelope glycoprotein gp41, meothelin, HERV-K, or ERBB2.

[0093] 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 proteolysis and later, artificial constructs have been generated by genetic engineering.

[0094] 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 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 (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)).

[0095] A subgroup of antibody derivatives are antibody fragments. As used herein, the term "antibody fragments" refers to (i) monovalent and monospecific antibody derivatives which 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 below 60 kDa. In one embodiment, the antibody derivative is an antibody fragment, preferably a humanized antibody fragment.

[0096] 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 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.

[0097] 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.

[0098] 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.

[0099] 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.

[0100] 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.

[0101] 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.

[0102] 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 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-limiting examples of multivalent antibodies; an exemplary multivalent antibody comprises two binding sites, i.e. the antibody is bivalent.

[0103] 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 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.

[0104] Preferably, the antibody derivative above is an scFv (a "single chain variable fragment" or 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 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.

[0105] 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.

[0106] 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.

[0107] 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.

[0108] 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.

[0109] 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.

[0110] 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.

[0111] 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.

[0112] 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 Mar. 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.

[0113] 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.

[0114] In some embodiments, the antibody is fully human.

[0115] 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.

[0116] In preferred embodiments, the antibody comprises

[0117] 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,

[0118] 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.

[0119] Preferably, the antibody comprises

[0120] i) at least one of VH sequence of SEQ ID NO: 2, and/or

[0121] ii) at least one VL sequence of SEQ ID NO: 1, or variants thereof, respectively.

[0122] In some embodiments, said antibody is a scFv comprising SEQ ID NO.: 9 or a variant thereof.

[0123] 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 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.

[0124] Variants of the antibodies provided herein may be prepared by introducing appropriate 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.

[0125] 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, 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.

[0126] 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:

[0127] 1. nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, methionine),

[0128] 2. uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, proline, cysteine, tryptophan),

[0129] 3. basic side chains (e.g., lysine, arginine, histidine, proline),

[0130] 4. acidic side chains (e.g., aspartic acid, glutamic acid),

[0131] 5. beta-branched side chains (e.g., threonine, valine, isoleucine) and

[0132] 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:

[0133] 1. Substituting alanine (A) by valine (V);

[0134] 2. Substituting arginine (R) by lysine (K);

[0135] 3. Substituting asparagine (N) by glutamine (Q);

[0136] 4. Substituting aspartic acid (D) by glutamic acid (E);

[0137] 5. Substituting cysteine (C) by serine (S);

[0138] 6. Substituting glutamic acid (E) by aspartic acid (D);

[0139] 7. Substituting glycine (G) by alanine (A);

[0140] 8. Substituting histidine (H) by arginine (R) or lysine (K);

[0141] 9. Substituting isoleucine (I) by leucine (L);

[0142] 10. Substituting methionine (M) by leucine (L);

[0143] 11. Substituting phenylalanine (F) by tyrosine (Y);

[0144] 12. Substituting serine (S) by threonine (T);

[0145] 13. Substituting tryptophan (W) by tyrosine (Y);

[0146] 14. Substituting phenylalanine (F) by tryptophan (W); and/or

[0147] 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.

[0148] 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.

[0149] 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.

[0150] 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.

[0151] In some embodiments, the variant does not show any improvement over the parent antibody.

[0152] In some embodiments, a variant antibody as described herein

[0153] (i) retains specific binding to PD-L1, in particular to hPD-L1, preferably blocking the interaction between PD-L1 and PD-1; and/or

[0154] (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

[0155] (iv) competes with the antibody disclosed herein for binding to PD-L1; and/or

[0156] (v) has at least 80%, preferably at least 85%, 90%, 95% or 97% sequence identity to the sequences disclosed herein.

[0157] 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.

[0158] 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.

[0159] 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®.

[0160] Such KinExA®.measurements are preferably done at room temperature, more preferably under the conditions as described in example 1.

[0161] 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.

[0162] 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.

[0163] Further provided are antibodies that have no cross-reactivity to other member of the B7 family, such as PD-L2 and B7-H3.

[0164] Further contemplated are engineered immune cells as described above expressing antibodies that compete with the antibodies disclosed herein for binding to PD-L1.

[0165] 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.

[0166] 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-lalpha and are preferably of human origin, i.e. hIL-2, hIL-4, hIL-7, hIL-15, hIL-21, or hMIP-1 alpha. 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.

[0167] 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.

[0168] 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.

[0169] 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.

[0170] 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.

[0171] 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.

[0172] 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).

[0173] Further contemplated are isolated nucleic acids hybridizing with the nucleic acids described herein under stringent conditions.

[0174] 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.

[0175] 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.

[0176] 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 hIgGl. In some embodiments, the signal peptide comprises SEQ ID NO: 15.

[0177] 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.

[0178] 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.

