CLAIMS

What is claimed is:

1. Use of an effective amount of a composition comprising nucleated cells in the manufacture of a first medicament and use of an effective amount of an immunoconjugate in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy to treat a disease in an individual, wherein:

(a) the nucleated cells comprise an exogenous antigen associated with the disease; and

(b) the immunoconjugate comprises a mutant IL-2 polypeptide and a second polypeptide, wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A, and L72G, with numbering according to the human IL-2 sequence set forth in SEQ ID NO:19, and wherein the second polypeptide is capable of specific binding to a T cell, a tumor cell, or the tumor cell environment.

2. Use of an effective amount of nucleated cells in the manufacture of a medicament for use in combinatorial therapy for treating a disease in an individual, wherein said medicament is to be administered in combination with an effective amount of an immunoconjugate, wherein:

(a) the nucleated cells comprise an exogenous antigen associated with the disease; and

(b) the immunoconjugate comprises a mutant IL-2 polypeptide and a second polypeptide, wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A, and L72G, with numbering according to the human IL-2 sequence set forth in SEQ ID NO:19, and wherein the second polypeptide is capable of specific binding to a T cell, a tumor cell, or the tumor cell environment.

3. Use of an effective amount of an immunoconjugate in the manufacture of a medicament for use in combinatorial therapy for treating a disease in an individual, wherein said medicament is to be administered in combination with an effective amount of a nucleated cells, wherein:

(a) the nucleated cells comprise an exogenous antigen associated with the disease; and

(b) the immunoconjugate comprises a mutant IL-2 polypeptide and a second polypeptide, wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A, and L72G, with numbering according to the human IL-2 sequence set forth in SEQ ID NO:19, and wherein the second polypeptide is capable of specific binding to a T cell, a tumor cell, or the tumor cell environment.

4. A combination of an effective amount of a composition comprising nucleated cells and an effective amount of an immunoconjugate for use as a medicament, wherein:

(a) the nucleated cells comprise an exogenous antigen; and

(b) the immunoconjugate comprises a mutant IL-2 polypeptide and a second polypeptide, wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A, and L72G, with numbering according to the human IL-2 sequence set forth in SEQ ID NO:19, and wherein the second polypeptide is capable of specific binding to a T cell, a tumor cell, or the tumor cell environment.

5. The use or combination for use of any one of claims 1-3, wherein the disease is cancer, an infectious disease, or a viral-associated disease.

6. The use or combination for use of any one of claims 1-5, wherein the second polypeptide binds a T cell, optionally wherein the second polypeptide binds PD-1 expressed on the T cell, further optionally wherein the second polypeptide is an antigen-binding moiety that specifically binds PD-1.

7. The use or combination for use of claim 6, wherein the second polypeptide is an antigen-binding moiety that specifically binds PD-1, wherein the anti-PD-1 antigen-binding moiety comprises

(a) a heavy chain variable region (VH) comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO:1, a HVR-H2 comprising the amino acid sequence of SEQ ID NO:2, a HVR-H3 comprising the amino acid sequence of SEQ ID NO:3, and a FR-H3 comprising the amino acid sequence of SEQ ID NO:7 at positions 71-73 according to Kabat numbering, and a light chain variable region (VL) comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO:4, a

HVR-L2 comprising the amino acid sequence of SEQ ID NO:5, and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:6; or

(b) a heavy chain variable region (VH) comprising a HVR-H1 comprising the amino acid sequence of SEQ ID NO:8, a HVR-H2 comprising the amino acid sequence of SEQ ID NO:9, and a HVR-H3 comprising the amino acid sequence of SEQ ID NO:10, and a light chain variable region (VL) comprising a HVR-L1 comprising the amino acid sequence of SEQ ID NO:11, a HVR-L2 comprising the amino acid sequence of SEQ ID NO:12, and a HVR-L3 comprising the amino acid sequence of SEQ ID NO:13. .

