METHODS FOR ENHANCING EFFICACY OF THERAPEUTIC IMMUNE CELLS

BACKGROUND OF THE INVENTION

[0001] Immune cells can be potent and specific "living drugs". Immune cells have the potential to target tumor cells while sparing normal tissues; several clinical observations indicate that they can have major anti-cancer activity. Thus, in patients receiving allogeneic hematopoietic stem cell transplantation (HSCT), T-cell-mediated graft-versus-host disease (GvHD) (Weiden, PL *et al.*, *N. Engl. J. Med.* 1979;300(19):1068-1073; Appelbaum, FR *Nature*, 2001;411(6835):385-389; Porter, DL *et al.*, *N. Engl. J. Med.* 1994;330(2):100-106; Kolb, HJ *et al. Blood.* 1995;86(5):2041-2050; Slavin, S. *et al.*, *Blood.* 1996;87(6):2195-2204), and donor natural killer (NK) cell alloreactivity (Ruggeri L, *et al. Science.* 2002;295(5562):2097-2100; Giebel S, *et al. Blood.* 2003;102(3):814-819; Cooley S, *et al. Blood.* 2010;116(14):2411-2419) are inversely related to leukemia recurrence. Besides the HSCT context, administration of antibodies that release T cells from inhibitory signals (Sharma P, *et al.*, *Nat Rev Cancer.* 2011;11(11):805-812.; Pardoll DM., *Nat Rev Cancer.* 2012;12(4):252-264), or bridge them to tumor cells (Topp MS, *et al. J. Clin.Oncol.* 2011;29(18):2493-2498) produced major clinical responses in patients with either solid tumors or leukemia. Finally, infusion of genetically-modified autologous T lymphocytes induced complete and durable remission in patients with refractory leukemia and lymphoma (Maude SL, *et al. N Engl J Med.* 2014;371(16):1507-1517).

[0002] Nevertheless, there is a significant need for improving immune cell therapy by broadening its applicability and enhancing its efficacy.

SUMMARY

[0003] The present disclosure relates to engineered immune cells having enhanced therapeutic efficacy for, *e.g.*, cancer therapy.

In one aspect, there is provided an engineered immune cell that comprises a nucleic acid comprising a nucleotide sequence encoding an immune activating receptor, and a nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain.

[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 an engineered immune cell, the method comprising introducing into an immune cell a nucleic acid comprising a nucleotide sequence encoding an immune activating receptor, and a nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain, thereby producing an engineered immune 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 immune cell that comprises a nucleic acid comprising a nucleotide sequence encoding an immune activating receptor and a nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain.

2. The engineered immune cell of claim 1, wherein the engineered immune cell is an engineered T cell, an engineered natural killer (NK) cell, an engineered NK/T cell, an engineered monocyte, an engineered macrophage, or an engineered dendritic cell.

3. The engineered immune cell of claim 1, wherein the receptor is a chimeric antigen receptor (CAR).

4. The engineered immune cell of claim 1, wherein the CAR is anti-CD19-4-1BB-CD3ζ CAR.

5. The engineered immune cell of claim 1, wherein the target-binding molecule is an antibody.

6. The engineered immune cell of claim 5, wherein the antibody is a single-chain variable fragment (scFv).

7. The engineered immune cell of claim 5, wherein the antibody binds to a factor in a CD3/T-cell receptor (TCR) complex, a cytokine, a human leukocyte antigen (HLA) Class I molecule, or a receptor that downregulates immune response.

8. The engineered immune cell of claim 7, wherein the factor in a CD3/TCR complex is CD3ε, TCRα, TCRβ, TCRγ, TCRδ, CD3δ, CD3γ, or CD3ζ.

9. The engineered immune cell of claim 7, wherein the HLA Class I molecule is β2-microglobulin, α1-microglobulin, α2-microglobulin, or α3-microglobulin.

10. The engineered immune cell of claim 7, wherein the receptor that downregulates immune response is 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 receptors (KIRs), CD94, NKG2A, or a protein tyrosine phosphatase.

11. The engineered immune cell of claim 7, wherein the cytokine is interleukin (IL)-6, IL-2, IL-4, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21, IL-27, IL-35, interferon (IFN)-γ, IFN-β, IFN-α, tumor necrosis factor (TNF)-α, or transforming growth factor (TGF)-β.

12. The engineered immune cell of claim 1, wherein the target-binding molecule binds to CD2, CD4, CD5, CD7, CD8, CD30, CD38, CD45, CD52, or CD127.

13. The engineered immune cell of claim 7, wherein the localizing domain comprises an endoplasmic reticulum (ER) or Golgi retention sequence; a proteosome localizing sequence; a transmembrane domain sequence derived from CD8α, CD8β, 4-1BB, CD28, CD34, CD4, FcεRIγ, CD16, OX40, CD3ζ, CD3ε, CD3γ, CD3δ, TCRα, CD32, CD64, VEGFR2, FAS, or FGFR2B.

14. The engineered immune cell of claim 13, 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.

15. The engineered immune cell of claim 13, wherein the proteasome localizing sequence comprises a PEST motif.

16. The engineered immune cell of claim 1, wherein the immune activating receptor is a non-naturally occurring molecule.

17. The engineered immune cell of claim 1, wherein the target-binding molecule linked to a localizing domain is a non-naturally occurring molecule.

18. Use of the engineered immune cell of claim 1 in the manufacture of a medicament for treating cancer in a subject in need thereof.

19. The use of claim 18, wherein the engineered immune cell is 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.

20. The use of claim 18, wherein the cancer is a solid tumor or a hematologic malignancy.

21. A method for producing the engineered immune cell of claim 1, the method comprising:

introducing into an immune cell a nucleic acid comprising a nucleotide sequence encoding an immune activating receptor and a nucleic acid comprising a nucleotide sequence encoding a target-binding molecule linked to a localizing domain, thereby producing an engineered immune cell.

22. The method of claim 21, wherein the engineered immune cell is produced ex vivo.