

CLAIMS

What is claimed is:

1. A method for stimulating an immune response in an individual, the method comprising
 - a) administering an effective amount of a composition comprising nucleated cells to an individual, wherein the nucleated cells comprise an exogenous antigen; and
 - b) administering an effective amount of an immunoconjugate to the individual, wherein 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 (numbering relative to the human IL-2 sequence 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. A method for stimulating an immune response to a tumor antigen in an individual, the method comprising
 - a) administering an effective amount of a composition comprising nucleated cells to an individual, wherein the nucleated cells comprise an exogenous tumor antigen; and
 - b) administering an effective amount of an immunoconjugate to the individual, wherein 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 (numbering relative to the human IL-2 sequence 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. A method for enhancing a nucleated cell-based immunotherapy, the method comprising administering an effective amount of an immunoconjugate in conjunction with the nucleated cell-based immunotherapy, wherein 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 (numbering relative to the human IL-2 sequence 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 method for treating a disease in an individual, the method comprising
 - a) administering an effective amount of a composition comprising nucleated cells to an individual, wherein the nucleated cells comprise an exogenous antigen associated with the disease; and
 - b) administering an effective amount of an immunoconjugate to the individual, wherein 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 (numbering relative to the human IL-2 sequence 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. A method of vaccinating an individual in need thereof, the method comprising
 - a) administering an effective amount of a composition comprising nucleated cells to an individual, wherein the nucleated cells comprise an exogenous antigen; and
 - b) administering an effective amount of an immunoconjugate to the individual, wherein 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 (numbering relative to the human IL-2 sequence 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.
6. The method of claim 5, wherein the individual has a disease responsive to vaccination.
7. The method of any one of claims 4-6, wherein the disease is cancer, an infectious disease, or a viral-associated disease.

8. A method for reducing tumor growth in an individual, the method comprising
 - a) administering an effective amount of a composition comprising nucleated cells to an individual, wherein the nucleated cells comprise an exogenous tumor antigen; and
 - b) administering an effective amount of an immunoconjugate to the individual, wherein 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 (numbering relative to the human IL-2 sequence 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.
9. The method of any one of claims 1-8, wherein the second polypeptide binds a T cell.
10. The method of claim 11, wherein the second polypeptide binds PD-1 expressed on the T cell.
11. The method of claim 10, wherein the second polypeptide is an antigen-binding moiety that specifically binds PD-1.
12. The method of claim 11, 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. .

13. The method of claim 11 or 12, 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.

14. The method of any one of claims 11-13, wherein the anti-PD-1 antigen-binding moiety comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:14, and (b) a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18.

15. The method of any one of claims 11-14, wherein the anti-PD-1 antigen-binding moiety comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:14, and (b) a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO:15.

16. The method of any one of claims 11-15, 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.

17. The method of any one of claims 11-16, 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.
18. The method of any one of claims 1-8, wherein the second polypeptide specifically binds a target antigen presented on a tumor cell or in a tumor cell environment.
19. The method of claim 18, 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).
20. The method of any one of claims 1-8 and 18-19, wherein the second polypeptide binds FAP.
21. The method of claim 19 of 20, wherein the second polypeptide is an antigen-binding moiety that specifically binds FAP.
22. The method of claim 21, 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.
23. The method of claim 21 or 22, 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.

24. The method of any one of claims 21-23, 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.

25. The method of any one of claims 21-24, 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.

26. The method of any one of claims 1-25, wherein the mutant IL-2 polypeptide further comprises the amino acid substitution T3A and/or the amino acid substitution C125A.

27. The method of any one of claims 1-26, 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.

28. The immunoconjugate of any one of claims 1-27, wherein the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO:20.
29. The method of any one of claims 1-28, wherein the nucleated cells are immune cells.
30. The method of any one of claims 1-29, wherein the nucleated cells are a plurality of peripheral blood mononuclear cells (PBMCs).
31. The method of claim 30, wherein the plurality of PBMCs comprise two or more of T cell, B cell, NK cell, monocytes, dendritic cells or NK-T cells.
32. The method of any one of claims 1-31, wherein the nucleated cells are one or more of T cells, B cells, NK cells, monocytes, dendritic cells and/or NK-T cells.
33. The method of any one of claims 1-32, wherein the exogenous antigen is delivered to the nucleated cells intracellularly.
34. The method of any one of claims 1-33, wherein the exogenous antigen is a disease-associated antigen.
35. The method of claim 34, wherein the disease-associated antigen is a cancer antigen, an infectious disease antigen, or a viral-associated disease antigen.
36. The method of any one of claims 1-35, wherein the exogenous antigen is a human papillomavirus (HPV) antigen.
37. The method of any one of claims 1-36, wherein the composition further comprises an adjuvant.

