

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 CD14+/CD16- cells, wherein the *ex vivo* population of CD14+/CD16- cells is an engineered *ex vivo* population of cells and/or comprises an exogenous agent.~~
- ~~2. The composition of claim 1, wherein the *ex vivo* population of CD14+/CD16- cells is a population of human cells.~~
- ~~3. The composition of claim 1, wherein the *ex vivo* population of CD14+/CD16- cells is a population of unpolarized or undifferentiated myeloid cells.~~
- ~~4. The composition of claim 1, wherein the exogenous agent is comprising a recombinant nucleic acid polynucleic acid comprises a sequence encoding a protein or a peptide that is expressed in a cell of the population of CD14+/CD16- cells.~~
- ~~5. 1. The composition of any one of the claims 1-4, wherein the , wherein the recombinant nucleic acid polynucleic acid comprises a sequence encoding a chimeric fusion protein (CFP), wherein:~~
- ~~6. The composition of any one of the claims 1-5, wherein the CFP comprises: (a) an extracellular domain comprising an antigen binding domain and (b) a transmembrane domain operatively linked to the extracellular domain.~~
- ~~7. The composition of any one of the claims 1-6, wherein the recombinant nucleic acid comprises a sequence encoding a protein or a peptide that is expressed on the membrane surface of a cell in the population of CD14+/CD16- cells.~~
- ~~8. The composition of claim 7, wherein the protein or the peptide expressed on the membrane surface comprises an extracellular antigen binding domain that binds to an antigen or a disease-causing agent.~~
- ~~9. The composition of claim 8, wherein the antigen is a cancer antigen.~~
- ~~10. The composition of claim 8, wherein the antigen is a microbial pathogenic antigen.~~
- ~~11. The composition of claim 1, wherein the recombinant nucleic acid encodes a protein or a peptide that augments immune response of the cell.~~
- ~~12. The composition of claim 11, wherein the recombinant nucleic acid encodes a bi-specific or tri-specific engager.~~
- ~~13. The composition of claim 1-4, wherein the protein or the peptide comprises one or more antigens or fragments thereof.~~

- ~~14. The composition of claim 13, wherein an antigen of the one or more antigens is fused to an endosomal targeting sequence.~~
- ~~15. The composition of claim 13, wherein an antigen of the one or more antigens comprises secretory sequence.~~
- ~~16. The composition of any one of the claims 13-15, wherein the recombinant nucleic acid encodes a first antigen that is fused to an endosomal targeting sequence, and a second antigen comprising a secretory sequence.~~
- ~~17. The composition of any one of the claims 1-4 or 13-16, wherein the recombinant nucleic acid encodes a protein or a peptide that is secreted by a cell in the population of CD14+/CD16- cells.~~
- ~~18. The composition of claim 16, wherein the first antigen and the second antigen are encoded by the same gene.~~
- ~~19. The composition of any one of the claims 13-18, wherein the protein or the peptide is an endosomally processed protein.~~
- ~~20. The composition of any one of the claims 13-19, wherein the protein or the peptide is an epitope of an antigen that is displayed on the cell surface.~~
- ~~21. The composition of any one of the claims 13-20, wherein the antigen is a viral, bacterial, protozoan, or fungal antigen.~~
- ~~22. The composition of claim 1, wherein the exogenous agent is a recombinant nucleic acid comprising a sequence encoding a protein or a peptide that is a cytoplasmic protein or a peptide.~~
- ~~23. The composition of claim 1, wherein the exogenous agent is a recombinant nucleic acid comprising a sequence encoding a protein or a peptide that is immunogenic.~~
- ~~24. The composition of claim 1, wherein the exogenous agent is an antigen.~~
- ~~25. The composition of claim 1-24, wherein (i) at least 25% of the cells in the population of cells are CD14+ and CD16-, and (ii) less than 25% of the cells in the population of cells are dendritic cells.~~
- ~~26. (i) The composition of any one of the claims 1-25, wherein at least 50% of the cells in the *ex vivo* population of human cells are CCR2CD14+ and/or CCR5+, 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 ~~1-26~~, 1, wherein at least 50% of the cells in the *ex vivo* population of human cells are CCR2+ and/or CCR5+.