[0179] The invention also provides a method of generating an immune cell as described herein, comprising the steps of:

[0180] (a) Providing an immune cell,

[0181] (b) Introducing into said cell at least one nucleic acid encoding said antigen receptor and at least one nucleic acid encoding said antibody; and

[0182] (c) Expressing said nucleic acids by said cell.

[0183] In some embodiments, step (b) comprises introducing the expression vector as described above into said cell.

[0184] The method may comprise the additional step of:

[0185] (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).

[0186] The invention also relates to a pharmaceutical composition comprising

[0187] i) an effective amount of the engineered immune cell described herein or of the expression vector described herein, and

[0188] ii) a pharmaceutically acceptable excipient.

[0189] 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) 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 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 TWEENTM, PLURONICSTM or polyethylene glycol (PEG), (xiii) cryopreservatives such as dimethyl sulfoxide (DMSO).

[0190] 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.

[0191] Also provided is a method of treating a subject in need thereof comprising:

[0192] (a) Providing the engineered immune cell described herein; and

[0193] (b) Administrating said immune cells to said subject.

[0194] 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.

[0195] 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.

[0196] 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.

[0197] 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.

[0198] 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.

[0199] The cells, the vector or the composition are administered one or more times to said subject.

[0200] 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.

[0201] 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 allogeneic NKT cells can be used to treat multiple cancer patients with minimal risk of GvHD.

[0202] 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), 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 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.

[0203] 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.

[0204] In some embodiments, the kit may further comprise an inducer of a safety switch.

SEQUENCES

[0205] The sequences disclosed herein are

-VL of scFv

SEQ ID NO: 1

EIVMTQSPSTLSASVGDRVIITCQASEDIYSLLAWYQQKPGKAPKLLIYDASDLASG
VPSRFSGSGSGAEFTLTISSLQPDDFATYYCQGNYGSSSSSSYGAVFGQGTKLTVLG

-VH of scFv

SEQ ID NO: 2

EVQLVESGGGLVQPGGSLRLSCTVSGIDLSSYTMGWVRQAPGKGLEWVGIISSGG
RTYYASWAKGRFTISRDTSKNTVYLQMNSLRAEDTAVYYCARGRYTGYPYYFAL
WGQGTLVTVSS

-CDR-L1 of scFv

SEQ ID NO: 3

QASEDIYSLLA

-CDR-L2 of scFv

SEQ ID NO: 4

DASDLAS

-CDR-L3 of scFv

SEQ ID NO: 5

QGNYGSSSSSSYGAV

-CDR-H1 of scFv

SEQ ID NO: 6

IDLSSYTMG

-CDR-H2 of scFv

SEQ ID NO: 7

IISSGGRTYYASWAKG

-CDR-H3 of scFv

SEQ ID NO: 8

GRYTGYPYYFAL

-scFv

SEQ ID NO: 9

EIVMTQSPSTLSASVGDRVIITCQASEDIYSLLAWYQQKPGKAPKLLIYDASDLASG

VPSRFSGSGSGAEFTLTISSLQPDDFATYYCQGNYGSSSSSSYGAVFGQGTKLTVLG

GGGGSGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCTVSGIDLSSYTMG

-continued

WVRQAPGKGLEWVGIISSGGRTYYASWAKGRFTISRDTSKNTVYLQMNSLRAED

TAVYYCARGRYTGYPYYFALWGQGTLVTVSS

-linker

SEQ ID NO: 10

GGGGSGGGGSGGGGSGGGGS

-forward primer

SEQ ID NO: 11

TAACCATGGAGTTTGGGCTGAG

-reverse primer

SEQ ID NO: 12

GACGCATGCTCAGCTCGACACGGTGACC

-VL sequence of 14g2a scFv

SEQ ID NO: 13

DVVMTQTPLSLPVSLGDQASISC**RSSQSLVHRNGNTYLH**WYLQKPGQSPKLLIH**K**

VSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC**SQSTHVPPLT**FGAGTKLE

LKR

(CDR sequences highlighted in bold)

-VH sequence of 14g2a scFv

SEQ ID NO: 14

EVQLLQSGPELEKPGASVMISCKASG**SSFTGYNMN**WVRQNIGKSLEWIG**AIDPYY**

GGTSYNQKFKGRATLTVDKSSSTAYMHLKSLTSEDSAVYYCVS**GMEY**WGQGTS

VTVSS

(CDR sequences highlighted in bold)