38. The method of claim 37, 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.

39. The method of any one of claims 1-38, 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.

40. The method of claim 39, wherein the width of the constriction is about 10% to about 99% of the mean diameter of the input nucleated cells.

41. The method of claim 39 or 40, wherein the width of the constriction is about 4.2 μm to about 6 μm .

42. The method of any one of claims 39-41, wherein the width of the constriction is about 4.2 μm to about 4.8 μm .

43. The method of any one of claims 39-42, wherein the width of the constriction is about 4.5 μm .

44. The method of any one of claims 39-43, 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.

45. The method of any one of claims 39-44, 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.
46. The method of any one of claims 1-45, wherein the nucleated cells are conditioned with an adjuvant to form conditioned cells.
47. The method of claim 46, 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.
48. The method of claim 46 or 47, wherein the nucleated cells are conditioned before or after introducing the exogenous antigen into the nucleated cells.
49. The method of any one of claims 46-48, 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.
50. The method of any one of claims 46-49, wherein the adjuvant is a CpG oligodeoxynucleotide (ODN).
51. The method of any one of claims 46-50, wherein the adjuvant is CpG 7909.
52. The method of any one of claims 46-51, wherein the conditioned cells are a conditioned plurality of modified PBMCs.
53. The method of claim 52, wherein the plurality of modified PBMCs are further modified to increase expression of one or more of co-stimulatory molecules.

54. The method of claim 53, 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.
55. The method of any one of claims 52-54, wherein the plurality of modified PBMCs are further modified to increase expression of one or more cytokines.
56. The method of claim 55, wherein the cytokine is IL-15, IL-12, IL-2, IFN- α , or IL-21.
57. The method of any one of claim 52-56, 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.
58. The method of any one of claims 52-57, wherein the plurality of modified PBMCs have increased expression of one or more of IFN- γ , IL-6, MCP-1, MIP-1 β , IP-10, or TNF- α compared to a plurality of unconditioned PBMCs.
59. The method of claim 58, wherein the expression of one or more of IFN- γ , IL-6, MCP-1, MIP-1 β , 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.
60. The method of any one of claims 1-59, wherein the immunoconjugate is administered before, at the same time, or after administration of the composition comprising nucleated cells.
61. The method of any one of claims 1-60, wherein the composition comprising nucleated cells is administered a plurality of times.

62. The method of any one of claims 1-61, wherein the immunoconjugate is administered a plurality of times after administration of the composition comprising nucleated cells.
63. The method of any one of claims 1-62, wherein the composition and/or the immunoconjugate is administered intravenously.
64. The method of any one of claims 1-63, wherein the immunoconjugate is administered subcutaneously or intratumorally.
65. The method of claim 64, wherein the immunoconjugate is administered subcutaneously in combination with hyaluronidase.
66. The method of any one of claims 1-65, wherein the individual is a human.
67. The method of any one of claims 1-66, wherein the individual has cancer, an infectious disease, or a viral associated disease.
68. The method of any one of claims 1-67, wherein the composition of nucleated cells and/or the immunoconjugate is administered prior to, concurrently with, or following administration of another therapy.
69. The method of claim 68, wherein the another therapy is a chemotherapy or a radiation therapy.
70. A composition comprising nucleated cells comprising an exogenous antigen for use in a method for treating a disease in an individual, wherein the composition is used in combination with an immunoconjugate comprising 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).

71. The composition of claim 70, wherein the disease is cancer, an infectious disease, or a viral-associated disease.

72. The composition of claim 70 or 71, wherein the composition comprising nucleated cells is administered before, at the same time, or after the immunoconjugate.

73. The composition of any one of claims 70-72, wherein the immunoconjugate is administered a plurality of times after administration of the composition comprising nucleated cells.

74. The composition of any one of claims 70-73, wherein the second polypeptide binds a T cell.

75. The composition of any one of claims 70-74, wherein the second polypeptide binds PD-1 expressed on the T cell.

76. The composition of any one of claims 70-75, wherein the second polypeptide is an antigen-binding moiety that specifically binds PD-1.

77. The composition of claim 76, 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 .

78. The composition of claim 76 or 77, 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.

79. The composition of any one of claims 76-78, wherein the anti-PD-1 antigen-binding moiety comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:14, and (b) a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18.

80. The composition of any one of claims 76-79, wherein the anti-PD-1 antigen-binding moiety comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:14, and (b) a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO:15.

81. The composition of any one of claims 70-80, 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:24, 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.