- ~~27.3.~~ The composition of claim 1, wherein at least 50% of the cells in the *ex vivo* population of human cells are CD63+.
- ~~28.4.~~ The composition of ~~any one of the claims 1-27,~~claim 1, wherein at least 50% of the cells in the *ex vivo* population of human cells are CD56-, CD3-, and/or CD19-.
- ~~29.~~ ~~The composition of any one of the claims 1-28;~~The composition of claim 1, wherein less than 40% of the cells in the ~~population of cells are macrophage cells.~~
- ~~30.~~ ~~The composition of claim 1-29, wherein the ex vivo population of human cells comprises at least 1×10^7 cells.~~
- ~~31.~~ ~~A pharmaceutical composition comprising the composition of any one of claims 1-30 and a pharmaceutically acceptable excipient.~~
- ~~32.~~ ~~A pharmaceutical composition comprising:~~
- ~~(a) a population of human cells comprising a recombinant nucleic acid, wherein the recombinant nucleic acid comprises a sequence encoding a chimeric fusion protein (CFP), wherein: (i) at least 25% of the cells in the population of human cells are CD14+ and CD16-, and (ii) less than 25% of the cells in the population of human cells are dendritic cells; and~~
- ~~(b) a pharmaceutically acceptable excipient.~~
- ~~33.~~ ~~The pharmaceutical composition of claim 32, wherein the CFP comprises: (i) an extracellular antigen binding domain and (ii) a transmembrane domain, wherein the extracellular antigen binding domain and the transmembrane domain are operably linked.~~
- ~~34.~~ ~~The pharmaceutical composition of claim 32 or 33, wherein the CFP further comprises one or more intracellular signaling domains, operably linked to the transmembrane domain.~~
- ~~35.~~ ~~The pharmaceutical composition of any one of the claims 32-34, wherein the CFP comprises an intracellular domain derived from a phagocytic receptor or a scavenger receptor.~~
- ~~36.~~ ~~The pharmaceutical composition of any one of the claims 32-35, wherein the antigen binding domain is a CD5 binding domain or a HER2 binding domain.~~
- ~~37.~~ ~~The pharmaceutical composition of any one of the claims 32-36, wherein the transmembrane domain is derived from a CD8 transmembrane domain, a CD28 transmembrane domain or a CD68 transmembrane domain.~~
- ~~38.~~ ~~The pharmaceutical composition of any one of the claims 32-37, wherein the one or more intracellular signaling domains comprises a kinase recruitment domain.~~
- ~~39.(A)~~ The pharmaceutical composition of any one of the claims 32-38, wherein
- the CFP comprises: (a) an extracellular domain comprising: (i) a scFv that specifically binds CD5 or HER2, and (ii) a hinge domain derived from CD8, or CD28 or an extracellular domain of CD68 or a portion thereof; (b) a CD8 transmembrane domain, a CD28 transmembrane domain or a CD68

~~transmembrane domain; and (c) an intracellular domain comprising 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, an (ii) a second intracellular signaling domain that: (A) comprises a PI3-kinase (PI3K) recruitment domain, or (B) is derived from CD40.~~

~~40.5. The pharmaceutical composition of claim 32, wherein the population of human cells are *ex vivo* engineered~~population of human cells are macrophage cells.

~~41. A pharmaceutical~~The composition, comprising:

~~6. (a) a population of human cells comprising a recombinant nucleic acid~~claim 1, wherein the recombinant nucleic acid comprises a sequence encoding a sequence encoding an antigenic peptide wherein: (i):

(a) at least 25~~50~~% of the cells in the *ex vivo* population of human cells are ~~CD14~~CCR2+ and ~~CD16-~~, and (ii) less than ~~25~~/or CCR5+;

(b) at least 50% of the cells in the *ex vivo* population of human cells are ~~dendritic cells;~~ and CD63+;

~~(b) a pharmaceutically acceptable excipient.~~

(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.

~~42. The pharmaceutical composition of claim 41, wherein a CD14+ and CD16- cell of the population of human cells displays a microbial antigen or fragment thereof in association with an MHC class I or MHC class II molecule on the cell surface.~~

~~43.7. The pharmaceutical composition of claim 41 or 42, 1, wherein the recombinant nucleic acid comprises a sequence encoding an antigenic peptide selected from a CMVpp65 peptide, a mutant R132H an isocitrate dehydrogenase 1 (IDH) peptide, a TRP2 peptide, and a SARS-CoV-2 antigenic peptide~~polynucleic acid is an mRNA.

