alanine, the tyrosine residue at position 45 by alanine and the leucine residue at position 72 of IL-2 by glycine essentially abolishes binding of this mutant IL-2 polypeptide to the α-subunit of the IL-2 receptor (CD25).

[0009] Further to the above-mentioned approaches, IL-2 immunotherapy may be improved by selectively targeting IL-2 to tumors, *e.g*. in the form of immunoconjugates comprising an antibody that binds to an antigen expressed on tumor cells. Several such immunoconjugates have been described (*see e.g*. Ko *et al*., *J Immunother* (2004) 27, 232-239; Klein *et al*., *Oncoimmunology* (2017) 6(3), e1277306).

[0010] Tumors may be able, however, to escape such targeting by shedding, mutating or downregulating the target antigen of the antibody. Moreover, tumor-targeted IL-2 may not come into optimal contact with effector cells such as cytotoxic T lymphocytes (CTLs), in tumor microenvironments that actively exclude lymphocytes. Thus, there remains a need to further improve IL-2 immunotherapy. An approach, which may circumvent the problems of tumor-targeting, is to target IL-2 directly to effector cells, in particular CTLs. A fusion protein of IL-2 and a PD-1 antigen-binding protein is described in WO 2018/184964 A1.

[0011] There remains a need for improved methods for inducing an effective endogenous T cell response.

[0012] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0013] In some examples, the disclosure provides 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.

[0014] In some examples, the disclosure provides 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.

[0015] In some examples, the disclosure provides 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.

[0016] In some examples, the disclosure provides 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. In some examples, the disease is cancer, an infectious disease, or a viral-associated disease.

[0016a] In one aspect, the present disclosure refers to 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.

[0016b] In one aspect, the present disclosure refers to 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.

[0016c] In one aspect, the present disclosure refers to 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.

[0016d] In one aspect, the present disclosure refers to 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.

[0017] In some examples, the disclosure provides 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. In some examples, the individual has a disease responsive to vaccination. In some examples, the disease is cancer, an infectious disease, or a viral-associated disease.

[0018] In some examples, the disclosure provides 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.

[0019] In some examples of the disclosure, the second polypeptide binds a T cell. In some examples, the second polypeptide binds PD-1 expressed on the T cell. In some examples, the second polypeptide is an antigen-binding moiety that specifically binds PD-1. In some examples, 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. In some examples, 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. In some examples, 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. In some examples, 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. In some examples, 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. In some examples, 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.

[0020] In some examples of the disclosure, the second polypeptide specifically binds a target antigen presented on a tumor cell or in a tumor cell environment. In some examples, 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). In some examples, the second polypeptide binds FAP. In some examples, the second polypeptide is an antigen-binding moiety that specifically binds FAP. In some examples, 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. In some examples, 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. In some examples, 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. In some examples, 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.

[0021] In some examples of the disclosure, the mutant IL-2 polypeptide further comprises the amino acid substitution T3A and/or the amino acid substitution C125A. In some examples, 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. In some examples, the mutant IL-2 polypeptide comprises the amino acid sequence of SEQ ID NO:20.

[0022] In some examples of the disclosure, the nucleated cells are immune cells. In some examples, the nucleated cells are a plurality of peripheral blood mononuclear cells (PBMCs). In some examples, the plurality of PBMCs comprise two or more of T cell, B cell, NK cell, monocytes, dendritic cells or NK-T cells. In some examples, the nucleated cells are one or more of T cells, B cells, NK cells, monocytes, dendritic cells and/or NK-T cells.

[0023] In some examples of the disclosure, the exogenous antigen is delivered to the nucleated cells intracellularly. In some examples, the exogenous antigen is a disease-associated antigen. In some examples, the disease-associated antigen is a cancer antigen, an infectious disease antigen, or a viral-associated disease antigen. In some examples, the exogenous antigen is a human papillomavirus (HPV) antigen.