82. The composition of any one of claims 70-81, wherein the immunoconjugate comprises a polypeptide sequence of SEQ ID NO:22, a polypeptide sequence of SEQ ID NO:24, and a polypeptide sequence of SEQ ID NO:25.

83. The composition of any one of claims 70-73, wherein the second polypeptide specifically binds a target antigen presented on a tumor cell or in a tumor cell environment.

84. The composition of claim 83, 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).

85. The composition of any one of claims 70-73 and 83-84, wherein the second polypeptide binds FAP.

86. The composition of any one of claims 70-73 and 83-85, wherein the second polypeptide is an antigen-binding moiety that specifically binds FAP.

87. The composition of any one of claims 70-73 and 83-86, 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.

88. The composition of any one of claims 70-73 and 83-87, wherein the antigen-binding moiety the specifically bind FAP comprises (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.

89. The composition of any one of claims 70-73 and 83-88, 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.

90. The composition of any one of claims 70-73 and 83-89, 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.

91. The composition of any one of claims 70-90, wherein the mutant IL-2 polypeptide further comprises the amino acid substitution T3A and/or the amino acid substitution C125A.

92. The composition of any one of claims 70-91, 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.

93. The composition of any one of claims 70-92, wherein the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO:20.

94. The composition of any one of claims 70-93, wherein the nucleated cells are immune cells.

95. The composition of any one of claims 70-94, wherein the nucleated cells are a plurality of peripheral blood mononuclear cells (PBMCs).

96. The composition of claim 95, wherein the plurality of PBMCs comprise two or more of T cell, B cell, NK cell, monocytes, dendritic cells or NK-T cells.

97. The composition of any one of claims 70-94, wherein the nucleated cells are one or more of T cells, B cells, NK cells, monocytes, dendritic cells and/or NK-T cells.

98. The composition of any one of claims 70-97, wherein the exogenous antigen is delivered to the nucleated cells intracellularly.

99. The composition of any one of claims 70-98, wherein the exogenous antigen is a disease-associated antigen.

100. The composition of any one of claims 70-99, wherein the disease-associated antigen is a cancer antigen, an infectious disease antigen, or a viral-associated disease antigen.

101. The composition of any one of claims 70-100, wherein the exogenous antigen is a human papillomavirus (HPV) antigen.

102. The composition of any one of claims 70-101, wherein the composition further comprises an adjuvant.

103. The composition of claim 102, 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.

104. The composition of any one of claims 70-103, 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.

105. The composition of claim 104, wherein the width of the constriction is about 10% to about 99% of the mean diameter of the input nucleated cells.

106. The composition of claim 104 or 105, wherein the width of the constriction is about 4.2 μm to about 6 μm .

107. The composition of any one of claims 104-106, wherein the width of the constriction is about 4.2 μm to about 4.8 μm .

108. The composition of any one of claims 104-107, wherein the width of the constriction is about 4.5 μm .

109. The composition of any one of claims 104-108, 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.
110. The composition of any one of claims 104-109, 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.
111. The composition of any one of claims 70-110, wherein the nucleated cells are conditioned with an adjuvant to form conditioned cells.
112. The composition of claim 111, 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.
113. The composition of claim 111 or 112, wherein the nucleated cells are conditioned before or after introducing the exogenous antigen into the nucleated cells.
114. The composition of any one of claims 111-113, 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.
115. The composition of any one of claims 111-114, wherein the adjuvant is a CpG oligodeoxynucleotide (ODN).
116. The composition of any one of claims 111-115, wherein the adjuvant is CpG 7909.
117. The composition of any one of claims 111-116, wherein the conditioned cells are a conditioned plurality of modified PBMCs.

118. The composition of claim 117, wherein the plurality of modified PBMCs are further modified to increase expression of one or more of co-stimulatory molecules.
119. The composition of claim 118, 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.
120. The composition of any one of claims 117-119, wherein the plurality of modified PBMCs are further modified to increase expression of one or more cytokines.
121. The composition of claim 120, wherein the cytokine is IL-15, IL-12, IL-2, IFN- α , or IL-21.
122. The composition of any one of claims 117-123, 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.
123. The composition of any one of claims 117-124, wherein the plurality of modified PBMCs have increased expression of one or more of IFN- γ , IL-6, MCP-1, MIP-1 β , IP-10, or TNF- α compared to a plurality of unconditioned PBMCs.
124. The composition of any one of claims 117-123, wherein the expression of one or more of IFN- γ , IL-6, MCP-1, MIP-1 β , 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.
125. The composition of any one of claims 70-124, wherein the composition and/or the immunoconjugate is administered intravenously.

