CLAIMS

What is claimed is:

1. A nanoparticle comprising:

a targeting ligand that binds to a professional phagocyte; and

a nucleic acid that encodes a protein molecule having at least a first binding domain and a second binding domain,

wherein the first binding domain is specific to a cell surface protein expressed by an immune cell, and

wherein the second binding domain is specific to a cell surface protein expressed by a cancer cell.

2. The nanoparticle of claim 1, wherein the targeting ligand binds to a cell surface protein expressed by a monocyte, a macrophage, or both.
3. The nanoparticle of claim 1, wherein the targeting ligand comprises di-mannose.
4. The nanoparticle of claim 1, wherein the nucleic acid comprises ribonucleic acid (RNA).
5. The nanoparticle of claim 4, wherein the RNA comprises messenger RNA (mRNA).
6. The nanoparticle of claim 5, wherein the mRNA comprises synthetic RNA or in vitro transcribed RNA (IVT RNA).
7. The nanoparticle of claim 1, wherein the first binding domain is specific to a cell surface protein of a lymphocyte.
8. The nanoparticle of claim 7, wherein the lymphocyte is selected from the group consisting of a T-cell, a B-cell, a natural killer (NK) cell, and a tumor-infiltrating lymphocyte (TIL) cell.
9. The nanoparticle of claim 1, wherein the first binding domain is specific to a cell surface protein of a T-cell selected from the group consisting of a CD8+ T cell, CD4+ T cell, a gamma delta T cell, and an NK T-cell.
10. The nanoparticle of claim 9, wherein the first binding domain is specific to CD3.
11. The nanoparticle of claim 1, wherein the protein molecule is a bi-specific T-cell engager.
12. The nanoparticle of claim 11, wherein the protein molecule is an EpCAM-CD3 bi-specific T-cell engager.
13. The nanoparticle of claim 1, wherein the second binding domain is specific to an antigen expressed by the cancer cell.
14. The nanoparticle of claim 1, further comprising a second nucleic acid that encodes one or more interferon regulatory factors (IRFs).
15. The nanoparticle of claim 1, further comprising a tumor cell proliferation inhibitor or a nucleic acid encoding a tumor cell proliferation inhibitor.

16. The nanoparticle of claim 15, wherein the nucleic acid encodes an antibody, or an antigen-binding fragment of an antibody.
17. The nanoparticle of claim 15, wherein the nanoparticle comprises a nucleic acid encoding a CD40-CD40L inhibitor or a TGFβ inhibitor.
18. The nanoparticle of claim 1, wherein the nanoparticle is a liposome, a liposomal nanoparticle, a lipid nanoparticle, or a solid lipid nanoparticle.
19. A composition comprising:

a first plurality of nanoparticles, wherein each of the first plurality of nanoparticles comprises:

a targeting ligand that binds to a professional phagocyte; and

a nucleic acid encoding a protein molecule having a first binding domain specific to a cell surface protein expressed by an immune cell, and a second binding domain is specific to a cell surface protein expressed by a cancer cell.

20. The composition of claim 19, wherein the targeting ligand binds to a cell surface protein expressed by a monocyte, a macrophage, or both.
21. The composition of claim 19, wherein the targeting ligand comprises di-mannose.
22. The composition of claim 19, wherein the nucleic acid comprises RNA.
23. The composition of claim 22, wherein the RNA comprises mRNA.
24. The composition of claim 23, wherein the mRNA comprises synthetic RNA or IVT RNA.
25. The composition of claim 19, wherein the first binding domain is specific to a cell surface protein of a lymphocyte.
26. The composition of claim 25, wherein the lymphocyte is selected from the group consisting of a T-cell, a B-cell, an NK cell, and a TIL cell.
27. The composition of claim 19, wherein the first binding domain is specific to a cell surface protein of a T-cell selected from the group consisting of a CD8+ T cell, CD4+ T cell, a gamma deltaT cell, and an NK T-cell.
28. The composition of claim 27, wherein the first binding domain is specific to CD3.
29. The composition of claim 19, wherein the protein molecule is a bi-specific T-cell engager.
30. The composition of claim 29, wherein the protein molecule is an EpCAM-CD3 bi-specific T-cell engager.
31. The composition of claim 19, wherein the second binding domain is specific to an antigen expressed by the cancer cell.
32. The composition of claim 19, further comprising a pharmaceutically acceptable carrier.
33. The composition of any of claim Nos. 19-32, wherein at least a subset of the first plurality of

nanoparticles further comprises one or more of (a) a nucleic acid encoding one or more interferon regulatory factors (IRFs), and (b) a nucleic acid encoding IKKβ.

34. The composition of any of claim Nos. 19-32, further comprising:

a second plurality of nanoparticles, wherein at least a subset of the second plurality of nanoparticles comprise one or more of (a) a nucleic acid encoding one or more interferon regulatory factors (IRFs), and (b) a nucleic acid encoding IKKβ.

35. The composition of any of claim Nos. 19-34, further comprising a tumor cell proliferation inhibitor.

36. The composition of any of claim Nos. 19-35, wherein at least a subset of the first or second plurality of nanoparticles further comprise a nucleic acid encoding a tumor cell proliferation inhibitor.

37. The composition of any of claim 34, wherein at least a subset of the first or second plurality of nanoparticles further comprise a nucleic acid encoding an antigen-binding fragment of an antibody of a tumor cell proliferation inhibitor.

38. The composition of any of claim Nos. 19 or 34-36, further comprising a third plurality of nanoparticles, wherein at least a subset of the third plurality of nanoparticles comprise a nucleic acid encoding an antigen-binding fragment of an antibody of a tumor cell proliferation inhibitor.

39. The composition of any of claim Nos. 35-38, wherein the tumor cell proliferation inhibitor is a CD40-CD40L inhibitor or a TGFβ inhibitor.

40. The composition of claim 38, comprising the first plurality of nanoparticles and the third plurality of nanoparticles in the absence of the second plurality of nanoparticles.

41. The composition of claim 38, wherein the first, second, and/or third plurality of nanoparticles comprise a liposome, a liposomal nanoparticle, a lipid nanoparticle, or a solid lipid nanoparticle.

