suggested herein that combination therapies using IMPs and cancer therapeutics that can target both tumor cells and overcome the immunosuppressive tumor microenvironment by targeting MDSCs, TAMs, neutrophils, other monocyte-derived cells, and the tumor-associated stroma may be able to provide enhanced therapeutic benefit compared to monotherapy with cancer therapeutics, such as immune checkpoint inhibitors.

**[0008]** In various examples, the disclosure provides a method of treating cancer in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering alters the population of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), neutrophils, monocytes, dendritic cells, and/or stroma at the tumor site.

**[0008a]** In one aspect, the present disclosure refers to use of negatively charged particles in the manufacture of a medicament for use in combinatorial therapy for treating cancer or a proliferative disease in a subject, wherein the particle is free from attached peptide or antigenic moieties or other bioactive agents, and wherein the medicament is to be administered in combination with a cancer therapeutic.

**[0009]** In various examples, the disclosure provides a method of treating cancer in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering alters the tumor-associated stroma.

**[0010]** In various examples, the disclosure provides a method of treating cancer in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering alters stromal connective tissue, fibroblasts, endothelium, adipose tissue, extracellular matrix, pericytes, cancer stem cells, mesenchymal stem cells, and/or mesenchymal stromal cells.

**[0011]** In various examples, the disclosure provides a method of treating cancer in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering reduces tumor size and/or tumor growth. In various examples, the administering induces tumor cell death, apoptosis, and/or necrosis via direct particle uptake by tumor cells.

**[0012]** In various examples, the disclosure provides a method of treating cancer in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering regulates the anti-tumor immune response.

**[0013]** In various examples, the disclosure provides a method of treating cancer in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering alters or modulates the tumor-specific immune response.

**[0014]** In various examples, the disclosure provides a method of treating a proliferative disease in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering alters the population of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), dendritic cells, neutrophils and/or monocytes at the tumor site.

**[0015]** In various examples, the disclosure provides a method of treating a proliferative disease in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering alters the tumor-associated stroma.

**[0016]** In various examples, the disclosure provides a method of treating a proliferative disease in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering alters stromal connective tissue, fibroblasts, endothelium, adipose tissue, extracellular matrix, pericytes, mesenchymal stem cells, and/or mesenchymal stromal cells.

**[0017]** In various examples, the disclosure provides a method of treating a proliferative disease in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering reduces tumor size and/or tumor growth.

**[0018]** In various examples, the disclosure provides a method of treating a proliferative disease in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering regulates an anti-tumor immune response.

**[0019]** In various examples, the disclosure provides a method of treating a proliferative disease in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering regulates a tumor-specific immune response.

**[0020]** In various examples, the disclosure provides a method of treating a cancer or a proliferative disorder in a subject comprising administering to the subject negatively charged particles in combination with a cancer therapeutic, wherein said particle is free from attached peptide or antigenic moieties or other bioactive agents and wherein the administering regulates cancer stem cells.

**[0021]** In various examples, the negatively charged particles are polyglycolic acid (PGA) particles, polylactic acid (PLA) particles, polystyrene particles, poly (lactic-co-glycolic acid) (PLGA) particles, diamond particles, or iron, zinc, cadmium, gold, or silver particles, or combinations thereof.

**[0022]** In some examples, the negatively charged particles are poly (lactic-co-glycolic acid) (PLGA) particles. In various examples, the particle comprises about 50:50, about 80:20 to about 100:0 polylactic acid : polyglycolic acid or from about 50:50, about 80:20 to about 100:0 polyglycolic acid: polylactic acid. In various examples, the particle comprises 50:50 polylactic acid: polyglycolic acid. In various examples, the particle comprises polylactic acid : polyglycolic acid from about 99:1 to about 1:99, including all values and ranges that lie in between these values.

**[0023]** In various examples the particles are surface functionalized. In various examples, surface functionalization is achieved by carboxylation. In further examples, surface functionalization is achieved by the addition of targeting agents. In some examples, the targeting agent comprises polypeptides, antibodies, nucleic acids, lipids, small-molecules, carbohydrates, and surfactants. In various examples, surface functionalized nanoparticles are targeted preferentially to monocytes, neutrophils, macrophages, dendritic cells, T-cells, B-cells, NK cells, NK T-cells, fibroblasts, cancer associated fibroblasts, endothelial cells, adipocytes, pericytes, endothelium, vasculature, lymphatic vessels, tumor-associated vasculature, mesenchymal stromal cells, mesenchymal stem cells, and/or extracellular matrix.