126. The composition of any one of claims 70-125, wherein the immunoconjugate is administered subcutaneously or intratumorally.
127. The composition of claim 126, wherein the immunoconjugate is administered subcutaneously in combination with hyaluronidase.
128. The composition of any one of claims 70-127, wherein the individual is a human.
129. The composition of any one of claims 70-128, wherein the individual has cancer, an infectious disease, or a viral associated disease.
130. The composition of any one of claims 70-129, wherein the composition is administered prior to, concurrently with, or following administration of another therapy.
131. The composition of claim 130, wherein the another therapy is a chemotherapy or a radiation therapy.
132. An immunoconjugate comprising 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 for use in a method for treating a disease in an individual, wherein the immunoconjugate is used in combination with a composition comprising nucleated cells comprising an exogenous antigen.
133. The immunoconjugate of claim 132, wherein the disease is cancer, an infectious disease, or a viral-associated disease.
134. The immunoconjugate of claim 132 or 133, wherein the composition comprising the nucleated cells is administered before, at the same time, or after the immunoconjugate.

135. The immunoconjugate of any one of claims 132-134, wherein the immunoconjugate is administered a plurality of times after administration of the composition comprising nucleated cells.
136. The immunoconjugate of any one of claims 132-135, wherein the second polypeptide binds a T cell.
137. The immunoconjugate of any one of claims 132-136, wherein the second polypeptide binds PD-1 expressed on the T cell.
138. The immunoconjugate of any one of claims 132-137, wherein the second polypeptide is an antigen-binding moiety that specifically binds PD-1.
139. The immunoconjugate of claim 138, 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. .

140. The immunoconjugate of claim 138 or 139, 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.

141. The immunoconjugate of any one of claims 138-140, wherein the anti-PD-1 antigen-binding moiety comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:14, and (b) a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18.

142. The immunoconjugate of any one of claims 138-141, wherein the anti-PD-1 antigen-binding moiety comprises (a) a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO:14, and (b) a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO:15.

143. The immunoconjugate of any one of claims 132-142, 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.

144. The immunoconjugate of any one of claims 132-143, wherein the immunoconjugate comprises a polypeptide sequence of SEQ ID NO:22, a polypeptide sequence of SEQ ID NO:24, and a polypeptide sequence of SEQ ID NO:25.

145. The immunoconjugate of any one of claims 132-135, wherein the second polypeptide specifically binds a target antigen presented on a tumor cell or in the tumor cell environment.

146. The immunoconjugate of any one of claims 132-135 and 145, 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), the Carcinoembryonic Antigen (CEA) and the Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP).

147. The immunoconjugate of any one of claims 132-135 and 145-146, wherein the second polypeptide binds FAP.

148. The immunoconjugate of any one of claims 132-135 and 145-147, wherein the second polypeptide is an antigen-binding moiety that specifically binds FAP.

149. The immunoconjugate of claim 148, 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.

150. The immunoconjugate of claim 148 or 149, 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.

151. The immunoconjugate of any one of claims 148-150, 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.

152. The immunoconjugate of any one of claims 132-135 and 145-151, 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.

153. The immunoconjugate of any one of claims 132-152, wherein the mutant IL-2 polypeptide further comprises the amino acid substitution T3A and/or the amino acid substitution C125A.

154. The immunoconjugate of any one of claims 132-153, 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.

155. The immunoconjugate of any one of claims 132-154, wherein the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO:20.

156. The immunoconjugate of any one of claims 132-155, wherein the nucleated cells are immune cells.
157. The immunoconjugate of any one of claims 132-156, wherein the nucleated cells are a plurality of peripheral blood mononuclear cells (PBMCs).
158. The immunoconjugate of claim 157, wherein the plurality of PBMCs comprise two or more of T cell, B cell, NK cell, monocytes, dendritic cells or NK-T cells.
159. The immunoconjugate of any one of claims 132-156, wherein the nucleated cells are one or more of T cells, B cells, NK cells, monocytes, dendritic cells and/or NK-T cells.
160. The immunoconjugate of any one of claims 132-159, wherein the exogenous antigen is delivered to the nucleated cells intracellularly.
161. The immunoconjugate of any one of claims 132-160, wherein the exogenous antigen is a disease-associated antigen.
162. The immunoconjugate of any one of claims 132-161, wherein the disease-associated antigen is a cancer antigen, an infectious disease antigen, or a viral-associated disease antigen.
163. The immunoconjugate of any one of claims 132-162, wherein the exogenous antigen is a human papillomavirus (HPV) antigen.
164. The immunoconjugate of any one of claims 132-163, wherein the composition further comprises an adjuvant.