42. A composition for treating cancer in a human subject, the composition comprising:

a first plurality of nanoparticles, wherein each of the plurality of nanoparticles comprises

(i) a targeting ligand that binds to a monocyte, macrophage, or both; and

(ii) an mRNA encoding a protein molecule having at least a first binding domain specific to a cell surface protein expressed by a lymphocyte, and a second binding domain specific to a cell surface protein expressed by a cancer cell;

wherein the first plurality of nanoparticles stimulates or enhances an immune response in the human subject, thereby treating cancer.

43. The composition of claim 42, wherein the targeting ligand comprises di-mannose.

44. The composition of claim 42, wherein the mRNA comprises synthetic RNA or IVT RNA.
45. The composition of claim 42, wherein the first binding domain is specific to a cell surface protein of a lymphocyte.
46. The composition of claim 42, wherein the lymphocyte is selected from the group consisting of a T-cell, a B-cell, an NK cell and a TIL cell.
47. The composition of claim 42, wherein the first binding domain is specific to a cell surface protein of a T-cell selected from the group consisting of a CD8+ T cell, CD4+ T cell, a gamma delta T cell, and an NK T-cell.
48. The composition of claim 47, wherein the first binding domain is specific to CD3.
49. The composition of claim 42, wherein the protein molecule is a bi-specific T-cell engager.
50. The composition of claim 49, wherein the protein molecule is an EpCAM-CD3 bi-specific T-cell engager.
51. The composition of claim 42, wherein the second binding domain is specific to an antigen expressed by the cancer cell.
52. The composition of claim 42, further comprising a pharmaceutically acceptable carrier.
53. The composition of any of claim Nos. 42-52, wherein the at least a subset of the first plurality of nanoparticles further comprise one or more of (a) an mRNA encoding one or more interferon regulatory factors (IRFs), (b) an mRNA encoding IKKβ, or (c) an mRNA encoding one or more IRFs and an mRNA encoding IKKβ, and (c) an mRNA encoding a tumor cell proliferation inhibitor.
54. The composition of any of claim Nos. 42-53, further comprising:

a second plurality of nanoparticles, wherein each of the second plurality of nanoparticles comprises

a targeting ligand that binds to a monocyte, a macrophage, or both, and

one or more of (a) an mRNA encoding one or more interferon regulatory factors (IRFs), (b) an mRNA encoding IKKβ, and (c) an mRNA encoding a tumor cell proliferation inhibitor.

55. The composition of claim 54, wherein the second plurality of nanoparticles comprise an mRNA encoding an antigen-binding fragment of an antibody of a tumor cell proliferation inhibitor.
56. The composition of any of claim Nos. 53-55, wherein the tumor cell proliferation inhibitor is a CD40-CD40L inhibitor or a TGFβ inhibitor.
57. The composition of claim 54, wherein the first and/or second plurality of nanoparticles comprise a liposome, a liposomal nanoparticle, a lipid nanoparticle, or a solid lipid nanoparticle.
58. A method for treating cancer in a human subject, the method comprising:

administering to the human subject a composition comprising a first plurality of nanoparticles, wherein each of the first plurality of nanoparticles comprises:

(i) a targeting ligand that binds to a monocyte, a macrophage, or both; and

(ii) an mRNA encoding a protein molecule having at least a first binding domain specific to a cell surface protein expressed by a lymphocyte, and a second binding domain specific to a cell surface protein expressed by a cancer cell;

wherein the plurality of nanoparticles stimulates or enhances an immune response in the human subject, thereby treating cancer.

59. The method of claim 58, wherein the targeting ligand comprises di-mannose.
60. The method of claim 58, wherein the mRNA comprises synthetic RNA or IVT RNA.
61. The method of claim 58, wherein the lymphocyte is selected from the group consisting of a T-cell, a B-cell, an NK cell, and a TIL cell.
62. The method of claim 58, wherein the first binding domain is specific to a cell surface protein of a T-cell selected from the group consisting of a CD8+ T cell, CD4+ T cell, a gamma delta T cell, and an NK T-cell.
63. The method of claim 62, wherein the first binding domain is specific to CD3.
64. The method of claim 58, wherein the protein molecule is a bi-specific T-cell engager.
65. The method of claim 64, wherein the protein molecule is an EpCAM-CD3 bi-specific T-cell engager.
66. The method of claim 58, wherein the second binding domain is specific to an antigen expressed by the cancer cell.
67. The method of claim 58, wherein the composition further comprising a pharmaceutically acceptable carrier.
68. The method of any of claim Nos. 58-67, wherein at least a subset of the first plurality of nanoparticles further comprise one or more of (a) a nucleic acid encoding one or more interferon regulatory factors (IRFs), (b) a nucleic acid encoding IKKβ, and (c) a nucleic acid encoding a tumor cell proliferation inhibitor.
69. The method of any of claim Nos. 58-68, further comprising:

administering to the human subject a composition comprising a second plurality of nanoparticles, wherein each of the second plurality of nanoparticles comprises

a targeting ligand that binds to a monocyte, a macrophage, or both, and

one or more of (a) an mRNA encoding one or more interferon regulatory factors (IRFs), and (b) an mRNA encoding IKKβ.

70. The method of claim 68 or 69, wherein at least a subset of the first or second plurality of

nanoparticles further comprise an mRNA encoding a tumor cell proliferation inhibitor.

71. The method of any of claim Nos. 58-70, further comprising:

administering to the human subject a composition comprising a third plurality of nanoparticles, wherein each of the third plurality of nanoparticles comprises

a targeting ligand that binds to a monocyte, a macrophage, or both, and

an mRNA encoding a tumor cell proliferation inhibitor.

72. The method of claim 70 or 71, wherein, an mRNA encoding a tumor cell proliferation inhibitor encodes an antigen-binding fragment of an antibody of a tumor cell proliferation inhibitor.

73. The method of claim 72, wherein the tumor cell proliferation inhibitor is a CD40-CD40L inhibitor or a TGFβ inhibitor.

74. The composition of claim 71, wherein the first, second, and/or third plurality of nanoparticles comprise a liposome, a liposomal nanoparticle, a lipid nanoparticle, or a solid lipid nanoparticle.