-hIgG1 signal peptide

SEQ ID NO: 15

MEFGLSWLFLVAILKGVQ

-CDR-L1 of anti-GD2-CAR

SEQ ID NO: 16

RSSQSLVHRNGNTYLH

-CDR-L2 of anti-GD2-CAR

SEQ ID NO: 17

KVSNRFS

-CDR-L3 of anti-GD2-CAR

SEQ ID NO: 18

SQSTHVPPLT

-CDR-H1 of anti-GD2-CAR

SEQ ID NO: 19

SSFTGYNMN

-CDR-H2 of anti-GD2-CAR

SEQ ID NO: 20

AIDPYYGGTSYNQKFKG

-CDR-H3 of anti-GD2-CAR

SEQ ID NO: 21

GMEY

-CDR-L1 of anti-CSPG4 CAR

SEQ ID NO: 22

RASQTIYKNLH

-CDR-L2 of anti-CSPG4 CAR

SEQ ID NO: 23

YGSDSIS

-CDR-L3 of anti-CSPG4 CAR

SEQ ID NO: 24

LQGYSTPWT

-continued

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

-continued

-CDR-H1 of anti-5T4 CAR

SEQ ID NO: 42

KASQSVSNDVA

-CDR-H2 of anti-5T4 CAR

SEQ ID NO: 43

YTSSRYA

-CDR-H3 of anti-5T4 CAR

SEQ ID NO: 44

QQDYNSPPT

-VL of anti-5T4 CAR

SEQ ID NO: 45

SIVMTQTPTFLLVSAGDRVTITCKASQSVSNDVAWYQQKPGQSPTLLISYTSSRYA
GVPDRFIGSGYGTDFTFTISTLQAEDLAVYFCQQDYNSPPTFGGGTKLEIKR

-VH of anti-5T4 CAR

SEQ ID NO: 46

EVQLQQSGPDLVKPGASVKISCKASGYSFTGYYMHWVKQSHGKSLEWIGRINPNN
GVTLYNQKFKDKAILTVDKSSTTAYMELRSLTSEDSAVYYCARSTMITNYVMDY
WGQVTSVTVSS

-41BB costimulatory domain

SEQ ID NO: 47

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

-CD3 zeta intracellular domain

SEQ ID NO: 48

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHM
QALPPR

-CD28 costimulatory domain

SEQ ID NO: 49

RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS

-Survivin specific TCR, beta chain

SEQ ID NO: 50

DAMVIQNPRYQVTQFGKPVTLSCSQTLNHNVMYWYQQKSSQAPKLLFHYYDKD
FNNEADTPDNFQSRRPNTSFCFLDIRSPGLGDAAMYLCATSRGDSTAEPQHFGDGT
RLSIL

-Survivin specific TCR, alpha chain

SEQ ID NO: 51

GESVGLHLPTLSVQEGDNSIINCAYSNSASDYFIWYKQESGKGPQFIIDIRSNMDKR
QGQRVTVLLNKTVKHLSLQIAATQPGDSAVYFCAETVTDSWGKLQFGAGTQVVV
TPD

-WT-1 specific TCR. CDR1 alpha

SEQ ID NO: 52

SSYSPS

-WT-1 specific TCR. CDR2 alpha

SEQ ID NO: 53

YTSAATL

-WT-1 specific TCR. CDR3 alpha

SEQ ID NO: 54

WSPFSGGGADGLT

-WT-1 specific TCR. CDR3 alpha

SEQ ID NO: 55

SPFSGGGADGLT

-WT-1 specific TCR. CDR1 beta

SEQ ID NO: 56

DFQATT

-WT-1 specific TCR. CDR2 beta

SEQ ID NO: 57

SNEGSKA

-WT-1 specific TCR. CDR3 beta

SEQ ID NO: 58

SARDGGEG

-continued

-WT-1 specific TCR. CDR3 beta

SEQ ID NO: 59

RDGGEGSETQY

-WT specific TCR, alpha chain

SEQ ID NO: 60

MLLLLVPVLEVIFTLGGTRAQSVTQLDSHVSVSEGTPVLLRCNYSSSYSPSLFWYV
QHPNKGLQLLLKYTSAATLVKGINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCV
VSPFSGGGADGLT

-WT-1 specific TCR, beta chain

SEQ ID NO: 61

MLLLLLLLGPGSGLGAVVSQHPSWVICKSGTXVKIECRSLDFQATTMFWYRQFPK
QSLMLMATSNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYIC
SARDGGEG

-WT-1 specific TCR, alpha chain

SEQ ID NO: 62

MLLLLVPVLEVIFTLGGTRAQSVTQLDSHVSVSEGTPVLLRCNYSSSYSPSLFWYV
QHPNKGLQLLLKYTSAATLVKGINGFEAEFKKSETSFHLTKPSAHMSDAAEYFCV
VSPFSGGGADGLT

-WT-1 specific TCR, beta chain

SEQ ID NO: 63

MLLLLLLLGPGSGLGAVVSQHPSWVICKSGTSVKIECRSLDFQATTMFWYRQFPK
QSLMLMATSNEGSKATYEQGVEKDKFLINHASLTLSTLTVTSAHPEDSSFYICSAR
DGGEGSETQY

[0206] 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

[0207] Cell lines

[0208] 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, Tex.). 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 (Gibco) in a humidified atmosphere containing 5% CO_2 at 37° C.