~~8. The pharmaceutical composition of any one of the claims 41-42,~~The composition of claim 1, wherein the recombinant ~~nucleic~~polynucleic acid ~~encodes~~comprises a first sequence encoding a CFP, wherein the CFP comprises:

~~44.(a) an extracellular domain comprising an antigen that is fused to an endosomal targeting sequence, and a second antigen~~binding domain comprising a ~~secretory sequence.~~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

- ~~45. The pharmaceutical composition of any one of the claims 41-44, wherein the antigen is endosomally processed by the CD14+ and CD16+ cell.~~
- ~~46. The composition of claim 1, or the pharmaceutical compositions of any one of the claims 32-45, wherein the cells of the population of human cells are isolated from a biological sample from a subject.~~
- ~~47. The composition of claim 1, or the pharmaceutical compositions of any one of the claims 32-46, wherein the cells are isolated from peripheral blood.~~
- ~~48. The composition of claim 1, or the pharmaceutical compositions of any one of the claims 32-47, wherein the cells are engineered *ex vivo*.~~
- (b) The pharmaceutical composition of any one of the claims 32-48, wherein: (a) 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 ~~25% of the cells in the population of cells are CCR2+ and/or CCR5+;~~ (b) 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. ~~25%~~ The composition of claim 1, wherein the ~~cells in the~~ *ex vivo* population of human cells comprises at least 1×10^7 cells ~~are CD63+;~~ (c).
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-, CD3-, and/or CD19-; and (d) CD120a+ (TNFR1+) or CD120b+ (TNFR2+);
- ~~49. (B) less than 40~~50% of the cells in the population of cells ~~are macrophage cells express~~ one or more of CD64, CD68, CD80, CD86, CD163, CD206, CD200R, CD31, CD71, CLEC9A, CD1C and AXL/SIGLEC6;

- ~~The pharmaceutical composition of any one of the claims 32-49, wherein (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~~
- ~~50. (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 is a population of unpolarized or undifferentiated myeloid cells.~~
- ~~51. The pharmaceutical composition of any one of the claims 32-50, wherein the pharmaceutical composition comprises about 10⁷ cells to about 10¹⁰ cells.~~
- ~~52. The pharmaceutical composition of any one of the claims 32-51, wherein the cells of the composition have been exposed to two or less freeze/thaw cycles.~~
- ~~53. The composition of claim 1, or the pharmaceutical composition of any one of the claims 32-52, wherein the cells have been cultured *ex vivo* for less than 48 hours post isolation or enrichment from a biological sample.~~
- ~~54. The composition of claim 1, or the pharmaceutical composition of any one of the claims 32-52, wherein the viability of cells prior administration is to a greater than 80%, greater than 85%, greater than 90% or greater than 95%.~~
- ~~55. A method of treating a microbial infection in a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition of any one of the claims 41-54.~~
- ~~56. The method of claim 55, further comprising, preparing a CD14⁺ and CD16⁻ cell expressing a microbial antigen prior to administering, comprising: (i) isolating or enriching from a biological sample, a CD14⁺ and CD16⁻ cell, (ii) introducing into the cell from (i) a recombinant nucleic acid comprising a sequence encoding at least one antigen for expression and presentation of the antigen on the surface of the cell.~~
- ~~57. A method of treating a disease or condition in a subject in need thereof, the method comprising administering extent compared to the subject a pharmaceutical composition of any one of the claims 31-40.~~
- ~~58. The method of claim 57, wherein the disease or condition is cancer.~~
- ~~59. The method of claim 57, further comprising, preparing a CD14⁺ and CD16⁻ cell expressing a CFP prior to administering, comprising: (i) isolating or enriching from a biological sample, a CD14⁺ and CD16⁻ cell, (ii) introducing into the cell from (i) a recombinant nucleic acid a corresponding population of cells without the polynucleic acid comprising a sequence encoding the CFP.~~