75. The method of claim Nos. 58 or 69, wherein the step of administering a composition comprising the first plurality of nanoparticles and the step of administering a composition comprising the second plurality of nanoparticles are performed concurrently or sequentially.

76. The method of any of claim Nos. 58 or 69, wherein the step of administering a composition comprising the first plurality of nanoparticles is performed after the step of administering a composition comprising the second plurality of nanoparticles.

77. The method of claim 71, wherein the step of administering a composition comprising the third plurality of nanoparticles is performed concurrently or sequentially with the step of administering the first plurality of nanoparticles.

78. The method of claim 71, wherein the step of administering a composition comprising the third plurality of nanoparticles is performed concurrently or sequentially with the step of administering the second plurality of nanoparticles.

79. The method of claim 69, comprising the steps of administering a composition comprising the first plurality of nanoparticles and administering a composition comprising the third plurality of nanoparticles in the absence of the step of administering a composition comprising the second plurality of nanoparticles.

80. A modified professional phagocyte comprising:

a nanoparticle loaded with a nucleic acid encoding a protein molecule having at least a first binding domain specific to a cell surface protein expressed by an immune cell and a second binding domain specific for a cell surface protein expressed by cancer cell,

wherein the nanoparticle is adhered to the surface of the phagocyte or has been internalized by

the phagocyte.

81. The modified professional phagocyte of claim 80, wherein the phagocyte is a monocyte or a macrophage.
82. The modified professional phagocyte of claim 80, where the phagocyte is a tumor-associated macrophage.
83. The modified professional phagocyte of claim 80, wherein the nucleic acid comprises ribonucleic acid (RNA).
84. The modified professional phagocyte of claim 83, wherein the RNA comprises messenger RNA (mRNA).
85. The modified professional phagocyte of claim 84, wherein the mRNA comprises synthetic RNA or in vitro transcribed RNA (IVT RNA).
86. The modified professional phagocyte of claim 80, wherein the first binding domain is specific to a cell surface protein of a lymphocyte.
87. The modified professional phagocyte of claim 86, wherein the lymphocyte is selected from the group consisting of a T-cell, a B-cell, an NK cell, and a TIL cell.
88. The modified professional phagocyte of claim 80, wherein the first binding domain is specific to a cell surface protein of a T-cell selected from the group consisting of a CD8+ T cell, CD4+ T cell, a gamma delta T cell, and an NK T-cell.
89. The modified professional phagocyte of claim 80, wherein the first binding domain is specific to CD3.
90. The modified professional phagocyte of claim 80, wherein the protein molecule is a bi-specific T-cell engager.
91. The modified professional phagocyte of any of claim Nos.80-90, wherein the protein molecule is an EpCAM-CD3 bi-specific T-cell engager.
92. The modified professional phagocyte of any of claim Nos. 80-91, wherein the nanoparticle is further loaded with one or more of (a) a nucleic acid encoding one or more interferon regulatory factors (IRFs), (b) a nucleic acid encoding IKKβ, and (c) a nucleic acid encoding a tumor cell proliferation inhibitor.
93. The modified professional phagocyte of any of claim Nos. 80-92, further comprising:
    a second nanoparticle loaded with one or more of (a) a nucleic acid encoding one or more interferon regulatory factors (IRFs), (b) a nucleic acid encoding IKKβ, and (c) a nucleic acid encoding a tumor cell proliferation inhibitor,

wherein the second nanoparticle is adhered to the surface of the phagocyte or has been internalized by the phagocyte.

94. The modified professional phagocyte of claim No. 92 or 93, wherein the first or second nanoparticle is loaded with a nucleic acid encoding an antibody or an antigen-binding fragment of an antibody of a tumor cell proliferation inhibitor.
95. The modified professional phagocyte of claim 94, wherein the tumor cell proliferation inhibitor is a CD40-CD40L inhibitor or a TGFβ inhibitor.
96. The modified professional phagocyte of claim 80, further comprising at least one of
a second nanoparticle loaded with one or more of (a) a nucleic acid encoding one or more interferon regulatory factors (IRFs), (b) a nucleic acid encoding IKKβ, or (c) a nucleic acid encoding a tumor cell proliferation inhibitor; and
a third nanoparticle loaded with a nucleic acid encoding a tumor cell proliferation inhibitor, wherein each of the second and third nanoparticles is adhered to the surface of the phagocyte or has been internalized by the phagocyte.
97. The modified professional phagocyte of claim 96, wherein the first, second, and/or third nanoparticle comprises a liposome, a liposomal nanoparticle, a lipid nanoparticle, or a solid lipid nanoparticle.
98. A nanoparticle comprising a positively-charged polymer core and a neutral or negatively-charged coating around the polymer core, wherein the positively-charged polymer core encapsulates nucleotides encoding at least one binding domain that binds an immune cell activating epitope and/or at least one binding domain that binds a cancer antigen.
99. The nanoparticle of claim 98, wherein the nanoparticles are <130 nm.
100. The nanoparticle of claim 98, wherein the positively charged polymer comprises poly(β-amino ester, poly(L-lysine), poly(ethylene imine) (PEI), poly-(amidoamine) dendrimers (PAMAMs), poly(amine-co-esters), poly(dimethylaminoethyl methacrylate) (PDMAEMA), chitosan, poly-(L-lactide-co-L-lysine), poly[α-(4-aminobutyl)-L-glycolic acid] (PAGA), or poly(4-hydroxy-L-proline ester) (PHP).
101. The nanoparticle of claim 100, wherein the positively charged polymer comprises poly(β-amino ester).
102. The nanoparticle of claim 98, wherein the neutral or negatively-charged coating comprises polyglutamic acid (PGA), poly(acrylic acid), alginic acid, or cholesteryl hemisuccinate/1,2-dioleoyl-sn-glycero-3-phosphoethanolamine.
103. The nanoparticle of claim 102, wherein the neutral or negatively-charged coating comprises polyglutamic acid (PGA).
104. The nanoparticle of claim 98, wherein the neutral or negatively-charged coating comprises a zwitterionic polymer.

105. The nanoparticle of claim 98, wherein the neutral or negatively-charged coating comprises a liposome.

106. The nanoparticle of claim 105, wherein the liposome comprises 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), 3ß-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol), dioctadecyl-amidoglycylspermine (DOGS), cholesterol, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC).