[0209] Example 1-Characterization of the scFv

[0210] Neutralization of Human PD-L1

[0211] 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.

[0212] 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.

[0213] Stability

[0214] 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.

[0215] 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.

[0216] 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.

[0217] 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 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.

[0218] Kinetic Exclusion Assay of the scFv and the full-length antibody

[0219] The affinity of a monovalent and a bivalent antibody to PD-L1 was determined by Kinetic 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.

[0220] a) Monovalent antibody

[0221] 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 KinExA® Pro software version 4.1.9 or 4.2.10.

[0222] b) Bivalent antibody

[0223] 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 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 around three times tighter than the affinity of the scFv to PD-L1.

[0224] Selectivity

[0225] 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-RO2H) 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.

[0226] 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.

[0227] Binding to Cell Surface PD-L1

[0228] 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.

[0229] 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.

[0230] Example 2 - Generation of the retroviral construct encoding the anti-PD-L1 scFv and retrovirus production

[0231] DNA encoding the anti-PD-L1 scFv was amplified by PCR with the following primers: Forward: 5'-TAAC-CATGG AGTTTGGGCTGAG-3' (SEQ ID NO: 11) and Reverse: 5'-GACGCATGCTCAGCTCGACACGGT-GACC-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 Sphl at the 3' end (reverse primer). The PCR product and the retroviral backbone SFG(I)eGFP were digested with Ncol and Sphl 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 FIG. **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 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.

[0232] Example 3 - Generation and expansion of CAR T cells producing the anti-PD-L1 scFv

[0233] 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 (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 0.5×10^{6} cell/mL in T cells media without cytokines. Twenty four hours after 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 mL/well of anti-PD-L1 scFv). Non-Transduced (NT) T cells were plated at the same concentration (0.25×10^{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 1×10^6 cells/mL. T cells were expanded in vitro for 5 days and then analyzed by flow cytometry to assess transduction efficiency and T cell composition. Eleven to twelve days after initiation, T cells were tested in functional assays.

[0234] 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

[0235] 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 FIG. **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 (FIG. **3**). Transduction with the anti-PD-L1 scFv did not affect T cell proliferation (FIG. **4**). Moreover, expression of the anti-PD-L1 scFv did not affect T cell subset compositions (FIG. **5**). This example demonstrates the generation of CART cells co-expressing anti-GD2 CARs and anti-PD-L1 scFvs.

[0236] 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

[0237] 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 (1 μg/ml, Miltenyi) and anti-CD28 (1 μg/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 FIG. **6**A, the anti-PD-L1 scFv was released by transduced T cells after stimulation with immobilized anti-CD^3/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 (FIG. **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.

[0238] The anti-PD-L1 scFv produced by activated T cells is capable of binding to immobilized PD-L1 (FIG. **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). FIG. **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.

[0239] Example 6-T cells co-transduced with the GD2. CAR-4-1BB and SFG.scFv.anti-PD-L1 vectors have enhanced tumor-killing activity

[0240] Transduced and non-transduced T cells (0.5×10^5 cells/well) were co-cultured with the tumor cell line CHLA-255 (2.5×10^5 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.

[0241] 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) (FIG. **7**) and produced higher levels of IFNgamma in the second cycle of co-culture (14 days of culture) with tumor cells (FIG. **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 CART cells from exhaustion and may provide prolonged anti tumor activity in vivo and prevent tumor recurrence.

[0242] 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 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.

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<220> FEATURE:
<223> OTHER INFORMATION: VL of scFv

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

Asp Arg Val Ile Ile Thr Cys Gln Ala Ser Glu Asp Ile Tyr Ser Leu
            20   25   30

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

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

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

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gly Asn Tyr Gly Ser Ser Ser
                85   90   95

Ser Ser Ser Tyr Gly Ala Val Phe Gly Gln Gly Thr Lys Leu Thr Val
            100   105   110

Leu Gly

<210> SEQ ID NO 2
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH of scFv

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

Ser Leu Arg Leu Ser Cys Thr Val Ser Gly Ile Asp Leu Ser Ser Tyr
            20   25   30

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

Gly Ile Ile Ser Ser Gly Gly Arg Thr Tyr Tyr Ala Ser Trp Ala Lys
    50   55   60

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

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

Arg Gly Arg Tyr Thr Gly Tyr Pro Tyr Tyr Phe Ala Leu Trp Gly Gln
            100   105   110

Gly Thr Leu Val Thr Val Ser Ser
        115   120

<210> SEQ ID NO 3
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<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 3

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<210> SEQ ID NO 4
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<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 4

Asp Ala Ser Asp Leu Ala Ser
1               5


<210> SEQ ID NO 5
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 5

Gln Gly Asn Tyr Gly Ser Ser Ser Ser Ser Ser Tyr Gly Ala Val
1               5                   10                  15