8. The use or combination for use of claim 6 or claim 7, wherein the second polypeptide is an antigen-binding moiety that specifically binds PD-1, wherein the anti-PD-1 antigen-binding moiety comprises

(a) a heavy chain variable region (VH) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:14, and

(b) a light chain variable region (VL) comprising an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18.

9. The use or combination for use of any one of claims 6-8, wherein the immunoconjugate comprises a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:22, a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:24, and a polypeptide comprising an amino acid sequence that is at least about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the sequence of SEQ ID NO:25.

10. The use or combination for use of any one of claims 6-9, wherein the immunoconjugate comprises a polypeptide sequence of SEQ ID NO:22, a polypeptide sequence of SEQ ID NO:24, and two polypeptide sequences of SEQ ID NO:25.

11. The use or combination for use of any one of claims 1-10, wherein the second polypeptide specifically binds a target antigen presented on a tumor cell or in a tumor cell environment.

12. The use or combination for use of claim 11, wherein the target antigen is selected from the group consisting of a Fibroblast activation protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), Carcinoembryonic Antigen (CEA) and the Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

13. The use or combination for use of any one of claims 1-5 and 11-12, wherein the second polypeptide binds FAP.

14. The use or combination for use of claim 12 of 13, wherein the second polypeptide is an antigen-binding moiety that specifically binds FAP.

15. The use or combination for use of claim 14, wherein the antigen-binding moiety the specifically bind FAP comprising (i) the heavy chain variable region sequence of SEQ ID NO:29 and the light chain variable region sequence of SEQ ID NO: 28; (ii) the heavy chain variable region sequence of SEQ ID NO:31 and the light chain variable region sequence of SEQ ID NO:30; (iii) the heavy chain variable region sequence of SEQ ID NO: 33 and the light chain variable region sequence of SEQ ID NO:32; (iv) the heavy chain variable region sequence of SEQ ID NO:35 and the light chain variable region sequence of SEQ ID NO:34; or (v) the heavy chain variable region sequence of SEQ ID NO:37 and the light chain variable region sequence of SEQ ID NO:36.

16. The use or combination for use of claim 14 or 15, wherein the antigen-binding moiety the specifically bind FAP comprising (i) a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:42, a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:43, and a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:41; (ii) a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%,

98%, 99% or 100% identical to SEQ ID NO:38, a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:39, and a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 231; or (iii) a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:44, a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:45, and a polypeptide sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO:41.

17. The use or combination for use of any one of claims 14-16, wherein antigen-binding moiety the specifically bind FAP comprises the heavy chain variable region sequence of SEQ ID NO:33 and the light chain variable region sequence of SEQ ID NO:32.

18. The use or combination for use of any one of claims 14-17, wherein the immunoconjugate comprises a polypeptide sequence of SEQ ID NO:38, a polypeptide sequence of SEQ ID NO:39, and a polypeptide sequence of SEQ ID NO:40.

19. The use or combination for use of any one of claims 1-18, wherein the mutant IL-2 polypeptide further comprises the amino acid substitution T3A and/or the amino acid substitution C125A.

20. The use or combination for use of any one of claims 1-19, wherein the mutant IL-2 polypeptide comprises an amino acid sequence that is at least about 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO:20**,** wherein the mutant IL-2 polypeptide shows reduced affinity to the high-affinity IL-2 receptor and substantially similar affinity to the intermediate-affinity IL-2 receptor, each compared to a wild-type IL-2 polypeptide.

21. The use or combination for use of any one of claims 1-20, wherein the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO:20.

22. The use or combination for use of any one of claims 1-21, wherein the nucleated cells are immune cells.

23. The use or combination for use of any one of claims 1-22, wherein the nucleated cells are a plurality of peripheral blood mononuclear cells (PBMCs).

24. The use or combination for use of claim 23, wherein the plurality of PBMCs comprise two or more of T cell, B cell, NK cell, monocytes, dendritic cells or NK-T cells.

