[0006] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

SUMMARY

[0007] The present disclosure provides peripheral blood mononuclear cells (PBMCs) comprising an antigen for the stimulation of immune responses in individuals. In some examples, antigens are delivered intracellularly using the Cell Squeeze® platform. The present inventors have unexpectedly found that mixed populations of PBMCs have greater efficacy than pure B cell and T cell populations. In addition, the disclosure is based at least in part on the unexpected discovery that conditioning PBMCs with an adjuvant increased activation of antigen presenting cells of the PBMCs leading to increased immunostimulation when the PBMCs are administered to an individual.

[0008] In some examples, the disclosure provides a plurality of modified PBMCs comprising an antigen, wherein the antigen is exogenous to the modified PBMCs. In some examples, the disclosure provides a plurality of modified PMBCs comprising an antigen, wherein the antigen is exogenous to the modified PBMCs, wherein the antigen is a cancer antigen, an infectious disease antigen or a viral-disease associated antigen. In some examples, the disclosure provides a conditioned plurality of modified PBMCs comprising an antigen, wherein the antigen is exogenous to the modified PBMCs. In some examples, the disclosure provides a conditioned plurality of modified PMBCs comprising an antigen, wherein the antigen is exogenous to the modified PBMCs, wherein the antigen is a cancer antigen, an infectious disease antigen or a viral-disease associated antigen. In some examples, the disclosure provides a conditioned plurality of modified PBMCs comprising an antigen and an adjuvant, wherein the antigen is exogenous to the modified PBMCs. In one ~~aspect~~example, the ~~present~~ disclosure ~~refers to~~provides a conditioned plurality of modified PBMCs comprising an antigen, wherein the antigen is exogenous to the modified PBMCs.

[0008a] In one aspect, the present disclosure refers to a conditioned plurality of modified PBMCs comprising an antigen, wherein the antigen is exogenous to the modified PBMCs, wherein the plurality of modified PBMCs comprises two or more of T cells, B cells, NK cells, monocytes, or combinations thereof , and wherein the plurality of modified PBMCs have been incubated with an adjuvant for a sufficient time to condition the plurality of modified PBMCs.

[0009] In some examples, the disclosure provides a conditioned plurality of PBMCs comprising an antigen, prepared by incubating the plurality of PBMCs comprising the antigen with an adjuvant for a sufficient time for the PBMCs to condition, thereby generating the conditioned plurality of PBMCs comprising the antigen. In some examples, the disclosure provides a conditioned plurality of PBMCs comprising an antigen, prepared by incubating the plurality of PBMCs with an adjuvant for a sufficient time for the PBMCs to condition prior to introducing the antigen to the PBMCs, thereby generating the conditioned plurality of PBMCs comprising the antigen.

[0010] In some examples, the disclosure provides a plurality of modified PBMCs comprising an antigen, prepared by a process comprising the steps of: a) passing a cell suspension comprising a

plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the antigen for a sufficient time to allow the antigen to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen. In some examples, the disclosure provides a conditioned plurality of modified PBMCs comprising an antigen, prepared by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the antigen for a sufficient time to allow the antigen to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen; and c) incubating the plurality of modified PBMCs comprising the antigen with an adjuvant for a sufficient time for the modified PBMCs comprising the antigen to condition, thereby generating the conditioned plurality of modified PBMCs comprising the antigen. In some examples, the process further comprises: isolating the plurality of modified PBMCs comprising the antigen from the cell suspension before incubation with the adjuvant to condition the modified PBMCs. In some examples, the disclosure provides a plurality of modified PBMCs comprising an antigen and an adjuvant, prepared by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen and the adjuvant to pass through to form a plurality of perturbed input PBMCs; and b) incubating the plurality of perturbed input PBMCs with the antigen and the adjuvant for a sufficient time to allow the antigen and the adjuvant to enter the perturbed input PBMCs; thereby generating the plurality of modified PBMCs comprising the antigen and adjuvant. ~~In one aspect, the present disclosure refers to a plurality of modified PBMCs comprising an antigen, or an antigen and an adjuvant, prepared by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen or the antigen and the adjuvant to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the antigen, or the antigen and the adjuvant for a sufficient time to allow the antigen or the antigen and the adjuvant to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant. In another aspect, the present disclosure refers to a plurality of modified PBMCs comprising an antigen, prepared by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of~~

~~the input PBMCs large enough for a nucleic acid encoding the antigen to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the nucleic acid encoding the antigen for a sufficient time to allow the nucleic acid encoding the antigen to enter the perturbed input PBMCs, wherein the nucleic acid is expressed in the PBMCs to produce the antigen thereby generating a plurality of modified PBMCs comprising the antigen. In yet another aspect, the present disclosure refers to a plurality of modified PBMCs comprising an antigen and an adjuvant, prepared by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs comprising the antigen through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the adjuvant to pass through to form a plurality of perturbed input PBMCs; and b) incubating the plurality of perturbed input PBMCs with the adjuvant for a sufficient time to allow the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen and the adjuvant.~~