165. The immunoconjugate of claim 164, 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.

166. The immunoconjugate of any one of claims 132-165, 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.

167. The immunoconjugate of claim 166, wherein the width of the constriction is about 10% to about 99% of the mean diameter of the input nucleated cells.

168. The immunoconjugate of claim 166 or 167, wherein the width of the constriction is about 4.2 μm to about 6 μm .

169. The immunoconjugate of any one of claims 166-168, wherein the width of the constriction is about 4.2 μm to about 4.8 μm .

170. The immunoconjugate of any one of claims 166-169, wherein the width of the constriction is about 4.5 μm .

171. The immunoconjugate of any one of claims 166-170, 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.

172. The immunoconjugate of any one of claims 166-171, 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.
173. The immunoconjugate of any one of claims 132-172, wherein the nucleated cells are conditioned with an adjuvant to form conditioned cells.
174. The immunoconjugate of claim 173, 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.
175. The immunoconjugate of claim 173 or 174, wherein the nucleated cells are conditioned before or after introducing the exogenous antigen into the nucleated cells.
176. The immunoconjugate of any one of claims 173-175, 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.
177. The immunoconjugate of any one of claims 173-176, wherein the adjuvant is a CpG oligodeoxynucleotide (ODN).
178. The immunoconjugate of any one of claims 173-177, wherein the adjuvant is CpG 7909.
179. The immunoconjugate of any one of claims 173-178, wherein the conditioned cells are a conditioned plurality of modified PBMCs.
180. The immunoconjugate of claim 179, wherein the plurality of modified PBMCs are further modified to increase expression of one or more of co-stimulatory molecules.

181. The immunoconjugate of claim 180, 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.
182. The immunoconjugate of any one of claims 179-181, wherein the plurality of modified PBMCs are further modified to increase expression of one or more cytokines.
183. The immunoconjugate of claim 182, wherein the cytokine is IL-15, IL-12, IL-2, IFN- α , or IL-21.
184. The immunoconjugate of any one of claims 179-183, 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.
185. The immunoconjugate of any one of claims 179-184, wherein the plurality of modified PBMCs have increased expression of one or more of IFN- γ , IL-6, MCP-1, MIP-1 β , IP-10, or TNF- α compared to a plurality of unconditioned PBMCs.
186. The immunoconjugate of any one of claims 179-185, wherein the expression of one or more of IFN- γ , IL-6, MCP-1, MIP-1 β , 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.
187. The immunoconjugate of any one of claims 132-186, wherein the composition and/or the immunoconjugate is administered intravenously.

188. The immunoconjugate of any one of claims 132-186, wherein the immunoconjugate is administered subcutaneously or intratumorally.
189. The immunoconjugate of claim 188, wherein the immunoconjugate is administered subcutaneously in combination with hyaluronidase.
190. The immunoconjugate of any one of claims 132-189, wherein the individual is a human.
191. The immunoconjugate of any one of claims 132-190, wherein the individual has cancer, an infectious disease, or a viral associated disease.
192. The immunoconjugate of any one of claims 132-191, wherein the composition is administered prior to, concurrently with, or following administration of another therapy.
193. The immunoconjugate of claim 192, wherein the another therapy is a chemotherapy or a radiation therapy.
194. Use of an effective amount of an immunoconjugate in the manufacture of a medicament for stimulating an immune response 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; 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 formulated for administration in conjunction with a composition comprising nucleated cells, wherein the nucleated cells comprise an exogenous antigen.
195. The use of claim 194, wherein the immunoconjugate is administered before, at the same time, or after the composition comprising nucleated cells.

196. Use of an effective amount of a composition in the manufacture of a medicament for stimulating an immune response in an individual, wherein the composition comprises nucleated cells comprising an exogenous antigen;

wherein the composition is formulated for administration in conjunction with an immunoconjugate comprising 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).

197. The composition of claim 196, wherein the composition comprising the nucleated cells is administered before, at the same time, or after the immunoconjugate.

198. A kit for use in the method of any one of claims 1-69.

199. 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.

200. 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).

201. 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.

202. A method for producing an immunoconjugate for use in conjunction with a composition comprising nucleated cells for stimulating an immune response in an individual, the method comprising expressing a nucleic acid encoding the immunoconjugate in a cell under conditions to produce the 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 administering a composition comprising nucleated cells, wherein the nucleated cells comprise an exogenous antigen.

203. The method of claim 202, wherein the immunoconjugate is a fusion protein.

204. A method for producing a composition comprising nucleated cells for use in conjunction with an immunoconjugate for stimulating an immune response in an individual, the method comprising introducing an exogenous antigen intracellularly to a population of nucleated cells;

wherein the composition is for use in conjunction with administration of 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; 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).