<210> SEQ ID NO 6
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 6

Ile Asp Leu Ser Ser Tyr Thr Met Gly
1               5


<210> SEQ ID NO 7
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 7

Ile Ile Ser Ser Gly Gly Arg Thr Tyr Tyr Ala Ser Trp Ala Lys Gly
1               5                   10                  15


<210> SEQ ID NO 8
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Oryctolagus cuniculus

<400> SEQUENCE: 8

Gly Arg Tyr Thr Gly Tyr Pro Tyr Tyr Phe Ala Leu
1               5                   10


<210> SEQ ID NO 9
<211> LENGTH: 254
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: scFv

<400> SEQUENCE: 9

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

Asp Arg Val Ile Ile Thr Cys Gln Ala Ser Glu Asp Ile Tyr Ser Leu
            20                  25                  30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
```

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```
        35                  40                  45

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

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

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gly Asn Tyr Gly Ser Ser Ser
                85                  90                  95

Ser Ser Ser Tyr Gly Ala Val Phe Gly Gln Gly Thr Lys Leu Thr Val
            100                 105                 110

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

Ser Gly Gly Gly Gly Ser Glu Val Gln Leu Val Glu Ser Gly Gly Gly
    130                 135                 140

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

Ile Asp Leu Ser Ser Tyr Thr Met Gly Trp Val Arg Gln Ala Pro Gly
                165                 170                 175

Lys Gly Leu Glu Trp Val Gly Ile Ile Ser Ser Gly Gly Arg Thr Tyr
            180                 185                 190

Tyr Ala Ser Trp Ala Lys Gly Arg Phe Thr Ile Ser Arg Asp Thr Ser
        195                 200                 205

Lys Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr
    210                 215                 220

Ala Val Tyr Tyr Cys Ala Arg Gly Arg Tyr Thr Gly Tyr Pro Tyr Tyr
225                 230                 235                 240

Phe Ala Leu Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
                245                 250


<210> SEQ ID NO 10
<211> LENGTH: 20
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: linker

<400> SEQUENCE: 10

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1               5                   10                  15

Gly Gly Gly Ser
            20


<210> SEQ ID NO 11
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: forward primer

<400> SEQUENCE: 11

taaccatgga gtttgggctg ag                                               22


<210> SEQ ID NO 12
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: reverse primer
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<400> SEQUENCE: 12

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<210> SEQ ID NO 13
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<220> FEATURE:
<223> OTHER INFORMATION: VL sequence of 14g2a scFv

<400> SEQUENCE: 13

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

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

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

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

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

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

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

Lys Arg
```

<210> SEQ ID NO 14
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH sequence of 14g2a scFv

<400> SEQUENCE: 14

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

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

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

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

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

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

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

Ser
```

<210> SEQ ID NO 15
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: hIgG1 signal peptide

<400> SEQUENCE: 15

Met Glu Phe Gly Leu Ser Trp Leu Phe Leu Val Ala Ile Leu Lys Gly
1 5 10 15

Val Gln

<210> SEQ ID NO 16
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L1 of anti-GD2-CAR

<400> SEQUENCE: 16

Arg Ser Ser Gln Ser Leu Val His Arg Asn Gly Asn Thr Tyr Leu His
1 5 10 15

<210> SEQ ID NO 17
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L2 of anti-GD2-CAR

<400> SEQUENCE: 17

Lys Val Ser Asn Arg Phe Ser
1 5

<210> SEQ ID NO 18
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L3 of anti-GD2-CAR

<400> SEQUENCE: 18

Ser Gln Ser Thr His Val Pro Pro Leu Thr
1 5 10

<210> SEQ ID NO 19
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H1 of anti-GD2-CAR

<400> SEQUENCE: 19

Ser Ser Phe Thr Gly Tyr Asn Met Asn
1 5

<210> SEQ ID NO 20
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H2 of anti-GD2-CAR

<400> SEQUENCE: 20

Ala Ile Asp Pro Tyr Tyr Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

-continued

```
<210> SEQ ID NO 21
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H3 of anti-GD2-CAR

<400> SEQUENCE: 21

Gly Met Glu Tyr
1


<210> SEQ ID NO 22
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L1 of anti-CSPG4 CAR

<400> SEQUENCE: 22

Arg Ala Ser Gln Thr Ile Tyr Lys Asn Leu His
1               5                   10


<210> SEQ ID NO 23
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L2 of anti-CSPG4 CAR

<400> SEQUENCE: 23

Tyr Gly Ser Asp Ser Ile Ser
1               5


<210> SEQ ID NO 24
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L3 of anti-CSPG4 CAR

<400> SEQUENCE: 24

Leu Gln Gly Tyr Ser Thr Pro Trp Thr
1               5


<210> SEQ ID NO 25
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H1 of anti-CSPG4 CAR

<400> SEQUENCE: 25

Tyr Thr Phe Thr Asp Tyr Ser Met His
1               5


<210> SEQ ID NO 26
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H2 of anti-CSPG4 CAR