107. The nanoparticle of claim 98, wherein the nucleotides comprise ribonucleic acid (RNA).

108. The nanoparticle of claim 107, wherein the RNA comprises synthetic RNA.

109. The nanoparticle of claim 107, wherein the RNA comprises in vitro transcribed mRNA.

110. The nanoparticle of claim 98, wherein the nucleotides comprise integrating or non-integrating double-stranded DNA.

111. The nanoparticle of claim 98, wherein the nucleotides are in the form of a plasmid, a minicircle plasmid, or a closed-ended linear ceDNA.

112. The nanoparticle of claim 98, wherein the cancer antigen is expressed by an ovarian cancer cell, a melanoma cell, a glioblastoma cell, a multiple myeloma cell, a melanoma cell, a prostate cancer cell, a breast cancer cell, a stem cell cancer cell, a mesothelioma cell, a renal cell carcinoma cell, a pancreatic cancer cell, a lung cancer cell, a cholangiocarcinoma cell, a bladder cancer cell, a neuroblastoma cell, a colorectal cancer cell, or a merkel cell carcinoma cell.

113. The nanoparticle of claim 98, wherein the cancer antigen comprises B-cell maturation antigen (BCMA), carboxy-anhydrase-IX (CAIX), CD19, CD24, CD56, CD133, CEA, disialoganglioside, EpCam, EGFR, EGFR variant III (EGFRvIII), ERBB2, folate receptor (FOLR), GD2, glypican-2, HER2, Lewis Y, L1-CAM, mesothelin, MUC16, PD-L1, PSMA, Prostate Stem Cell antigen (PSCA), ROR1, TYRP1/gp75, SV40 T, or WT-1.

114. The nanoparticle of claim 98, wherein the binding domain that binds the cancer antigen comprises the complementarity determining regions (CDRs) of antibody adecatumumab, anetumab, ravtansine, amatuximab, HN1, oregovomab, ovarex, abagovomab, edrecolomab, farletuzumab. flanvotumab, TA99, 20D7, Cetuximab, FMC63, SJ25C1, HD37, R11, R12, 2A2, Y31, 4D5, 3G10 atezolizumab, avelumab, or durvalumab.

115. The nanoparticle of claim 98, wherein the binding domains that binds a cancer antigen is a protein molecule.

116. The nanoparticle of claim 115, wherein the different protein molecules within the nanoparticle comprise binding domains that bind different cancer antigens.

117. The nanoparticle of claim 116, wherein the different cancer antigens are expressed by the same cancer type.

118. The nanoparticle of claim 117, wherein the cancer type is ovarian cancer, melanoma, or glioblastoma.

119. The nanoparticle of claim 116, wherein the different cancer antigens comprise

at least two cancer antigens selected from EpCam, L1-CAM, MUC16, folate receptor (FOLR), Lewis Y, ROR1, mesothelin, WT-1, PD-L1, EGFR, and CD56;

at least two cancer antigens selected from Tyrosinase related protein 1 (TYRP1/gp75); GD2, PD-L1, and EGFR; or two cancer antigens selected from EGFR variant III (EGFRvIII) and IL13Ra2.

120. The nanoparticle of claim 98, wherein the at least one binding domain of the protein molecule binds an immune cell activating epitope expressed by a T cell or a natural killer (NK) cell.

121. The nanoparticle of claim 120, wherein the immune cell activating epitope is expressed by a T cell.

122. The nanoparticle of claim 121, wherein the immune cell activating epitope expressed by the T cell comprises CD2, CD3, CD7, CD8, CD27, CD28, CD30, CD40, CD83, 4-1BB, OX40, lymphocyte function-associated antigen-1 (LFA-1), LIGHT, NKG2C, or B7-H3.

123. The nanoparticle of claim 122, wherein the immune cell activating epitope expressed by the T cell comprises CD3, CD28, or 4-1BB.

124. The nanoparticle of claim 98, wherein the binding domains that bind an immune cell activating epitope comprise a protein molecule.

125. The nanoparticle of claim 124, wherein the different protein molecules within the nanoparticle comprise binding domains that bind different immune cell activating epitopes.

126. The nanoparticle of claim 125, wherein the different immune cell activating epitopes comprise CD3 and CD28 or CD3 and 4-1BB.

127. The nanoparticle of claim 126, wherein at least one binding domain comprises the CDRs of antibody OKT3, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, TGN1412, 9D7, 9.3, KOLT-2, 15E8, 248.23.2, EX5.3D10, OKT8 or the SK1.

128. The nanoparticle of claim 120, wherein the immune cell activating epitope is expressed by a NK cell.

129. The nanoparticle of claim 128, wherein the immune cell activating epitope expressed by the NK cell comprises NKG2D, CD8, CD16, KIR2DL4, KIR2DS1, KIR2DS2, KIR3DS1, NKG2C, NKG2E, NKG2D, NKp30, NKp44, NKp46, NKp80, or DNAM-1.

130. The nanoparticle of claim 129, wherein at least one binding domain comprises the CDRs of antibody 5C6, 1D11, mAb 33, P44-8, SK1, or 3G8.

131. The nanoparticle of claim 98, wherein the binding domains are linked through a protein linker.

132. The nanoparticle of claim 131, wherein the protein linker comprises a Gly-Ser linker.

133. The nanoparticle of claim 131, wherein the protein linker comprises a proline-rich linker.

134. The nanoparticle of claim 124, wherein the protein molecule comprises a single chain variable fragment (scFv).

135. The nanoparticle of claim 124, wherein the protein molecule comprises

at least one binding domain binds CEA and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds EGFR and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds EpCam and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds HER2 and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds PD-L1 and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds PSMA and at least one binding domain binds CD3, CD28, or 4-1BB; or

at least one binding domain binds [TYRP1/gp75] and at least one binding domain binds CD3, CD28, or 4-1BB.

136. The nanoparticle of claim 135, wherein the protein molecule comprises catumaxomab, MT110, ertumaxomab, MDX-447, MM-141, AMG211, RO6958688, RO6895882, TF2, BAY2010112, AMG701, solitomab, or blinatumomab.