**[0024]** In various examples, the particles have a zeta potential between -100 mV and -1 mV. In various examples, the particles have a zeta potential between -80 mV and -30 mV. In some examples, the zeta potential of the particle is from about -100 mV to about -40 mV,

from about -75 mV to about -40 mV, from about -70 mV to about -30 mV, from about -60 mV to about -35 mV, or from about -50 mV to about -40 mV. In various examples, the zeta potential is about -30 mV, -35 mV, -40 mV, -45 mV, -50 mV, -55 mV, -60 mV, -65 mV, -70 mV, -75 mV, -80 mV, -85 mV, -90 mV, -95 mV or -100 mV, including all values and subranges that lie between these values.

**[0025]** In various examples, the diameter of the negatively charged particle is between 0.1 μm to 10 μm. In various examples, the particle has an average diameter of between about 0.2 μm and about 2 μm; between about 0.3 μm to about 5 μm; between about 0.5 μm to about 3 μm; or between about 0.5 μm to about 1 μm. In some examples, the particle has a diameter of about 100 to 1500 nm, about 200 to 2000 nm, about 100 to 10000 nm, about 300 to 1000 nm, about 400 to 800 nm, or about 200 to 700 nm. In various examples, the particle has an average diameter of about 100nm, 200 nm, 300 nm, 400nm, 500 nm, 600 nm, 700 nm, 800 nm, 900 nm, 1000 nm, 1100 nm, 1200 nm, 1300 nm, 1400 nm, 1500nm, or 2000 nm, including all values and subranges that lie between these values. In some examples, the diameter of the negatively charged particle is between 400 nm to 800 nm.

**[0026]** In various examples, the subject has a cancer selected from the group consisting of brain cancer, skin cancer, eye cancer, breast cancer, prostate cancer, pancreatic cancer, lung cancer, esophageal cancer, head and neck cancer, cervical cancer, liver cancer, colon cancer, colorectal cancer, rectal cancer, bone cancer, uterine cancer, ovarian cancer, bladder cancer, stomach cancer, oral cancer, thyroid cancer, kidney cancer, testicular cancer, leukemia, lymphoma and mesothelioma. Additional cancers contemplated by the methods are disclosed in the Detailed Description.

**[0027]** In various examples, the cancer therapeutic is a chemotherapeutic selected from the group consisting of growth inhibitors, DNA-replication inhibitors, kinase inhibitors, signaling cascade inhibitors, angiogenesis inhibitors, metabolic inhibitors, amino acid synthesis inhibitors, selective inhibitors of oncogenic proteins, inhibitors of metastasis, inhibitors of anti-apoptosis factors, apoptosis inducers, nucleoside signaling inhibitors, enzyme inhibitors and DNA-damaging agents.

**[0028]** In various examples, the cancer therapeutic comprises one or more biologic agents selected from the group consisting of cytokines, angiogenesis inhibitors, enzymes, immune checkpoint modulators and monoclonal antibodies.

**[0029]** In various examples, cytokines are selected form the group consisting of transforming growth factors, tumor necrosis factor, interferons and interleukins. Exemplary

cytokines include, but are not limited to, IFN-alpha, IFN-beta, IFN-gamma, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IL-11, IL-12, IL-13, IL-15, IL-17, IL-18, IL-21, members of the transforming growth factor beta superfamily, including TGF-β1, TGF-β2 and TGF-β3, tumor necrosis factor alpha, Granulocyte colony-stimulating factor (G-CSF), and Granulocyte macrophage colony-stimulating factor (GM-CSF).

**[0030]** In various examples the monoclonal antibodies are mono-specific, bi-specific, tri-specific or bispecific T-cell engaging (BiTE) antibodies.

**[0031]** In various examples the monoclonal antibodies are immune cell co-stimulatory molecule agonists that induce an anti-tumor immune response. Exemplary co-stimulatory molecules include, but are not limited to, ICOS (Inducible T cell Co-stimulator) (CD278), OX40 (CD134), 41 BB, GITR (Glucocorticoid-induced Tumor Necrosis Factor Receptor), CD40 and CD27.