<400> SEQUENCE: 26
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```
Trp Ile Asn Thr Ala Thr Gly Glu Pro Thr Tyr Ala Asp Asp Phe Lys
1               5                   10                  15

Gly


<210> SEQ ID NO 27
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H3 of anti-CSPG4 CAR

<400> SEQUENCE: 27

Tyr Tyr Asp Tyr
1


<210> SEQ ID NO 28
<211> LENGTH: 109
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VL sequence of anti-CSPG4 CAR

<400> SEQUENCE: 28

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

Gly Glu Thr Val Ser Leu Ser Cys Arg Ala Ser Gln Thr Ile Tyr Lys
            20                  25                  30

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

Ile Lys Tyr Gly Ser Asp Ser Ile Ser Gly Ile Pro Ser Arg Phe Thr
    50                  55                  60

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Asn Ile Asn Ser Val Lys
65                  70                  75                  80

Pro Glu Asp Glu Gly Ile Tyr Tyr Cys Leu Gln Gly Tyr Ser Thr Pro
                85                  90                  95

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


<210> SEQ ID NO 29
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH sequence of anti-CSPG4 CAR

<400> SEQUENCE: 29

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

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

Ser Met His Trp Val Lys Lys Thr Pro Gly Lys Gly Leu Lys Trp Leu
        35                  40                  45

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

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

Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Thr Ala Thr Tyr Phe Cys
                85                  90                  95
```

-continued

```
Phe Ser Tyr Tyr Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
            100                 105                 110

Ser


<210> SEQ ID NO 30
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L1 of anti-GPC3 CAR

<400> SEQUENCE: 30

Arg Ser Ser Gln Ser Leu Val His Ser Asn Arg Asn Thr Tyr Leu His
1               5                   10                  15


<210> SEQ ID NO 31
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L2 of anti-GPC3 CAR

<400> SEQUENCE: 31

Lys Val Ser Asn Arg Phe Ser
1               5


<210> SEQ ID NO 32
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L3 of anti-GPC3 CAR

<400> SEQUENCE: 32

Ser Gln Asn Thr His Val Pro Pro Thr
1               5


<210> SEQ ID NO 33
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H1 of anti-GPC3 CAR

<400> SEQUENCE: 33

Tyr Thr Phe Thr Asp Tyr Glu Met His
1               5


<210> SEQ ID NO 34
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H2 of anti-GPC3 CAR

<400> SEQUENCE: 34

Ala Leu Asp Pro Lys Thr Gly Asp Thr Ala Tyr Ser Gln Lys Phe Lys
1               5                   10                  15

Gly


<210> SEQ ID NO 35
<211> LENGTH: 6
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H3 of anti-GPC3 CAR

<400> SEQUENCE: 35

Phe Tyr Ser Tyr Thr Tyr
1               5


<210> SEQ ID NO 36
<211> LENGTH: 113
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VL of anti-GPC3 CAR

<400> SEQUENCE: 36

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

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

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

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

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

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

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

Arg


<210> SEQ ID NO 37
<211> LENGTH: 115
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH of anti-GPC3 CAR v

<400> SEQUENCE: 37

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

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

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

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

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

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

Thr Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

Val Ser Ser
        115
```

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<210> SEQ ID NO 38
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VH of anti-GPC3 CAR

<400> SEQUENCE: 38

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

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

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

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

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

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

Thr Arg Phe Tyr Ser Tyr Thr Tyr Trp Gly Gln Gly Thr Leu Val Thr
            100                 105                 110

Val Ser Ala
        115


<210> SEQ ID NO 39
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L1 of anti-5T4 CAR

<400> SEQUENCE: 39

Tyr Ser Phe Thr Gly Tyr Tyr Met His
1               5


<210> SEQ ID NO 40
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L2 of anti-5T4 CAR

<400> SEQUENCE: 40

Arg Ile Asn Pro Asn Asn Gly Val Thr Leu Tyr Asn Gln Lys Phe Lys
1               5                   10                  15

Asp


<210> SEQ ID NO 41
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-L3 of anti-5T4 CAR

<400> SEQUENCE: 41

Ser Thr Met Ile Thr Asn Tyr Val Met Asp Tyr
1               5                   10
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<210> SEQ ID NO 42
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H1 of anti-5T4 CAR

<400> SEQUENCE: 42

Lys Ala Ser Gln Ser Val Ser Asn Asp Val Ala
1 5 10

<210> SEQ ID NO 43
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H2 of anti-5T4 CAR

<400> SEQUENCE: 43

Tyr Thr Ser Ser Arg Tyr Ala
1 5

<210> SEQ ID NO 44
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CDR-H3 of anti-5T4 CAR

<400> SEQUENCE: 44

Gln Gln Asp Tyr Asn Ser Pro Pro Thr
1 5

<210> SEQ ID NO 45
<211> LENGTH: 108
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: VL of anti-5T4 CAR

<400> SEQUENCE: 45

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

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

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

Ser Tyr Thr Ser Ser Arg Tyr Ala Gly Val Pro Asp Arg Phe Ile Gly
50 55 60

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

Glu Asp Leu Ala Val Tyr Phe Cys Gln Gln Asp Tyr Asn Ser Pro Pro
85 90 95

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

<210> SEQ ID NO 46
<211> LENGTH: 120
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: VH of anti-5T4 CAR

<400> SEQUENCE: 46

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

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

Tyr Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile
        35                  40                  45

Gly Arg Ile Asn Pro Asn Asn Gly Val Thr Leu Tyr Asn Gln Lys Phe
    50                  55                  60

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

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

Ala Arg Ser Thr Met Ile Thr Asn Tyr Val Met Asp Tyr Trp Gly Gln
            100                 105                 110

Val Thr Ser Val Thr Val Ser Ser
        115                 120
```