25. The use or combination for use of any one of claims 1-24, wherein the nucleated cells are one or more of T cells, B cells, NK cells, monocytes, dendritic cells and/or NK-T cells.

26. The use or combination for use of any one of claims 1-25, wherein the exogenous antigen is delivered to the nucleated cells intracellularly.

27. The use or combination for use of any one of claims 1-26, wherein the exogenous antigen is a disease-associated antigen.

28. The use or combination for use of claim 27, wherein the disease-associated antigen is a cancer antigen, an infectious disease antigen, or a viral-associated disease antigen.

29. The use or combination for use of any one of claims 1-28, wherein the exogenous antigen is a human papillomavirus (HPV) antigen.

30. The use or combination for use of any one of claims 1-29, wherein the composition further comprises an adjuvant.

31. The use or combination for use of claim 30, wherein the adjuvant is a CpG oligodeoxynucleotide (ODN), LPS, IFN-α, STING agonists, RIG-I agonists, poly I:C, R837, R848, a TLR3 agonist, a TLR4 agonist or a TLR 9 agonist.

32. The use or combination for use of any one of claims 1-31, wherein the nucleated cells comprising the exogenous antigen are prepared by a process comprising the steps of:

a) passing a cell suspension comprising input nucleated cells through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input nucleated cells in the suspension, thereby causing perturbations of the input nucleated cells large enough for the exogenous antigen to pass through to form perturbed input nucleated cells;

b) incubating the perturbed input nucleated cells with the exogenous antigen for a sufficient time to allow the exogenous antigen to enter the perturbed input nucleated cells, thereby generating nucleated cells comprising the exogenous antigen.

33. The use or combination for use of claim 32, wherein the width of the constriction is about 10% to about 99% of the mean diameter of the input nucleated cells.

34. The use or combination for use of claim 32 or claim 33, wherein the width of the constriction is about 4.2 μm to about 6 μm.

35. The use or combination for use of any one of claims 32-34, wherein the width of the constriction is about 4.2 μm to about 4.8 μm.

36. The use or combination for use of any one of claims 32-35, wherein the width of the constriction is about 4.5 μm.

37. The use or combination for use of any one of claims 32-36, wherein the cell suspension comprising the plurality of input nucleated cells are passed through multiple constrictions wherein the multiple constrictions are arranged in series and/or in parallel.

38. The use or combination for use of any one of claims 32-37, wherein the exogenous antigen is present in at least about 70% of the nucleated cells following incubation of the perturbed input nucleated cells with the exogenous antigen.

39. The use or combination for use of any one of claims 1-38, wherein the nucleated cells are conditioned with an adjuvant to form conditioned cells.

40. The use or combination for use of claim 39, wherein the nucleated cells are incubated with the adjuvant for about 1 hour to about 24 hours, about 2 hours to about 10 hours, about 3 hours to about 6 hours, or about 4 hours for the cells to condition.

41. The use or combination for use of claim 39 or claim 40, wherein the nucleated cells are conditioned before or after introducing the exogenous antigen into the nucleated cells.

42. The use or combination for use of any one of claims 39-41, wherein the adjuvant is a CpG oligodeoxynucleotide (ODN), LPS, IFN-α, STING agonists, RIG-I agonists, poly I:C, R837, R848, a TLR3 agonist, a TLR4 agonist or a TLR 9 agonist.

43. The use or combination for use of any one of claims 39-42, wherein the adjuvant is a CpG oligodeoxynucleotide (ODN).

44. The use or combination for use of any one of claims 39-43, wherein the adjuvant is CpG 7909.

45. The use or combination for use of any one of claims 39-44, wherein the conditioned cells are a conditioned plurality of modified PBMCs.

46. The use or combination for use of claim 45, wherein the plurality of modified PBMCs are further modified to increase expression of one or more of co-stimulatory molecules.

47. The use or combination for use of claim 46, wherein the co-stimulatory molecule is B7-H2 (ICOSL), B7-1 (CD80), B7-2 (CD86), CD70, LIGHT, HVEM, CD40, 4-1BBL, OX40L, TL1A, GITRL, CD30L, TIM4, SLAM, CD48, CD58, CD155, or CD112.