**[0032]** In various examples, the immune checkpoint modulators target Programmed cell death protein 1 (PD-1), Programmed cell death protein ligand-1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T-cell Immunoglobulin and mucin-domain containing-3 (TIM-3), Lymphocyte-activation Gene 3 (LAG-3) and/or TIGIT (T cell immunoreceptor with Ig and ITIM domains). In various examples, the immune checkpoint modulator is an antibody selected from the group consisting of ipilimumab, tremelimumab, pembrolizumab, nivolumab, atezolizumab, avelumab, cemiplimab and durvalumab.

**[0033]** In various examples, monoclonal antibodies useful in the methods are selected from the group comprising Alemtuzumab, Bevacizumab, Brentuximab, Cetuximab, Denosumab, Ibritumomab, Trastuzumab, Panitumumab, Pertuzumab, and Rituximab. In various examples, monoclonal antibodies useful in the methods target receptor tyrosine kinase, EGFR, VEGF, VEGFR, PDGF, PDGFR, TGF-β, TGF-β-LAP, SIRP-α, CD47, CD39, CD73, and fibroblast activating protein (FAP).

**[0034]** In various examples, the cancer therapeutic comprises an enzyme. In various examples, the cancer therapeutic comprises an enzyme that targets T-cells, B-cells, APCs, macrophages, dendritic cells, monocytes, MDSCs, TAMs, neutrophils, other monocyte-derived cells, tumor-associated stroma, cancer stem cells, mesenchymal stem cells, extracellular matrix, and amino acids. In various examples, the cancer therapeutic comprises an enzyme selected from the group comprising asparaginase, kynurininase, L-arginine deiminase, L-methionine-γ-lyase, one or more amino acid degrading enzymes, and one or more nucleoside degrading enzymes.

**[0035]** In various examples, the cancer therapeutic comprises one or more cell-based therapies selected from the group consisting of adoptive cell transfer, tumor-infiltrating leukocyte therapy, chimeric antigen receptor T-cell therapy (CAR-T), NK-cell therapy and stem cell therapy.

**[0036]** In various examples the cell-based therapy is the adoptive transfer of autologous patient-derived cells. In various examples the cell-based therapy is the adoptive transfer of allogenic donor-derived cells.

**[0037]** In various examples, the cell-based therapy is the transfer of universal donor-derived or induced pluripotent stem cell-derived cells that are not patient specific and amenable to long-term storage. Such therapies are also referred to as 'off-the-shelf' therapies.

**[0038]** In various examples, the cancer therapeutic is a hormone therapy. In various examples, the cancer therapeutic comprises one or more antibody-drug conjugates. In various examples, the cancer therapeutic comprises one or more cancer vaccines. In various examples, the cancer vaccine is a protein, polypeptide, nucleic acid vaccine and/or a dendritic cell vaccine.

**[0039]** In various examples, the cancer therapeutic is an immunotherapy selected from the group comprising oncolytic virus, bacteria, oncolytic bacteria or other bacterial compositions, Bacillus Calmette-Guerin (BCG), a microbiome modulator, and/or a toll-like receptor (TLR) agonist. In various examples, the TLR agonist is a TLR3, TLR4, TLR5, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12, and/or TLR13 agonist. In various examples, the TLR agonist is derived from virus, bacteria and/or made synthetically. In various examples, the immunotherapy is a STING pathway modulator.

**[0040]** In various examples, the cancer therapeutic comprises a viral or a bacterial vector. In various examples, the viral vector is selected from the group comprising adenovirus, adeno-associated virus (AAV), herpes simplex virus, lentivirus, retrovirus, alphavirus, flavivirus, rhabdovirus, measles virus, Newcastle disease virus, poxvirus, vaccinia virus, modified Ankara virus, vesicular stomatitis virus, picornavirus, tobacco mosaic virus, potato virus x, comovirus or cucumber mosaic virus. In various examples, the virus is an oncolytic virus. In various examples the virus is a chimeric virus, a synthetic virus, a mosaic virus or a pseudotyped virus.

**[0041]** Additional cancer therapeutics contemplated for use in the methods are set out in the Detailed Description.

**[0042]** In various examples, the particle and/or the cancer therapeutic is administered twice weekly, once weekly, once every two weeks, once every three weeks, once every 4 weeks, once every two months, once every three months, once every 6 months or once per year.

**[0043]** In various examples, the particle and/or the cancer therapeutic is administered intravenously, orally, nasally, intramuscularly, ocularly, transdermally, or subcutaneously.

