

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 a recombinant nucleic acid comprises a sequence encoding a protein or a peptide that is expressed in a cell of the population of CD14+/CD16- cells.
5. The composition of any one of the claims 1-4, wherein the recombinant nucleic acid comprises a sequence encoding a chimeric fusion protein (CFP).
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. The composition of any one of the claims 1-25, wherein at least 50% of the cells in the population of cells are CCR2+ and/or CCR5+.
27. The composition of claim 1-26, wherein at least 50% of the cells in the population of cells are CD63+.
28. The composition of any one of the claims 1-27, wherein at least 50% of the cells in the population of cells are CD56-, CD3-, and/or CD19-.
29. The composition of any one of the claims 1-28, 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. 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. The pharmaceutical composition of claim 32, wherein the population of human cells are *ex vivo* engineered cells.
41. A pharmaceutical composition, comprising:
- (a) a population of human cells comprising a recombinant nucleic acid, wherein the recombinant nucleic acid comprises a sequence encoding an antigenic peptide 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.

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. The pharmaceutical composition of claim 41 or 42, 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.
44. The pharmaceutical composition of any one of the claims 41-42, 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.
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*.
49. The pharmaceutical composition of any one of the claims 32-48, wherein: (a) at least 25% of the cells in the population of cells are CCR2⁺ and/or CCR5⁺; (b) at least 25% of the cells in the population of cells are CD63⁺; (c) at least 50% of the cells in the population of cells are CD56⁻, CD3⁻, and/or CD19⁻; and (d) less than 40% of the cells in the population of cells are macrophage cells.
50. The pharmaceutical composition of any one of the claims 32-49, wherein 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 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 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 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 55-60, wherein the recombinant nucleic acid is an RNA.
62. The method of claim 61, wherein the recombinant nucleic acid is an mRNA.
63. The method of any one of the claims 55-62, 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 55-63, 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 55-64, wherein the cells in the pharmaceutical composition have been cultured for less than 48 hours *ex vivo* prior to administration.
66. The method of any one of the claims 55-65, wherein the pharmaceutical 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 55-66, 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 55-67, wherein the pharmaceutical composition comprises at least 10^7 cells in the population of cells.
69. The method of any one of the claims 55-68, wherein the pharmaceutical composition comprises at least 10^8 cells in the population of cells.

70. The method of any one of the claims 55-69, wherein the pharmaceutical composition comprises at least 10^9 cells in the population of cells.
71. The method of any one of the claims 55-68, wherein the pharmaceutical composition is administered once.
72. The method of any one of the claims 55-71, wherein the pharmaceutical composition is administered more than once.
73. The method of any one of the claims 55-72, 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 55-73, wherein the cells are autologous to the subject.
75. The method of any one of the claims 55-74, 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.
76. Use of the composition or pharmaceutical composition of any one of the claims 1-54 for preparing cell-based therapeutic for the treatment of a disease or condition.
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.
80. A method of negatively selecting cells for preparing a pharmaceutical composition, the method comprising: (a) contacting a biological sample from a human subject with an anti-CD16 antibody and one or more antibodies selected from anti-CD56 antibody, anti-CD3 antibody and 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.
81. The method of claim 80, wherein the method comprises flow cytometry or fluorescence activated cell sorting (FACS).
82. A vaccine composition comprising a population of CD14+/CD16- cells comprising a recombinant nucleic acid, wherein the cells have been cultured *ex vivo* for less than 48 hours.

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 (GMCSF) 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.