48. The use or combination for use of any one of claims 45-47, wherein the plurality of modified PBMCs are further modified to increase expression of one or more cytokines.

49. The use or combination for use of claim 48, wherein the cytokine is IL-15, IL-12, IL-2, IFN-α, or IL-21.

50. The use or combination for use of any one of claim 45-49, wherein one or more co-stimulatory molecules is upregulated in the B cells of the conditioned plurality of modified PBMCs compared to the B cells in the plurality of nonmodified PBMCs, wherein the co-stimulatory molecule is CD80 and/or CD86.

51. The use or combination for use of any one of claims 45-50, wherein the plurality of modified PBMCs have increased expression of one or more of IFN-γ, IL-6, MCP-1, MIP-1β, IP-10, or TNF-α compared to a plurality of unconditioned PBMCs.

52. The use or combination for use of claim 51, wherein the expression of one or more of IFN-γ, IL-6, MCP-1, MIP-1β, IP-10, or TNF-α is increased by more than about 1.2-fold, 1.5-fold, 1.8-fold, 2-fold, 3-fold, 4-fold, 5-fold, 8-fold, or more than 10-fold compared to the plurality of unconditioned PBMCs.

53. The use or combination for use of any one of claims 1-52, wherein the immunoconjugate is to be administered before, at the same time, or after administration of the composition comprising nucleated cells.

54. The use or combination for use of any one of claims 1-53, wherein the composition comprising nucleated cells is to be administered a plurality of times.

55. The use or combination for use of any one of claims 1-54, wherein the immunoconjugate is to be administered a plurality of times after administration of the composition comprising nucleated cells.

56. The use or combination for use of any one of claims 1-55, wherein the composition and/or the immunoconjugate is to be administered intravenously.

57. The use or combination for use of any one of claims 1-56, wherein the immunoconjugate is to be administered subcutaneously or intratumorally.

58. The use or combination for use of claim 57, wherein the immunoconjugate is to be administered subcutaneously in combination with hyaluronidase.

59. The use or combination for use of any one of claims 1-58, wherein the individual is a human.

60. The use or combination for use of any one of claims 1-59, wherein the individual has cancer, an infectious disease, or a viral associated disease.

61. The use or combination for use of any one of claims 1-60, wherein the composition of nucleated cells and/or the immunoconjugate is to be administered prior to, concurrently with, or following administration of another therapy.

62. The use or combination for use of claim 61, wherein the another therapy is a chemotherapy or a radiation therapy.

63. A kit comprising a composition of nucleated cells comprising an exogenous antigen and an immunoconjugate, wherein the immunoconjugate comprises a mutant IL-2 polypeptide and a second polypeptide capable of specific binding to a T cell, a tumor cell, or the tumor cell environment; wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 19); wherein the composition and the immunoconjugate are for use in combination for stimulating an immune response to the exogenous antigen in an individual.

64. A kit comprising a composition of nucleated cells comprising an exogenous antigen , wherein the composition is for use in conjunction with an immunoconjugate for stimulating an immune response to the exogenous antigen in an individual;

wherein the immunoconjugate comprises a mutant IL-2 polypeptide and a second polypeptide capable of specific binding to a T cell, a tumor cell, or the tumor cell environment; and wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 19).

65. A kit comprising an immunoconjugate, wherein the immunoconjugate comprises a mutant IL-2 polypeptide and a second polypeptide capable of specific binding to a T cell, a tumor cell, or the tumor cell environment; wherein the mutant IL-2 polypeptide is a human IL-2 molecule comprising the amino acid substitutions F42A, Y45A and L72G (numbering relative to the human IL-2 sequence SEQ ID NO: 19);

wherein the immunoconjugate is for use in conjunction with a composition of nucleated cells comprising an exogenous antigen for stimulating an immune response to the exogenous antigen in an individual.