

THERAPEUTIC CELL COMPOSITIONS AND METHODS FOR MANUFACTURE AND USES THEREOF

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Application No. 16/826,708, filed on March 23, 2020, U.S. Provisional Application No. 62/946,896, filed on December 11, 2019, U.S. Provisional Application No. 63/003,617, filed on April 1, 2020, and U.S. Provisional Application No. 63/014,068, filed on April 22, 2020, each of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Circulating monocytes represent a versatile and dynamic cell population, composed of multiple subsets which differ in phenotype, size, morphology, and transcriptional profiles and are defined by their location in the blood. These discrete monocyte subsets can be distinguished by the expression of CD14 and CD16 in humans and Ly6C, CCR2, and CX3CR1 in mice. In humans, CD14⁺ CD16⁻ (classical) monocytes make up ~85% of the circulating monocyte pool, whereas the remaining ~15% consist of CD14⁺ CD16⁺ (intermediate) and CD14^{lo} CD16⁺ (nonclassical) monocytes. Similarly, in mice, two populations of monocytes have been described: Ly6Chi CCR2⁺ CX3CR1^{int} and Ly6Cl^o CCR2⁻ CX3CR1^{hi}, representing classical and nonclassical monocytes, respectively. Monocyte egression from the bone marrow requires expression of the chemokine receptor CCR2, which is restricted to CD14⁺CD16⁻ classical monocytes.

[0003] Myeloid cells are effectively recruited to a site of infection or pathogenesis. Classical monocytes are rapidly recruited to sites of cancer, infection, autoimmunity and injury, where they exhibit considerable functional plasticity, differentiating into a number of downstream cells such as dendritic cells and macrophages. Classical monocytes replenish resident peripheral monocyte-derived cells under steady-state conditions. However, several endogenous and tissue environment factors affect and control the effectivity of macrophages in the eradication of a pathogen and mounting a strong immune response with immunological memory.

SUMMARY

[0003a] In one aspect, provided herein is a composition comprising an *ex vivo* population of human cells comprising a recombinant polynucleic acid, wherein the recombinant polynucleic acid comprises a sequence encoding a chimeric fusion protein (CFP), wherein: i) at least 50% of the cells in the *ex vivo* population of human cells are CD14⁺ and CD16⁻, and ii) less than 10% of the cells in the *ex vivo* population of human cells are dendritic cells.

[0003b] In one aspect, provided herein is a pharmaceutical composition for treating cancer, comprising the composition as described herein.

[0003c] In one aspect, provided herein is a method for preparing the composition as described herein, the method comprising: (a) isolating a population of cells from a biological sample, and (b) introducing the recombinant polynucleic acid comprising a sequence encoding the CFP into the population of cells, wherein the *ex vivo* population of human cells is cultured *in vitro* for less than 24 hours.

[0003d] In one aspect, provided herein is a use of the composition as described herein for the manufacture of a medicament for the treatment of cancer,

[0003e] In one aspect, provided herein is a composition as described herein for use in therapy.

[0004] The development of cell therapies using myeloid cells, including macrophages and dendritic cells is appealing as these cells act as the bridge between innate and adaptive immunity. On one hand, harnessing the inflammatory abilities of these cells including, for example, phagocytosis, cytokine production, chemokine production, antigen presentation/cross-presentation, and T cell activation has the potential to revolutionize treatment for cancer. Harnessing the immune regulatory abilities of these cells also have the potential to treat numerous autoimmune disorders as well as neurodegenerative disease such as Alzheimer's and other protein accumulation disorders.

CLAIMS

What is claimed is:

1. A composition comprising an *ex vivo* population of human cells comprising a recombinant polynucleic acid, wherein the recombinant polynucleic acid comprises a sequence encoding a chimeric fusion protein (CFP), wherein:
 - (i) at least 50% of the cells in the *ex vivo* population of human cells are CD14+ and CD16-, and
 - (ii) less than 10% of the cells in the *ex vivo* population of human cells are dendritic cells.
2. The composition of claim 1, wherein at least 50% of the cells in the *ex vivo* population of human cells are CCR2+ and/or CCR5+.
3. The composition of claim 1, wherein at least 50% of the cells in the *ex vivo* population of human cells are CD63+.
4. The composition of claim 1, wherein at least 50% of the cells in the *ex vivo* population of human cells are CD56-, CD3-, and/or CD19-.
5. The composition of claim 1, wherein less than 40% of the cells in the *ex vivo* population of human cells are macrophage cells.
6. The composition of claim 1, wherein:
 - (a) at least 50% of the cells in the *ex vivo* population of human cells are CCR2+ and/or CCR5+;
 - (b) at least 50% of the cells in the *ex vivo* population of human cells are CD63+;
 - (c) at least 50% of the cells in the *ex vivo* population of human cells are CD56-, CD3-, and/or CD19-; and
 - (d) less than 40% of the cells in the *ex vivo* population of human cells are macrophage cells.
7. The composition of claim 1, wherein the recombinant polynucleic acid is an mRNA.
8. The composition of claim 1, wherein the recombinant polynucleic acid comprises a sequence encoding a CFP, wherein the CFP comprises:
 - (a) an extracellular domain comprising an antigen binding domain comprising a Fab, a Fab', a F(ab')₂, a Fd (consisting of VH and CH1), a single-chain variable fragment (scFv), a single-chain antibody, a disulfide-linked variable fragment (dsFv), or a VH domain capable of binding to an antigen, and
 - (b) a transmembrane domain operatively linked to the extracellular domain,and wherein, the *ex vivo* population of human cells lacks tonic signaling through the CFP.

9. The composition of claim 8, wherein the CFP further comprises an intracellular domain comprising an intracellular signaling domain.
10. The composition of claim 8, wherein the antigen binding domain comprises an scFv that binds to a CD5 antigen or a HER2 antigen.
11. The composition of claim 8, wherein the transmembrane domain is a CD8, CD28, or CD68 transmembrane domain.
12. The composition of any one of claims 9 - 11, wherein the intracellular domain comprises at least two intracellular signaling domains, wherein the at least two intracellular signaling domains comprise
 - (i) a first intracellular signaling domain derived from FcγR or FcεR, and
 - (ii) a second intracellular signaling domain that:
 - (A) comprises a PI3-kinase (PI3K) recruitment domain, or
 - (B) is derived from CD40.
13. The composition of claim 1, wherein the *ex vivo* population of human cells comprises at least 1×10^7 cells.
14. The composition of claim 1, wherein:
 - (A) at least 50% of the cells in the population of cells are CD16low, CCR2+ (CD192+), CCR5+ (CD195+), CD63+, CD56-, CD120a+ (TNFR1+) or CD120b+ (TNFR2+);
 - (B) less than 50% of the cells in the population of cells express one or more of CD64, CD68, CD80, CD86, CD163, CD206, CD200R, CD31, CD71, CLEC9A, CD1C and AXL/SIGLEC6;
 - (C) in the presence of a population of cells expressing a cell-surface antigen to which the CFP specifically binds the population of cells comprising the recombinant polynucleic acid secrete one or more of IL-1beta, TNF alpha, IFN-alpha, MIP-1alpha, IL-8, eotaxin, PIGF-1, CCL3, IL6 and IL10; and/or
 - (D) in the presence of GMCSF, IL-4, IL-10, TGFbeta, TCM and/or MCSF, the expression of MHCI or MHCII is upregulated in the population of cells to a greater extent compared to a corresponding population of cells without the polynucleic acid comprising a sequence encoding the CFP.
15. A pharmaceutical composition for treating cancer, comprising the composition of any one of claims 1-14.
16. Use of the composition of any one of claims 1-14 for the manufacture of a medicament for the treatment of cancer.
17. A method for preparing the composition of any one of claims 1-14, the method comprising:

- (a) isolating a population of cells from a biological sample, and
 - (b) introducing the recombinant polynucleic acid comprising a sequence encoding the CFP into the population of cells, wherein the *ex vivo* population of human cells is cultured *in vitro* for less than 24 hours.
18. The method of claim 17, wherein the biological sample is human peripheral blood, and wherein isolating comprises elutriation.
 19. The method of claim 17, wherein isolating comprises negative selection.
 20. The method of claim 19, wherein the negative selection comprises contacting the biological sample with an anti-CD16 antibody and one or more antibodies selected from an anti-CD56 antibody, an anti-CD3 antibody and an anti-CD19 antibody, and collecting cells in the biological sample that are not bound by the anti-CD16 antibody and not bound by the one or more antibodies.
 21. The method of claim 17, wherein the biological sample is an apheresis sample.
 22. The method of claim 17, wherein the recombinant polynucleic acid is mRNA and wherein introducing the recombinant polynucleic acid into the population of cells comprises electroporation.
 23. The method of claim 17, wherein the cells in the composition have been cultured for less than 72 hours or less than 48 hours *ex vivo*.
 24. The composition of any one of claims 1-14 for use in therapy.