[0024] In some examples of the disclosure, the composition further comprises an adjuvant. In some examples, 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.

[0025] In some examples of the disclosure, 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. In some examples, the width of the constriction is about 10% to about 99% of the mean diameter of the input nucleated cells. In some examples, the width of the constriction is about 4.2 μm to about 6 μm. In some examples, the width of the constriction is about 4.2 μm to about 4.8 μm. In some examples, the width of the constriction is about 4.5 μm. In some examples, 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. In some examples, 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.

[0026] In some examples of the disclosure, the nucleated cells are conditioned with an adjuvant to form conditioned cells. In some examples, 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. In some examples, the nucleated cells are conditioned before or after introducing the exogenous antigen into the nucleated cells. In some examples, 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. In some examples, the adjuvant is a CpG oligodeoxynucleotide (ODN). In some examples, the adjuvant is CpG 7909. In some examples, the conditioned cells are a conditioned plurality of modified PBMCs. In some examples, the plurality of modified PBMCs are further modified to increase expression of one or more of co-stimulatory molecules. In some examples, 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. In some examples, the plurality of modified PBMCs are further modified to increase expression of one or more cytokines. In some examples, the cytokine is IL-15, IL-12, IL-2, IFN-α, or IL-21. In some examples, 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. In some examples, 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. In some examples, 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.

[0027] In some examples of the disclosure, the immunoconjugate is administered before, at the same time, or after administration of the composition comprising nucleated cells. In some examples, the composition comprising nucleated cells is administered a plurality of times. In some examples, the immunoconjugate is administered a plurality of times after administration of the composition comprising nucleated cells. In some examples, the composition and/or the immunoconjugate is administered intravenously. In some examples, the immunoconjugate is administered subcutaneously or intratumorally. In some examples, the immunoconjugate is administered subcutaneously in combination with hyaluronidase.

[0028] In some examples of the disclosure, the individual is a human. In some examples, the individual has cancer, an infectious disease, or a viral associated disease. In some examples, the composition of nucleated cells and/or the immunoconjugate is administered prior to, concurrently with, or following administration of another therapy. In some examples, the another therapy is a chemotherapy or a radiation therapy.

[0029] In some examples, the disclosure provides 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). In some examples, the disease is cancer, an infectious disease, or a viral-associated disease. In some examples, the composition comprising nucleated cells is administered before, at the same time, or after the immunoconjugate.

[0030] In some examples, the disclosure provides 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. In some examples, the disease is cancer, an infectious disease, or a viral-associated disease. In some examples, the composition comprising the nucleated cells is administered before, at the same time, or after the immunoconjugate.

[0031] In some examples, the disclosure provides the 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. In some examples, the immunoconjugate is administered before, at the same time, or after the composition comprising nucleated cells.

[0032] In some examples, the disclosure provides the 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). In some examples, the composition comprising the nucleated cells is administered before, at the same time, or after the immunoconjugate.

[0033] In some examples, the disclosure provides a kit for use in any of the methods described herein. In some examples, the disclosure provides 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. In some examples, the disclosure provides 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). In some examples, the disclosure provides 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.

[0033a] In one aspect, the present disclosure refers to 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.

[0033b] In one aspect, the present disclosure refers to 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).

[0033c] In one aspect, the present disclosure refers to 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.

[0034] In some examples, the disclosure provides 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. In some examples, the immunoconjugate is a fusion protein.

[0035] In some examples, the disclosure provides 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).

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. 1 is a graph showing tumor volume over time in a TC1 mouse tumor model. Mice were treated with PD1-IL2v alone, with SQZ-PBMC (SQZ-PBMC comprising an HPV antigen delivered intracellularly) once with at three doses of cells or PD1-IL2v was given three times with a single dose range. Administration of SQZ-PBMC is indicated as “SQZ Prime.” Period of administration of PD1-IL2v is indicated by the gray bar.