<210> SEQ ID NO 47
<211> LENGTH: 42
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 41BB costimulatory domain

<400> SEQUENCE: 47

```
Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
1               5                   10                  15

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
            20                  25                  30

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
        35                  40
```

<210> SEQ ID NO 48
<211> LENGTH: 112
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD3 zeta intracellular domain

<400> SEQUENCE: 48

```
Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
1               5                   10                  15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
            20                  25                  30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
        35                  40                  45

Pro Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln Lys
    50                  55                  60

Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu Arg
65                  70                  75                  80

Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala
                85                  90                  95

Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro Arg
```

-continued

```
            100                 105                 110


<210> SEQ ID NO 49
<211> LENGTH: 41
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: CD28 costimulatory domain

<400> SEQUENCE: 49

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
1               5                   10                  15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
            20                  25                  30

Pro Arg Asp Phe Ala Ala Tyr Arg Ser
        35                  40


<210> SEQ ID NO 50
<211> LENGTH: 114
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Survivin specific TCR, beta chain

<400> SEQUENCE: 50

Asp Ala Met Val Ile Gln Asn Pro Arg Tyr Gln Val Thr Gln Phe Gly
1               5                   10                  15

Lys Pro Val Thr Leu Ser Cys Ser Gln Thr Leu Asn His Asn Val Met
            20                  25                  30

Tyr Trp Tyr Gln Gln Lys Ser Ser Gln Ala Pro Lys Leu Leu Phe His
        35                  40                  45

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

Gln Ser Arg Arg Pro Asn Thr Ser Phe Cys Phe Leu Asp Ile Arg Ser
65                  70                  75                  80

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

Asp Ser Thr Ala Glu Pro Gln His Phe Gly Asp Gly Thr Arg Leu Ser
            100                 105                 110

Ile Leu


<210> SEQ ID NO 51
<211> LENGTH: 114
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Survivin specific TCR, alpha chain

<400> SEQUENCE: 51

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

Asp Asn Ser Ile Ile Asn Cys Ala Tyr Ser Asn Ser Ala Ser Asp Tyr
            20                  25                  30

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

Asp Ile Arg Ser Asn Met Asp Lys Arg Gln Gly Gln Arg Val Thr Val
    50                  55                  60
```

-continued

```
Leu Leu Asn Lys Thr Val Lys His Leu Ser Leu Gln Ile Ala Ala Thr
65                  70                  75                  80

Gln Pro Gly Asp Ser Ala Val Tyr Phe Cys Ala Glu Thr Val Thr Asp
                85                  90                  95

Ser Trp Gly Lys Leu Gln Phe Gly Ala Gly Thr Gln Val Val Val Thr
            100                 105                 110

Pro Asp


<210> SEQ ID NO 52
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR1 alpha

<400> SEQUENCE: 52

Ser Ser Tyr Ser Pro Ser
1               5


<210> SEQ ID NO 53
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR2 alpha

<400> SEQUENCE: 53

Tyr Thr Ser Ala Ala Thr Leu
1               5


<210> SEQ ID NO 54
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR3 alpha

<400> SEQUENCE: 54

Trp Ser Pro Phe Ser Gly Gly Gly Ala Asp Gly Leu Thr
1               5                   10


<210> SEQ ID NO 55
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR3 alpha v

<400> SEQUENCE: 55

Ser Pro Phe Ser Gly Gly Gly Ala Asp Gly Leu Thr
1               5                   10


<210> SEQ ID NO 56
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR1 beta

<400> SEQUENCE: 56

Asp Phe Gln Ala Thr Thr
1               5
```

-continued

<210> SEQ ID NO 57
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR2 beta

<400> SEQUENCE: 57

Ser Asn Glu Gly Ser Lys Ala
1 5

<210> SEQ ID NO 58
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR3 beta

<400> SEQUENCE: 58

Ser Ala Arg Asp Gly Gly Glu Gly
1 5

<210> SEQ ID NO 59
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, CDR3 beta v

