

In one aspect, there is provided an engineered natural killer (NK) or T cell comprising a first nucleic acid comprising a nucleotide sequence encoding a chimeric antigen receptor (CAR), and a second nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain, wherein the target-binding molecule binds a target selected from programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (Tim3), killer immunoglobulin-like receptor 2DL1 (KIR1DL1), killer immunoglobulin-like receptor 2DL2/DL3 (KIR1DL2/DL3), CD94, NKG2A, Src homology region 2 domain-containing phosphatase-1 (SHP-1), and Src homology region 2 domain-containing phosphatase-2 (SHP-2), and wherein the target-binding molecule linked to the localizing domain is not a CAR and downregulates expression of the target in the engineered NK or T cell.

[0004] In another aspect, there is provided use of an engineered immune cell described herein in the manufacture of a medicament for treating cancer in a subject in need thereof. In other embodiments, the present disclosure provides the use of an engineered immune cell that comprises a gene encoding an immune activating receptor, and a gene encoding a target-binding molecule linked to a localizing domain for treating cancer, comprising administering a therapeutic amount of the engineered immune cell to a subject in need thereof.

[0005] In another aspect, there is provided also a method for producing the engineered NK or T cell as described herein, the method comprising introducing into an NK or T cell a first nucleic acid comprising a nucleotide sequence encoding a chimeric antigen receptor, and a second nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain, wherein the target-binding molecule binds a target selected from programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (Tim3), killer immunoglobulin-like receptor 2DL1 (KIR1DL1), CD94, NKG2A, Src homology region 2 domain-containing phosphatase-1 (SHP-1), and Src homology region 2 domain-containing phosphatase-2 (SHP-2), and wherein the target-binding molecule linked to the localizing domain is not a CAR and downregulates expression of the target in the engineered NK or T cell, thereby producing the engineered NK or T cell.

[0006] In some embodiments, the engineered immune cells possess enhanced therapeutic efficacy as a result of one or more of reduced graft-versus-host disease (GvHD) in a host, reduced or elimination of rejection by a host, extended survival in a host, reduced inhibition by the tumor in a host, reduced self-killing in a host, reduced inflammatory cascade in a host, or sustained natural/artificial receptor-mediated (*e.g.*, CAR-mediated) signal transduction in a host.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] The foregoing will be apparent from the following more particular description of example embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

[0008] FIGS. 1A-1B is a schematic representation of a strategy employed in the present invention. FIG. 1A is an overall mechanism of CAR mediated killing of cancer cells. FIG. 1B shows the combined expression of CAR with different formats of compartment-directed scFv (an example of a target-binding molecule linked to a localizing domain) and examples of possible targets. The CAR can be replaced by other receptors that can enhance immune cell capacity.

CLAIMS

What is claimed is:

1. An engineered natural killer (NK) or T cell comprising a first nucleic acid comprising a nucleotide sequence encoding a chimeric antigen receptor (CAR) and a second nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain, wherein the target-binding molecule binds a target selected from programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (Tim3), killer immunoglobulin-like receptor 2DL1 (KIR1DL1), killer immunoglobulin-like receptor 2DL2/DL3 (KIR1DL2/DL3), CD94, NKG2A, Src homology region 2 domain-containing phosphatase-1 (SHP-1), and Src homology region 2 domain-containing phosphatase-2 (SHP-2), and wherein the target-binding molecule linked to the localizing domain is not a CAR and downregulates expression of the target in the engineered NK or T cell.
2. The engineered immune cell of claim 1, wherein the CAR is anti-CD19-4-1BB-CD3 ζ CAR.
3. The engineered immune cell of claim 1, wherein the target-binding molecule is an antibody.
4. The engineered immune cell of claim 1, wherein the target-binding molecule is a single-chain variable fragment (scFv).

5. The engineered immune cell of claim 1, wherein the localizing domain comprises an endoplasmic reticulum (ER) or Golgi retention sequence.
6. The engineered immune cell of claim 5, wherein the ER or Golgi retention sequence comprises the amino acid sequence KDEL (SEQ ID NO: 4), KKXX (SEQ ID NO: 9), KXD/E (SEQ ID NO: 10), or YQRL (SEQ ID NO: 11), wherein X is any amino acid.
7. The engineered immune cell of claim 1, wherein the CAR is a non-naturally occurring molecule.
8. The engineered immune cell of claim 1, wherein the target-binding molecule linked to the localizing domain is a non-naturally occurring molecule.
9. Use of the engineered immune cell of claim 1 in the manufacture of a medicament for treating cancer in a subject in need thereof.
10. The use of claim 9, wherein the engineered immune cell is to be administered into the subject by intravenous infusion, intra-arterial infusion, direct injection into tumor and/or perfusion of tumor bed after surgery, implantation at a tumor site in an artificial scaffold, intrathecal administration, and intraocular administration.
11. The use of claim 9, wherein the cancer is a solid tumor or a hematologic malignancy.

12. A method for producing the engineered NK or T cell of claim 1, the method comprising:

introducing into an NK or T cell a first nucleic acid comprising a nucleotide sequence encoding a chimeric antigen receptor and a second nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain, wherein the target-binding molecule binds a target selected from programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell immunoglobulin and mucin-domain containing-3 (Tim3), killer immunoglobulin-like receptor 2DL1 (KIR1DL1), CD94, NKG2A, Src homology region 2 domain-containing phosphatase-1 (SHP-1), and Src homology region 2 domain-containing phosphatase-2 (SHP-2), and wherein the target-binding molecule linked to the localizing domain is not a CAR and downregulates expression of the target in the engineered NK or T cell,

thereby producing the engineered NK or T cell.
13. The method of claim 12, wherein the engineered immune cell is produced ex vivo.