137. A nanoparticle of claim 98, wherein the positively-charged polymer core further encapsulates nucleotides encoding one or more interferon regulatory factors (IRFs).

138. The nanoparticle of claim 137, wherein the one or more IRFs lack a functional autoinhibitory domain.

139. The nanoparticle of claim 137, wherein the one or more IRFs lack a functional nuclear export signal.

140. The nanoparticle of claim 137, wherein the one or more IRFs are selected from IRF1, IRF3, IRF5, IRF7, IRF8, and/or a fusion of IRF7 and IRF3.

141. The nanoparticle of claim 137, wherein the one or more IRFs are selected from a sequence having >90%, >95%, or greater than 98% identity to a sequence as set forth in SEQ ID NOs: 1-17.

142. The nanoparticle of claim 137, wherein the one or more IRFs comprise IRF5 selected from a sequence as set forth in SEQ ID NOs: 1-7.

143. The nanoparticle of claim 142, wherein the IRF5 comprises a sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3 with one or more mutations selected from S156D, S158D and T160D.

144. The nanoparticle of claim 142, wherein the IRF5 comprises a sequence as set forth in SEQ ID NO: 2 with one or more mutations selected from T10D, S158D, S309D, S317D, S451D, and S462D.

145. The nanoparticle of claim 142, wherein the IRF5 comprises a sequence as set forth in SEQ ID NO: 4 with one or more mutations selected from S425D, S427D, S430D, and S436D.

146. The nanoparticle of claim 137, wherein the one or more IRFs comprise IRF1 comprising a sequence as set forth in SEQ ID NOs: 8 or 12.

147. The nanoparticle of claim 137, wherein the one or more IRFs comprise IRF8 comprising a sequence as set forth in SEQ ID NOs: 11, 16, or 17.

148. The nanoparticle of claim 147, wherein the IRF8 comprises a sequence as set forth in SEQ ID NO: 11 with a K310R mutation.

149. The nanoparticle of claim 137, wherein the one or more IRFs comprise an IRF7/IRF3 fusion protein comprising an N-terminal IRF7 DNA binding domain, a constitutively active domain, and a C-terminal IRF3 nuclear export signal.

150. The nanoparticle of claim 149, wherein the IRF7/IRF3 fusion protein comprises a sequence as set forth in SEQ ID NO: 15.

151. The nanoparticle of claim 137, wherein the one or more IRFs comprise IRF4.

152. The nanoparticle of claim 137, wherein at least a subset of the nanoparticles comprise nucleotides encoding IKKβ.

153. The nanoparticle of claim 152, wherein the IKKβ is selected from a sequence having >90%, >95%, or >98% identity to a sequence as set forth in a sequence selected from SEQ ID NOs: 18-22.

154. The nanoparticle of claim 152, wherein the IKKβ comprises a sequence as set forth in a sequence selected from SEQ ID NOs: 18-22.

155. The nanoparticle of claim 152, wherein the nucleotides comprise a sequence as set forth in a sequence selected from SEQ ID NOs: 23-44.

156. The nanoparticle of claim 152, wherein the nucleotides encoding one or more IRFs and the nucleotides encoding IKKβ are encapsulated within the same nanoparticle.

157. The nanoparticle of claim 137, wherein the nucleotides encoding one or more IRFs and the nucleotides encoding IKKβ are encapsulated within the same nanoparticle core.

158. The nanoparticle of claim 137, wherein the nucleotides encoding one or more IRFs and the nucleotides encoding IKKβ are encapsulated in different nanoparticles.

159. The nanoparticle of claim 137, wherein the nucleotides encoding at least one or more binding domains are encapsulated within the same nanoparticle as the nucleotides encoding one or more IRFs and/or IKKβ.

160. The nanoparticle of claim 137, wherein the nucleotides encoding at least one or more binding domains are encapsulated within different nanoparticles than those encapsulating nucleotides encoding one or more IRFs and/or IKKβ.

161. The nanoparticle of claim 98, further comprising a transforming growth factor beta (TGFβ) inhibitor.

162. The nanoparticle of claim 161, wherein the TGFβ inhibitor comprises nucleotides encoding the TGFβ inhibitor.

163. The nanoparticle of claim 161, wherein the TGFβ inhibitor comprises the CDRs of an antibody that suppresses the activity of TGFβ.

164. The nanoparticle of claim 161, wherein the TGFβ inhibitor comprises an antibody that suppresses the activity of TGFβ.

165. The nanoparticle of claim 163 or 164, wherein the antibody comprises trabedersen, disitertide, metelimumab, fresolimumab, LY2382770, SIX-100, avotermin, and/or IMC-TR1.

166. The nanoparticle of claim 98, wherein the nanoparticles further comprise nucleotides encoding glucocorticoid-induced leucine zipper (GILZ).

167. The nanoparticle of claim 98, wherein the nanoparticles further comprise nucleotides comprising an anticancer gene selected from p53, RB, BRCA1, E1A, bcl-2, MDR-1, p21, p16, bax, bcl-xs, E2F, IGF-I, VEGF, angiostatin, oncostatin, endostatin, GM-CSF, IL-12, IL-2, IL-4, IL-7, IFN-γ, TNFα and/or HSV-tk.

168. A system comprising:

nanoparticles

wherein at least a subset of the nanoparticles comprise nucleotides encoding one or more interferon regulatory factors (IRFs) and

wherein at least a subset of the nanoparticles comprise nucleotides encoding a protein molecule having at least two binding domains

wherein one binding domain binds an antigen expressed by a cancer cell at a tumor site and

wherein one binding domain binds an immune cell activating epitope.

169. The system of claim 168, wherein the nanoparticles are <130 nm.

170. The system of claim 168, wherein the nanoparticles comprise a positively-charged core and a neutrally or negatively-charged coating on the outer surface of the core.

171. The system of claim 170, wherein the positively-charged core comprises a positively-charged lipid and/or a positively-charged polymer.