<400> SEQUENCE: 59

Arg Asp Gly Gly Glu Gly Ser Glu Thr Gln Tyr
1 5 10

<210> SEQ ID NO 60
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, alpha chain

<400> SEQUENCE: 60

Met Leu Leu Leu Leu Val Pro Val Leu Glu Val Ile Phe Thr Leu Gly
1 5 10 15

Gly Thr Arg Ala Gln Ser Val Thr Gln Leu Asp Ser His Val Ser Val
20 25 30

Ser Glu Gly Thr Pro Val Leu Leu Arg Cys Asn Tyr Ser Ser Ser Tyr
35 40 45

Ser Pro Ser Leu Phe Trp Tyr Val Gln His Pro Asn Lys Gly Leu Gln
50 55 60

Leu Leu Leu Lys Tyr Thr Ser Ala Ala Thr Leu Val Lys Gly Ile Asn
65 70 75 80

Gly Phe Glu Ala Glu Phe Lys Lys Ser Glu Thr Ser Phe His Leu Thr
85 90 95

Lys Pro Ser Ala His Met Ser Asp Ala Ala Glu Tyr Phe Cys Val Val
100 105 110

Ser Pro Phe Ser Gly Gly Gly Ala Asp Gly Leu Thr
115 120

<210> SEQ ID NO 61
<211> LENGTH: 116

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, beta chain
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 61

Met Leu Leu Leu Leu Leu Leu Leu Gly Pro Gly Ser Gly Leu Gly Ala
1 5 10 15

Val Val Ser Gln His Pro Ser Trp Val Ile Cys Lys Ser Gly Thr Xaa
20 25 30

Val Lys Ile Glu Cys Arg Ser Leu Asp Phe Gln Ala Thr Thr Met Phe
35 40 45

Trp Tyr Arg Gln Phe Pro Lys Gln Ser Leu Met Leu Met Ala Thr Ser
50 55 60

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

Phe Leu Ile Asn His Ala Ser Leu Thr Leu Ser Thr Leu Thr Val Thr
85 90 95

Ser Ala His Pro Glu Asp Ser Ser Phe Tyr Ile Cys Ser Ala Arg Asp
100 105 110

Gly Gly Glu Gly
115

<210> SEQ ID NO 62
<211> LENGTH: 124
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, alpha chain v

<400> SEQUENCE: 62

Met Leu Leu Leu Leu Val Pro Val Leu Glu Val Ile Phe Thr Leu Gly
1 5 10 15

Gly Thr Arg Ala Gln Ser Val Thr Gln Leu Asp Ser His Val Ser Val
20 25 30

Ser Glu Gly Thr Pro Val Leu Leu Arg Cys Asn Tyr Ser Ser Ser Tyr
35 40 45

Ser Pro Ser Leu Phe Trp Tyr Val Gln His Pro Asn Lys Gly Leu Gln
50 55 60

Leu Leu Leu Lys Tyr Thr Ser Ala Ala Thr Leu Val Lys Gly Ile Asn
65 70 75 80

Gly Phe Glu Ala Glu Phe Lys Lys Ser Glu Thr Ser Phe His Leu Thr
85 90 95

Lys Pro Ser Ala His Met Ser Asp Ala Ala Glu Tyr Phe Cys Val Val
100 105 110

Ser Pro Phe Ser Gly Gly Gly Ala Asp Gly Leu Thr
115 120

<210> SEQ ID NO 63
<211> LENGTH: 121
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: WT-1 specific TCR, beta chain v

-continued

```
<400> SEQUENCE: 63

Met Leu Leu Leu Leu Leu Leu Leu Gly Pro Gly Ser Gly Leu Gly Ala
1               5                   10                  15

Val Val Ser Gln His Pro Ser Trp Val Ile Cys Lys Ser Gly Thr Ser
            20                  25                  30

Val Lys Ile Glu Cys Arg Ser Leu Asp Phe Gln Ala Thr Thr Met Phe
        35                  40                  45

Trp Tyr Arg Gln Phe Pro Lys Gln Ser Leu Met Leu Met Ala Thr Ser
    50                  55                  60

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

Phe Leu Ile Asn His Ala Ser Leu Thr Leu Ser Thr Leu Thr Val Thr
                85                  90                  95

Ser Ala His Pro Glu Asp Ser Ser Phe Tyr Ile Cys Ser Ala Arg Asp
            100                 105                 110

Gly Gly Glu Gly Ser Glu Thr Gln Tyr
        115                 120
```

1. An engineered immune cell expressing:

i) an antigen receptor, and

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.

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,

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).

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.

6. The engineered immune cell of claim **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 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 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.

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.

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.

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, 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,

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 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 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.

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 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-lalpha, and/or 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 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-AL 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 gp120, 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 claim **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:

(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.

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**, 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 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-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 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.

* * * * *