- ~~60. The method of claim 55 or 57, wherein the introducing into the cell comprises performing an electroporation of the cell with the recombinant nucleic acid.~~
- ~~61. The method of any one of the claims 55-59, wherein the recombinant nucleic acid is an RNA.~~
- ~~62. The method of claim 60, wherein the recombinant nucleic acid is an mRNA.~~
- ~~63. The method of any one of the claims 55-61, wherein the recombinant nucleic acid is associated with one or more lipids in an aqueous mixture prior to incorporation in the cell.~~
- ~~64. The method of any one of the claims 55-62, wherein the CD14⁺/CD16⁻ cells are exposed to two or less freeze/thaw cycles prior to administration.~~
- ~~65. The method of any one of the claims 55-63, wherein the cells in the A pharmaceutical composition have been cultured for less than 48 hours *ex vivo* prior to administration.~~
- ~~66. The method of any one of the claims 55-64, wherein the pharmaceutical treating cancer, comprising the composition is administered to the human subject within 48 hours of incorporating the recombinant nucleic acid cells when cultured *in vitro*, or is frozen for future administering.~~
- ~~67. The method of any one of the claims 55-65, wherein the *ex vivo* population of human cells has been cultured for less than 36 hours, 24 hours, less than 18 hours, less than 12 hours or less than 6 hours *ex vivo* prior to administration.~~
- ~~68. The method of any one of the claims 55-66, wherein the pharmaceutical composition comprises at least 10⁷ cells in the population of cells.~~
- ~~69. The method of any one of the claims 55-67, wherein the pharmaceutical composition comprises at least 10⁸ cells in the population of cells.~~
- ~~70. The method of any one of the claims 55-68, wherein the pharmaceutical composition comprises at least 10⁹ cells in the population of cells.~~
- ~~71. The method of any one of the claims 55-67, wherein the pharmaceutical composition is administered once.~~
- ~~72. The method of any one of the claims 55-70, wherein the pharmaceutical composition is administered more than once.~~
- ~~73. The method of any one of the claims 55-71, wherein the pharmaceutical composition is administered once every two weeks, once every 4 weeks, once every 6 weeks, once every 8 weeks, once every 10 weeks, once every 12 weeks, once every 16 weeks, once every 20 weeks, once every 24 weeks or once annually.~~
- ~~74. The method of any one of the claims 55-72, wherein the cells are autologous to the subject.~~
- ~~75-15. The method of any one of the claims 55-73, wherein the pharmaceutical composition comprise cells that can: (a) differentiate into effector cells in the subject after~~

- administration; (b) infiltrate into a diseased site of the subject after administration or migrate to a diseased site of the subject after administration; or (c) have a life span of at least 5 days in the subject after administration of any one of claims 1-14.
- ~~76.~~ 16. Use of the composition ~~or pharmaceutical composition of any one of the claims 1-54 for preparing cell-based therapeutic of any one of claims 1-14 for the manufacture of a medicament~~ for the treatment of ~~a disease or condition~~ cancer.
- ~~77.~~ ~~The use of claim 76, wherein the disease or condition is a cancer, an infection or an autoimmune disease.~~
- ~~78.~~ ~~Use of the composition or pharmaceutical composition of any one of the claims 1-54 for treating a disease or condition.~~
- ~~79.~~ ~~The use of claim 78, wherein the disease or condition is a cancer, an infection or an autoimmune disease.~~
17. A method ~~of negatively selecting cells~~ for preparing ~~a pharmaceutical~~ the composition of any one of claims 1-14, the method comprising: ~~(a)~~
- (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.
- ~~80.~~ 20. The method of claim 19, wherein the negative selection comprises contacting ~~a~~ the biological sample ~~from a human subject~~ 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 ~~(b)~~ collecting cells in the biological sample that are not bound by the anti-CD16 antibody and not bound by the one or more antibodies, ~~(c) introducing a recombinant nucleic acid comprising a sequence encoding a CFP into cells collected from (b), thereby forming a population of cells, wherein: (i) at least 25% of the cells in the population of cells are CD14+ and CD16, and (ii) less than 25% of the cells in the population of cells are dendritic cells.~~
21. The method of claim ~~80~~, 17, wherein the biological sample is an apheresis sample.
- ~~81.~~ ~~The method comprises flow cytometry or fluorescence activated cell sorting (FACS).~~
22. ~~A vaccine composition comprising a~~ of claim 17, wherein the recombinant polynucleic acid is mRNA and wherein introducing the recombinant polynucleic acid into the population of CD14+/CD16- cells comprising a recombinant nucleic acid cells comprises electroporation.

~~82.~~23. The method of claim 17, wherein the cells in the composition have been cultured ~~*ex vivo*~~ for less than 72 hours or less than 48 hours *ex vivo*.

~~83.~~ The composition of claim 14 or 82, wherein the recombinant nucleic acid comprises an endosomal targeting sequence, the endosomal targeting sequence is a LAMP1 sequence.

~~84.~~ The composition of claim 15 or 82, wherein the recombinant nucleic acid comprises a secretory sequence that is a secretory signal peptide sequence of a Human Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) signaling sequence, a Human Immunoglobulin Heavy Chain signaling sequence, a Human Immunoglobulin Light Chain signaling sequence, a Human Serum Albumin signaling sequence, a Human Azurocidin signaling sequence, or a Human Cystatin.

~~85.~~ A method of treating a disease or condition in a human subject in need thereof, comprising: administering the pharmaceutical composition comprising the composition of any one of the claims 82-84 to the human subject.

24. The composition of any one of claims 1-14 for use in therapy.