[0010a] In one aspect, the present disclosure refers to a conditioned plurality of modified PBMCs comprising an antigen and a first adjuvant, wherein the antigen and the first adjuvant are exogenous to the modified PBMCs, wherein the plurality of modified PBMCs comprises two or more of T cells, B cells, NK cells, monocytes, or combinations thereof, and wherein the plurality of modified PBMCs have been incubated with a second adjuvant for a sufficient time to condition the plurality of modified PBMCs.

[0011] In some examples, the disclosure provides a conditioned plurality of modified PBMCs comprising an antigen, prepared by a process comprising the steps of: a) incubating a plurality of input PBMCs with an adjuvant for a sufficient time for the input PBMCs to condition, thereby generating a conditioned plurality of input PBMCs; b) passing a cell suspension comprising the conditioned plurality of input PBMCs through a cell-deforming constriction, wherein a diameter

PBMCs as compared to corresponding modified PBMCs prepared without the further incubation step.

[0012] In some examples, the disclosure provides a composition comprising the plurality of modified PBMCs as described herein for use in a method of treatment of the human or animal body by surgery, therapy or diagnosis. In some examples, the disclosure provides a composition comprising the plurality of modified PBMCs as described herein for use in the treatment of a cancer, an infectious disease or a viral-associated disease. In one aspect, the present disclosure refers to a composition comprising the conditioned plurality of modified PBMCs disclosed herein. In one aspect, the present disclosure refers to a composition comprising the conditioned plurality of modified PBMCs disclosed herein for use as a medicament. ~~In another aspect, the present disclosure refers to a composition comprising the plurality of modified PBMCs disclosed herein for use in the treatment of the human or animal body by surgery, therapy or diagnosis.~~ In yet another aspect, the present disclosure refers to the use of the~~a~~ composition comprising the conditioned plurality of modified PBMCs disclosed herein in the manufacture of a medicament for ~~the treatment of~~ treating a cancer~~,~~ or an infectious disease in an individual in need thereof~~for a viral-associated disease~~.

[0013] In some examples, the disclosure provides a composition comprising a conditioned plurality of modified PBMCs comprising an antigen for use as a medicament, wherein the conditioned plurality of modified PBMCs is prepared by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the antigen for a sufficient time to allow the antigen to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen; and c) incubating the plurality of modified PBMCs comprising the antigen with an adjuvant for a sufficient time for the modified PBMCs comprising the antigen to condition, thereby generating the conditioned plurality of modified PBMCs comprising the antigen. In some examples, the disclosure provides a composition comprising a conditioned plurality of modified PBMCs comprising an antigen for use in a method of treatment of the human or animal body by surgery, therapy or diagnosis, wherein the conditioned plurality of modified PBMCs is prepared by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the antigen a sufficient time to allow the antigen to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen; and c) incubating the plurality of modified PBMCs comprising the antigen with an adjuvant for a sufficient time for the modified PBMCs comprising the antigen to condition, thereby generating the conditioned plurality of modified PBMCs comprising the antigen. In some examples, the disclosure provides a composition comprising a conditioned plurality of modified PBMCs comprising an

input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen and adjuvant; and c) administering the plurality of modified PBMCs to the individual. ~~In one aspect, the present disclosure refers to the use of a conditioned plurality of PBMCs comprising an antigen in the manufacture of a medicament for stimulating an immune response in an individual, wherein the conditioned plurality of PBMCs is generated by a process comprising the step of: incubating a plurality of PBMCs with an adjuvant for a sufficient time for the PBMCs to condition, wherein the plurality of PBMCs comprises an antigen before the conditioning or wherein an antigen is introduced to the plurality of PBMCs after the conditioning, thereby generating the conditioned plurality of PBMCs comprising the antigen.~~