172. The system of claim 171, wherein the positively charged polymer comprises poly(β-amino ester, poly(L-lysine), poly(ethylene imine) (PEI), poly-(amidoamine) dendrimers (PAMAMs), poly(amine-co-esters), poly(dimethylaminoethyl methacrylate) (PDMAEMA), chitosan, poly-(L-lactide-co-L-lysine), poly[α-(4-aminobutyl)-L-glycolic acid] (PAGA), or poly(4-hydroxy-L-proline ester) (PHP).

173. The system of claim 172, wherein the positively charged polymer comprises poly(β-amino ester).

174. The system of claim 170, wherein the neutral or negatively-charged coating comprises polyglutamic acid (PGA), poly(acrylic acid), alginic acid, or cholesteryl hemisuccinate/1,2-dioleoyl-sn-glycero-3-phosphoethanolamine.

175. The system of claim 174, wherein the neutral or negatively-charged coating comprises polyglutamic acid (PGA).

176. The system of claim 170, wherein the neutral or negatively-charged coating comprises a zwitterionic polymer.

177. The system of claim 170, wherein the neutral or negatively-charged coating comprises a liposome.

178. The system of claim 177, wherein the liposome comprises 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), 3ß-[N-(N',N'-dimethylaminoethane)-carbamoyl]cholesterol (DC-Chol), dioctadecyl-amidoglycylspermine (DOGS), cholesterol, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC).

179. The system of claim 168, wherein the nucleotides comprise ribonucleic acid (RNA).

180. The system of claim 179, wherein the RNA comprises synthetic RNA.

181. The system of claim 179, wherein the RNA comprises in vitro transcribed mRNA.

182. The system of claim 168, wherein the nucleotides comprise integrating or non-integrating double-stranded DNA.

183. The system of claim 168, wherein the nucleotides are in the form of a plasmid, a minicircle plasmid, or a closed-ended linear ceDNA.

184. The system of claim 168, wherein the nucleotides are encapsulated within the positively-charged core.

185. The system of claim 168, wherein the one or more IRFs lack a functional autoinhibitory domain.

186. The system of claim 168, wherein the one or more IRFs lack a functional nuclear export signal.

187. The system of claim 168, wherein the one or more IRFs are selected from IRF1, IRF3, IRF5, IRF7, IRF8, and/or a fusion of IRF7 and IRF3.

188. The system of claim 168, wherein the one or more IRFs are selected from a sequence having >90%, >95%, or greater than 98% identity to a sequence as set forth in SEQ ID NOs: 1-17.

189. The system of claim 168, wherein the one or more IRFs comprise IRF5 selected from a sequence as set forth in SEQ ID NOs: 1-7.

190. The system of claim 189, wherein the IRF5 comprises a sequence as set forth in SEQ ID NO: 1 or SEQ ID NO: 3 with one or more mutations selected from S156D, S158D and T160D.

191. The system of claim 189, wherein the IRF5 comprises a sequence as set forth in SEQ ID NO: 2 with one or more mutations selected from T10D, S158D, S309D, S317D, S451D, and S462D.

192. The system of claim 189, wherein the IRF5 comprises a sequence as set forth in SEQ ID NO: 4 with one or more mutations selected from S425D, S427D, S430D, and S436D.

193. The system of claim 168, wherein the one or more IRFs comprise IRF1 comprising a sequence as set forth in SEQ ID NOs: 8 or 12.

194. The system of claim 168, wherein the one or more IRFs comprise IRF8 comprising a sequence as set forth in SEQ ID NOs: 11,16, or 17.

195. The system of claim 194, wherein the IRF8 comprises a sequence as set forth in SEQ ID NO: 11 with a K310R mutation.

196. The system of claim 168, wherein the one or more IRFs comprise an IRF7/IRF3 fusion protein comprising an N-terminal IRF7 DNA binding domain, a constitutively active domain, and a C-terminal IRF3 nuclear export signal.

197. The system of claim 196, wherein the IRF7/IRF3 fusion protein comprises a sequence as set forth in SEQ ID NO: 15.

198. The system of claim 168, wherein the one or more IRFs comprise IRF4.

199. The system of claim 168, wherein at least a subset of the nanoparticles comprise nucleotides encoding IKKβ.

200. The system of claim 199, wherein the IKKβ is selected from a sequence having >90%, >95%, or >98% identity to a sequence as set forth in a sequence selected from SEQ ID NOs: 18-22.

201. The system of claim 199, wherein the IKKβ comprises a sequence as set forth in a sequence selected from SEQ ID NOs: 18-22.

202. The system of claim 168, wherein the nucleotides comprise a sequence as set forth in a sequence selected from SEQ ID NOs: 23-44.

203. The system of claim 168, wherein the nucleotides encoding one or more IRFs and the nucleotides encoding IKKβ are encapsulated within the same nanoparticle.

204. The system of claim 199, wherein the nucleotides encoding one or more IRFs and the nucleotides encoding IKKβ are encapsulated within the same nanoparticle core.

205. The system of claim 168, wherein the nucleotides encoding one or more IRFs and the nucleotides encoding IKKβ are encapsulated in different nanoparticles.

206. The system of claim 168, wherein at least one binding domain of the protein molecule binds a cancer antigen expressed by an ovarian cancer cell, a melanoma cell, a glioblastoma cell, a multiple myeloma cell, a melanoma cell, a prostate cancer cell, a breast cancer cell, a stem cell cancer cell, a mesothelioma cell, a renal cell carcinoma cell, a pancreatic cancer cell, a lung cancer cell, a cholangiocarcinoma cell, a bladder cancer cell, a neuroblastoma cell, a colorectal cancer cell, or a merkel cell carcinoma cell.

207. The system of claim 206, wherein the cancer antigen comprises B-cell maturation antigen (BCMA), carboxy-anhydrase-IX (CAIX), CD19, CD24, CD56, CD133, CEA, disialoganglioside, EpCam, EGFR, EGFR variant III (EGFRvIII), ERBB2, folate receptor (FOLR), GD2, glypican-2, HER2, Lewis Y, L1-CAM, mesothelin, MUC16, PD-L1, PSMA, Prostate Stem Cell antigen (PSCA), ROR1, TYRP1/gp75, SV40 T, or WT-1.