**[0044]** In various examples, the subject is a mammal. In various examples, the subject is human.

**[0045]** In various examples, the administration improves one or more symptoms of the cancer or proliferative disorder. In various examples, the one or more symptoms are selected from the group consisting of tumor size or tumor burden in the subject, tumor metastasis, and levels of inflammatory cells in the tumor or in the tumor microenvironment. In various examples, the administration reduces the tumor size or tumor burden by 10%, 20%, 30% or more. In various examples, the administration alters monocytes, macrophages, granulocytes, dendritic cells and/or neutrophils at the tumor.

**[0046]** In various examples, the particle is formulated in a composition comprising a pharmaceutical acceptable carrier, diluent or excipient. In various examples, the cancer therapeutic is formulated in a composition comprising a pharmaceutical acceptable carrier, diluent or excipient. In various examples, the particle and cancer therapeutic can be formulated in the same composition or in separate compositions.

**[0047]** Also contemplated is a composition comprising any of the foregoing particles or cancer therapeutic compositions of the disclosure, or use thereof in preparation of a medicament, for treatment of any of the disorders described herein associated with inflammation and cancer and/or proliferative diseases.

**[0048]** It is understood that each feature or example, or combination, described herein is a non-limiting, illustrative example of any of the aspects of the disclosure and, as such, is meant to be combinable with any other feature or example, or combination, described herein. For example, where features are described with language such as “one example”, “some examples”, “further example”, “specific exemplary examples”, and/or “another example”, each of these types of examples is a non-limiting example of a feature that is intended to be combined with any other feature, or combination of features, described herein without having to list every possible combination. Such features or combinations of features

**WHAT IS CLAIMED:**

1. Use of negatively charged particles in the manufacture of a medicament for use in combinatorial therapy for treating cancer or a proliferative disease in a subject, wherein the particle is free from attached peptide or antigenic moieties or other bioactive agents, and wherein the medicament is to be administered in combination with a cancer therapeutic.

2. The use of claim 1, wherein the medicament alters myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), neutrophils and/or monocytes at the tumor site.

3. The use of claim 1 or claim 2, wherein the medicament reduces tumor size and/or tumor growth in the subject.

4. The use of any one of claims 1 to 3, wherein the medicament regulates the anti-tumor immune response.

5. The use of any one of claims 1 to 4, wherein the medicament alters cancer stem cells and/or mesenchymal stem cells.

6. The use of any one of claims 1 to 5, wherein the medicament alters the tumor-associated stroma.

7. The use of any one of claims 1 to 6, wherein the medicament alters fibroblasts, adipocytes, endothelial cells, mesenchymal stromal cells, and/or the ECM in the tumor-associated stroma.

8. The use of any one of the preceding claims wherein the negatively charged particles are polyglycolic acid polymers (PGA), polylactic acid (PLA), polystyrene particles, or poly (lactic-co-glycolic acid) (PLGA) particles, diamond particles, or iron, zinc, cadmium, gold, or silver particles.

9. The use of any one of the preceding claims, wherein the negatively charged particles are poly (lactic-co-glycolic acid) (PLGA) particles.

10. The use of claim 8 or 9, wherein the particle comprises about 50:50, about 80:20 to about 100:0 polylactic acid:polyglycolic acid or from about 50:50, about 80:20 to about 100:0 polyglycolic acid:polylactic acid.

11. The use of any one of the preceding claims, wherein the particle comprises 50:50 polylactic acid:polyglycolic acid.

12. The use of any one of the preceding claims, wherein the particle is carboxylated.

13. The use of any one of the preceding claims, wherein the particle has a zeta potential between -100 mV and -1 mV.

14. The use of any one of the preceding claims, wherein the particle has a zeta potential between -80 mV and -30 mV.

15. The use of any one of the preceding claims, wherein the diameter of the negatively charged particle is between 0.1 µm to 10 µm.

16. The use of any one of the preceding claims, wherein the diameter of the negatively charged particle is between 400 nm to 800 nm.

17. The use of any one of the preceding claims, wherein the subject has a cancer selected from the group consisting of brain cancer, skin cancer, eye cancer, breast cancer, pancreatic cancer, prostate cancer, lung cancer, esophageal cancer, head and neck cancer, cervical cancer, liver cancer, colon cancer, colorectal cancer, rectal cancer, bone cancer, uterine cancer, ovarian cancer, bladder cancer, stomach cancer, oral cancer, thyroid cancer, kidney cancer, testicular cancer, leukemia, lymphoma and mesothelioma.