[0022] In some examples, the disclosure provides a method for stimulating an immune response in an individual, comprising: a) incubating a plurality of input PBMCs with an adjuvant for a sufficient time for the input PBMCs to condition, thereby generating a conditioned plurality of input PBMCs; b) passing a cell suspension comprising the conditioned plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for an antigen to pass through to form a conditioned plurality of perturbed input PBMCs; c) incubating the conditioned plurality of perturbed input PBMCs with the antigen for a sufficient time to allow the antigen to enter the perturbed input PBMCs, thereby generating the conditioned plurality of modified PBMCs comprising the antigen; and d) administering the conditioned plurality of modified PBMCs to the individual. In some examples, the disclosure provides a method for stimulating an immune response in an individual, comprising: a) passing a cell suspension comprising a plurality of input PBMCs comprising an adjuvant through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for an antigen to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the antigen for a sufficient time to allow the antigen to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen and the adjuvant; and c) administering the plurality of modified PBMCs to the individual. In some examples, the disclosure provides a method for stimulating an immune response in an individual, comprising: a) passing a cell suspension comprising an input PBMCs comprising an antigen through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for an adjuvant to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the adjuvant for a sufficient time to allow the adjuvant to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen and the adjuvant; and c) administering the plurality of modified PBMCs to the individual. In some examples, the disclosure provides a method for stimulating an immune response in an individual, comprising: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the

constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for an antigen to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the antigen for a sufficient time to allow the antigen to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen; c) administering the plurality of modified PBMCs to the individual; and d) administering an adjuvant to the individual. In some examples, the disclosure provides a method for stimulating an immune response in an individual, comprising: a) passing a cell suspension comprising an input PBMCs comprising an antigen through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for an adjuvant to pass through to form a plurality of perturbed input PBMCs; b) incubating the plurality of perturbed input PBMCs with the adjuvant for a sufficient time to allow the adjuvant to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen and the adjuvant; and c) administering the plurality of modified PBMCs to the individual; and d) administering an adjuvant to the individual. ~~In one aspect, the present disclosure refers to the use of a plurality of modified PBMCs comprising an antigen or an antigen and an adjuvant in the manufacture of a medicament for stimulating an immune response in an individual, wherein the plurality of PBMCs is generated by a process comprising the steps of: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen or the antigen and the adjuvant to pass through to form a plurality of perturbed input PBMCs; and b) incubating the plurality of perturbed input PBMCs with the antigen or the antigen and the adjuvant for a sufficient time to allow the antigen or the antigen and the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant. In another aspect, the present disclosure refers to use of a plurality of modified PBMCs comprising an antigen and an adjuvant in the manufacture of a medicament for stimulating an immune response in an individual, wherein the plurality of modified PBMCs comprising the antigen and the adjuvant is generated by a process comprising the steps of: a) passing a cell suspension comprising an input PBMCs comprising the antigen through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the adjuvant to pass through to form a plurality of perturbed input PBMCs; and b) incubating the plurality of perturbed input PBMCs with the adjuvant for a sufficient time to allow the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen and the adjuvant.~~

[0023] In some examples, the disclosure provides a method for stimulating an immune response in an individual, comprising: administering to the individual a plurality of PBMCs associated with an antigen, wherein the plurality of modified PBMCs is prepared by a process comprising the steps of: a) incubating a plurality of input PBMCs with an antigen for a sufficient time to allow the antigen to associate with the cell surface of the input PBMCs,

thereby generating the plurality of PBMCs associated with the antigen; and b) administering the plurality of modified PBMCs to the individual. In some examples, the method further comprises administering an adjuvant to the individual. ~~In one aspect, the present disclosure refers to the use of a plurality of modified PBMCs associated with an antigen in the manufacture of a medicament for stimulating an immune response in an individual, wherein the plurality of modified PBMCs is prepared by a process comprising the step of: incubating a plurality of input PBMCs with an antigen for a sufficient time to allow the antigen to associate with the cell surface of the input PBMCs, thereby generating the plurality of modified PBMCs associated with the antigen.~~

[0023a] In one aspect, the present disclosure refers to use of the composition comprising the conditioned plurality of modified PBMCs disclosed herein in the manufacture of a medicament for stimulating an immune response in an individual in need thereof.

[0024] In some examples, the disclosure provides a method for generating a conditioned plurality of PBMCs comprising an antigen, comprising incubating a plurality of PBMCs comprising the antigen with an adjuvant for a sufficient time for the PBMCs to condition, thereby generating the conditioned plurality of PBMCs comprising the antigen. In some examples, the disclosure provides a method for generating a conditioned plurality of modified PBMCs comprising an antigen, comprising: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen to pass through to form a plurality of perturbed input PBMCs; b)

incubation step. In one aspect, the present disclosure refers to a method for generating a conditioned plurality of modified PBMCs comprising an antigen, wherein the method comprises ~~comprising~~ incubating a plurality of PBMCs comprising the antigen with ~~an~~ a first adjuvant for a sufficient time ~~for the PBMCs~~ to condition ~~before or after introducing the antigen to~~ the plurality of PBMCs, wherein the plurality of PBMCs comprises two or more of T cells, B cells, NK cells, monocytes, or combinations thereof, thereby generating the conditioned plurality of modified PBMCs comprising the antigen. ~~In another aspect, the present disclosure refers to a method for generating a plurality of modified PBMCs comprising an antigen, or an antigen and an adjuvant, comprising: a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen or the antigen and the adjuvant to pass through to form a plurality of perturbed input PBMCs; and b) incubating the plurality of perturbed input PBMCs with the antigen or the antigen and the adjuvant for a sufficient time to allow the antigen or the antigen and the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant.~~