208. The system of claim 168, wherein at least one binding domain of the protein molecule comprises the complementarity determining regions (CDRs) of antibody adecatumumab, anetumab, ravtansine, amatuximab, HN1, oregovomab, ovarex, abagovomab, edrecolomab, farletuzumab. flanvotumab, TA99, 20D7, Cetuximab, FMC63, SJ25C1, HD37, R11, R12, 2A2, Y31, 4D5, 3G10 atezolizumab, avelumab, or durvalumab.

209. The system of claim 168, wherein different protein molecules within the system comprise binding domains that bind different cancer antigens.

210. The system of claim 209, wherein the different cancer antigens are expressed by the same

cancer type.

211. The system of claim 210, wherein the cancer type is ovarian cancer, melanoma, or glioblastoma.

212. The system of claim 209, wherein the different cancer antigens comprise

at least two cancer antigens selected from EpCam, L1-CAM, MUC16, folate receptor (FOLR), Lewis Y, ROR1, mesothelin, WT-1, PD-L1, EGFR, and CD56;

at least two cancer antigens selected from Tyrosinase related protein 1 (TYRP1/gp75); GD2, PD-L1, and EGFR; or

two cancer antigens selected from EGFR variant III (EGFRvIII) and IL13Ra2.

213. The system of claim 168, wherein at least one binding domain of the protein molecule binds an immune cell activating epitope expressed by a T cell or a natural killer cell.

214. The system of claim 213, wherein the immune cell activating epitope is expressed by a T cell.

215. The system of claim 214, wherein the immune cell activating epitope expressed by the T cell comprises CD2, CD3, CD7, CD8, CD27, CD28, CD30, CD40, CD83, 4-1BB, OX40, lymphocyte function-associated antigen-1 (LFA-1), LIGHT, NKG2C, or B7-H3.

216. The system of claim 215, wherein the immune cell activating epitope expressed by the T cell comprises CD3, CD28, or 4-1BB.

217. The system of claim 168, wherein different protein molecules within the system comprise binding domains that bind different immune cell activating epitopes.

218. The system of claim 217, wherein the different immune cell activating epitopes comprise CD3 and CD28 or CD3 and 4-1BB.

219. The system of claim 218, wherein at least one binding domain comprises the CDRs of antibody OKT3, 20G6-F3, 4B4-D7, 4E7-C9, 18F5-H10, TGN1412, 9D7, 9.3, KOLT-2, 15E8, 248.23.2, EX5.3D10, OKT8 or the SK1.

220. The system of claim 213, wherein the immune cell activating epitope is expressed by a NK cell.

221. The system of claim 220, wherein the immune cell activating epitope expressed by the NK cell comprises NKG2D, CD8, CD16, KIR2DL4, KIR2DS1, KIR2DS2, KIR3DS1, NKG2C, NKG2E, NKG2D, NKp30, NKp44, NKp46, NKp80, or DNAM-1.

222. The system of claim 221, wherein at least one binding domain comprises the CDRs of antibody 5C6, 1D11, mAb 33, P44-8, SK1, or 3G8.

223. The system of claim 168, wherein the binding domains of the protein molecule are linked through a protein linker.

224. The system of claim 223, wherein the protein linker comprises a Gly-Ser linker.

225. The system of claim 223, wherein the protein linker comprises a proline-rich linker.

226. The system of claim 168, wherein the protein molecule comprises a single chain variable fragment (scFv).

227. The system of claim 168, wherein the protein molecule comprises

at least one binding domain binds CEA and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds EGFR and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds EpCam and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds HER2 and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds PD-L1 and at least one binding domain binds CD3, CD28, or 4-1BB;

at least one binding domain binds PSMA and at least one binding domain binds CD3, CD28, or 4-1BB; or

at least one binding domain binds [TYRP1/gp75] and at least one binding domain binds CD3, CD28, or 4-1BB.

228. The system of claim 227, wherein the protein molecule comprises catumaxomab, MT110, ertumaxomab, MDX-447, MM-141, AMG211, RO6958688, RO6895882, TF2, BAY2010112, AMG701, solitomab, or blinatumomab.

229. The system of claim 168, wherein the nucleotides encoding at least two binding domains are encapsulated within the same nanoparticle as the nucleotides encoding one or more IRFs and/or IKKβ.

230. The system of claim 168, wherein the nucleotides encoding at least two binding domains are encapsulated within the same nanoparticle core as the nucleotides encoding one or more IRFs and/or IKKβ.

231. The system of claim 168, wherein the nucleotides encoding at least two binding domains are encapsulated within different nanoparticles than those encapsulating nucleotides encoding one or more IRFs and/or IKKβ.

232. The system of claim 168, further comprising a transforming growth factor beta (TGFβ) inhibitor.

233. The system of claim 232, wherein the TGFβ inhibitor comprises nucleotides encoding the

TGFβ inhibitor.

234. The system of claim 232, wherein the TGFβ inhibitor comprises the CDRs of an antibody that suppresses the activity of TGFβ.

235. The system of claim 232, wherein the TGFβ inhibitor comprises an antibody that suppresses the activity of TGFβ.

236. The system of claim 234 or 235, wherein the antibody comprises trabedersen, disitertide, metelimumab, fresolimumab, LY2382770, SIX-100, avotermin, and/or IMC-TR1.

237. The system of claim 168, wherein the nanoparticles further comprise nucleotides encoding glucocorticoid-induced leucine zipper (GILZ).

238. The system of claim 168, wherein the nanoparticles further comprise nucleotides comprising an anticancer gene selected from p53, RB, BRCA1, E1A, bcl-2, MDR-1, p21, p16, bax, bcl-xs, E2F, IGF-I VEGF, angiostatin, oncostatin, endostatin, GM-CSF, IL-12, IL-2, IL-4, IL-7, IFN-γ, TNFα and/or HSV-tk.

239. The system of claim 168, further comprising a pharmaceutically acceptable carrier.

240. A monocyte or macrophage genetically modified to express the nucleotides of a system of claim 168.

241. A method of modulating the macrophage activation state at a tumor site within a subject, recruiting immune cells to the tumor site, and activating the recruited immune cells comprising: Administering the system of claim 168 to the subject, thereby modulating the macrophage activation state at the tumor site within the subject, recruiting immune cells to the tumor site, and activating the recruited immune cells.