18. The use of any one of the preceding claims, wherein the cancer therapeutic is a chemotherapeutic selected from the group consisting of growth inhibitors, DNA-replication inhibitors, kinase inhibitors, signaling cascade inhibitors, angiogenesis inhibitors, metabolic inhibitors, amino acid synthesis inhibitors, selective inhibitors of oncogenic proteins, inhibitors of metastasis, inhibitors of anti-apoptosis factors, apoptosis inducers, enzyme inhibitors, nucleoside signaling inhibitors and DNA-damaging agents.

19. The use of any one of claims 1-17, wherein the cancer therapeutic comprises one or more biologic agents selected from the group consisting of cytokines, enzymes, angiogenesis inhibitors, immune checkpoint modulators and monoclonal antibodies.

20. The use of claim 19, wherein the cytokines are selected from the group consisting of transforming growth factors, tumor necrosis factor, interferons and interleukins.

21. The use of claim 19, wherein the immune checkpoint modulators target Programmed cell death protein 1 (PD1), Programmed cell death protein ligand-1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T cell Immunoglobulin and Mucin-domain

containing-3 (TIM-3), Lymphocyte-activation Gene-3 (LAG-3) and/or TIGIT (T cell immunoreceptor with Ig and ITIM domains).

22. The use of claim 21, wherein the immune checkpoint modulator is an antibody selected from the group consisting of ipilimumab, tremelimumab, pembrolizumab, nivolumab, atezolizumab, avelumab,cemiplimab and durvalumab.

23. The use of claim 19, wherein the monoclonal antibody is mono-specific, bi-specific, tri-specific or bi-specific T-cell engager (BiTE).

24. The use of claim 19, wherein the monoclonal antibody comprises Alemtuzumab, Bevacizumab, Brentuximab, Cetuximab, Denosumab, Ibritumomab, Trastuzumab, Panitumumab, Pertuzumab, and Rituximab.

25. The use of claim 19, wherein the monoclonal antibody targets receptor tyrosine kinase, EGFR, VEGF, VEGFR, PDGF, PDGFR, TGF-β, TGF-β-LAP, SIRP-α, CD47, CD39, CD73 and/or fibroblast activating protein (FAP).

26. The use of any one of claims 1-17, wherein the cancer therapeutic comprises one or more cell-based therapies selected from the group consisting of adoptive cell transfer, tumor-infiltrating leukocyte therapy, chimeric antigen receptor T-cell therapy (CAR-T), NK-cell therapy and stem cell therapy.

27. The use of any one of claims 1-17, wherein the cancer therapeutic is a hormone therapy.

28. The use of any one of claims 1-17, wherein the cancer therapeutic comprises one or more antibody-drug conjugates.

29. The use of any one of claims 1-17, wherein the cancer therapeutic comprises one or more cancer vaccines.

30. The use of any one of claims 1-17, wherein the cancer therapeutic is an immunotherapy comprising oncolytic virus, oncolytic bacteria or other bacterial compositions, Bacillus Calmette-Guerin (BCG), a microbiome modulator, a STING pathway modulator and/or a toll-like receptor (TLR) agonist.

31. The use of any one of the preceding claims wherein the particle and or the cancer therapeutic is to be administered twice weekly, once weekly, once every two weeks, once every three weeks, once every 4 weeks, once every two months, once every three months, once every 6 months or once per year.

32. The use of any one of the preceding claims, wherein the particles are to be administered intravenously, orally, nasally, intramuscularly, ocularly, transdermally, or subcutaneously.

33. The use of any one of the preceding claims, wherein the subject is human.

34. The use of any one of the preceding claims wherein the administration improves one or more symptoms of the cancer of proliferative disorder.

35. The use of claim 34, wherein the one or more symptoms are selected from the group consisting of tumor size or tumor burden in the subject, tumor metastasis, and levels of inflammatory cells in the tumor.

36. The use of claim 35, wherein the administration reduces the tumor size or tumor burden by 10%, 20%, 30% or more.

37. The use of claim 34, wherein the administration reduces of the number of monocytes, macrophages, granulocytes and/or neutrophils at the tumor.

38. The use of any one of the preceding claims, wherein the particle is formulated in a composition comprising a pharmaceutical acceptable carrier, diluent or excipient.

39. The use of any one of the preceding claims, wherein the cancer therapeutic is formulated in a composition comprising a pharmaceutical acceptable carrier, diluent or excipient.