242. The method of claim 241, wherein the administering comprises intravenous administering and the nanoparticles are taken up by monocytes within the blood stream.

243. The method of claim 242, wherein the monocytes migrate to the tumor site and differentiate into macrophages.

244. The method of claim 243, wherein the differentiated macrophages are resistant to tumor suppression.

245. The method of claim 241, wherein the administering comprises locally administering at the tumor site and the nanoparticles are taken up by tumor-associated macrophages (TAM).

246. The method of claim 245, wherein the local administering comprises intraperitoneally administering or intracranially administering.

247. The method of claim 245, wherein the TAM undergo a phenotype transformation from a suppressed to an activated state.

248. The method of claim 245, wherein the tumor site comprises an ovarian cancer tumor site,

a glioblastoma tumor site, or a melanoma cancer tumor site.

249. The method of claim 241, wherein the recruited and activated immune cells are T cells or NK cells.

250. The method of claim 241, comprising administering nanoparticles comprising nucleotides encoding one or more IRFs before administering nanoparticles comprising nucleotides encoding at least two binding domains.

251. The method of claim 241, comprising administering nanoparticles comprising nucleic acids encoding one or more IRFs at least 24 hours before administering nanoparticles comprising nucleotides encoding at least two binding domains.

CLAIMS

1. A nanoparticle comprising:

a targeting ligand that binds to a professional phagocyte; and

a nucleic acid that encodes a protein molecule having at least a first binding domain and a second binding domain,

wherein the first binding domain is specific to a cell surface protein expressed by an immune cell, and

wherein the second binding domain is specific to a cell surface protein expressed by a cancer cell.

2. The nanoparticle of claim 1, wherein the targeting ligand binds to a cell surface protein expressed by a monocyte, a macrophage, or both.
3. The nanoparticle of claim 1, wherein the nucleic acid comprises ribonucleic acid (RNA).
4. The nanoparticle of claim 1, wherein the first binding domain is specific to a cell surface protein of a lymphocyte.
5. The nanoparticle of claim 1, wherein the first binding domain is specific to a cell surface protein of a T-cell selected from the group consisting of a CD8+ T cell, CD4+ T cell, a gamma delta T cell, and an NK T-cell.
6. The nanoparticle of claim 1, wherein the protein molecule is a bi-specific T-cell engager.
7. The nanoparticle of claim 1, wherein the second binding domain is specific to an antigen expressed by the cancer cell.
8. The nanoparticle of claim 1, further comprising a second nucleic acid that encodes one or more interferon regulatory factors (IRFs).
9. The nanoparticle of claim 1, further comprising a tumor cell proliferation inhibitor or a nucleic acid encoding a tumor cell proliferation inhibitor.
10. The nanoparticle of claim 1, wherein the nanoparticle is a liposome, a liposomal nanoparticle, a lipid nanoparticle, or a solid lipid nanoparticle.
11. A composition comprising:

at least one nanoparticle of any one of claim 1 to claim 10.

12. A composition for use in treating cancer in a human subject, the composition comprising:

a first plurality of nanoparticles comprising at least one nanoparticle of any one of claim 1 to claim 10,

wherein the first plurality of nanoparticles stimulates or enhances an immune response in the human subject, thereby treating cancer.

13. Use of a composition comprising the nanoparticle of any one of claims 1-10 in the preparation

of a medicament for treating cancer in a human subject.

14. The use of claim 13, further comprising:

a composition comprising a second plurality of nanoparticles to be administered to the human subject, wherein each of the second plurality of nanoparticles comprises

a targeting ligand that binds to a monocyte, a macrophage, or both, and

one or more of (a) an mRNA encoding one or more interferon regulatory factors (IRFs), and (b) an mRNA encoding IKKβ.

15. The use of claim 14, wherein at least a subset of the first or second plurality of nanoparticles further comprise an mRNA encoding a tumor cell proliferation inhibitor.

16. The use of any of claim 14, further comprising:

a composition comprising a third plurality of nanoparticles to be administered to the human subject, wherein each of the third plurality of nanoparticles comprises

a targeting ligand that binds to a monocyte, a macrophage, or both, and

an mRNA encoding a tumor cell proliferation inhibitor.

17. A modified professional phagocyte comprising:

a nanoparticle loaded with a nucleic acid encoding a protein molecule having at least a first binding domain specific to a cell surface protein expressed by an immune cell and a second binding domain specific for a cell surface protein expressed by cancer cell,

wherein the nanoparticle is adhered to the surface of the phagocyte or has been internalized by the phagocyte.

18. The modified professional phagocyte of claim 17, further comprising at least one of

a second nanoparticle loaded with one or more of (a) a nucleic acid encoding one or more interferon regulatory factors (IRFs), (b) a nucleic acid encoding IKKβ, or (c) a nucleic acid encoding a tumor cell proliferation inhibitor; and

a third nanoparticle loaded with a nucleic acid encoding a tumor cell proliferation inhibitor, wherein each of the second and third nanoparticles is adhered to the surface of the phagocyte or has been internalized by the phagocyte.

19. A nanoparticle comprising a positively-charged polymer core and a neutral or negatively-charged coating around the polymer core wherein the positively-charged polymer core encapsulates nucleotides encoding at least one binding domain that binds an immune cell activating epitope and/or at least one binding domain that binds a cancer antigen.

20. A system comprising:

nanoparticles

wherein at least a subset of the nanoparticles comprise nucleotides encoding one

or more interferon regulatory factors (IRFs) and

wherein at least a subset of the nanoparticles comprise nucleotides encoding a protein molecule having at least two binding domains

wherein one binding domain binds an antigen expressed by a cancer cell at a tumor site and

wherein one binding domain binds an immune cell activating epitope.

21. A monocyte or macrophage genetically modified to express the nucleotides of a system of claim 20.
22. Use of the system of claim 20 in the preparation of a medicament for modulating the macrophage activation state at a tumor site within a subject, comprising recruiting immune cells to the tumor site, and activating the recruited immune cells comprising:

the system to be administered to the subject, thereby modulating the macrophage activation state at the tumor site within the subject, recruiting immune cells to the tumor site, and activating the recruited immune cells.

23. The use of claim 22, wherein the medicament is for treating cancer in a human subject.