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIG. 1A is a schematic diagram of a representative cell within a plurality of PBMCs, displaying the SQZ-mediated delivery of E6 and/or E7 SLP; and the subsequent processing and presentation of E6 and E7 epitopes, respectively, on MHC-I. The representative cell can be any one of the PBMC cell types (such as T cells, monocytes, NK cells, and B cells).

[0026] FIG. 1B is a schematic representative of cohorts in the escalation phase in the monotherapy administration of PBMCs comprising an HPV antigen to an individual, with or without co-administration of a CpG adjuvant. The amount of circles depicts relative doses of modified PBMCs, arrows depict administrations, double helix represents CpG adjuvant, and "AVB" indicates additional vaccine boosts.

CLAIMS

What is claimed is:

1. A conditioned plurality of modified PBMCs comprising an antigen, wherein the antigen is exogenous to the modified PBMCs, wherein the plurality of modified PBMCs comprises two or more of T cells, B cells, NK cells, monocytes, or combinations thereof, and wherein the plurality of modified PBMCs have been incubated with an adjuvant for a sufficient time to condition the plurality of modified PBMCs.

2. The conditioned plurality of modified PBMCs of claim 1, wherein the antigen was intracellularly delivered to a plurality of input PBMCs to produce the plurality of modified PBMCs by passing a cell suspension comprising the plurality of input PBMCs through a cell-deforming constriction, thereby causing perturbations of the input PBMCs such that the antigen enters the input PBMCs through the perturbations when contacted with the input PBMCs.

3. A conditioned plurality of modified PBMCs comprising an antigen and a first adjuvant, wherein the antigen and the first adjuvant are exogenous to the modified PBMCs, wherein the plurality of modified PBMCs comprises two or more of T cells, B cells, NK cells, monocytes, or combinations thereof, and wherein the plurality of modified PBMCs have been incubated with a second adjuvant for a sufficient time to condition the plurality of modified PBMCs.

4~~2~~. The conditioned plurality of modified PBMCs of claim 3~~1~~, wherein the antigen and/or the first~~modified PBMCs further comprise an~~ adjuvant was intracellularly delivered to the plurality of input PBMCs to produce the plurality of modified PBMCs by passing a cell suspension comprising the plurality of input PBMCs through a cell-deforming constriction, thereby causing perturbations of the input PBMCs such that the antigen and/or the first adjuvant enters the input PBMCs through the perturbations when contacted with the input PBMCs.

~~3. The conditioned plurality of PBMCs of claim 1 or 2, prepared by incubating the plurality of PBMCs with an adjuvant for a sufficient time for the PBMCs to condition before or after introducing the antigen to the PBMCs, thereby generating the conditioned plurality of PBMCs comprising the antigen.~~

~~4. A plurality of modified PBMCs comprising an antigen, or an antigen and an adjuvant, prepared by a process comprising the steps of:~~

~~a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen or the antigen and the adjuvant to pass through to form a plurality of perturbed input PBMCs;~~

~~b) incubating the plurality of perturbed input PBMCs with the antigen, or the antigen and the adjuvant for a sufficient time to allow the antigen or the antigen and the adjuvant to enter the perturbed input PBMCs, thereby generating a plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant.~~

~~5. A plurality of modified PBMCs comprising an antigen, prepared by a process comprising the steps of:~~

~~a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for a nucleic acid encoding the antigen to pass through to form a plurality of perturbed input PBMCs;~~

~~b) incubating the plurality of perturbed input PBMCs with the nucleic acid encoding the antigen for a sufficient time to allow the nucleic acid encoding the antigen to enter the perturbed input PBMCs, wherein the nucleic acid is expressed in the PBMCs to produce the antigen thereby generating a plurality of modified PBMCs comprising the antigen.~~

~~6. The plurality of modified PBMCs of claim 4, wherein the input PBMCs comprise an adjuvant.~~

~~7. A plurality of modified PBMCs comprising an antigen and an adjuvant, prepared by a process comprising the steps of:~~

~~a) passing a cell suspension comprising a plurality of input PBMCs comprising the antigen through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of~~

~~the input PBMCs large enough for the adjuvant to pass through to form a plurality of perturbed input PBMCs; and~~

~~b) incubating the plurality of perturbed input PBMCs with the adjuvant for a sufficient time to allow the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen and the adjuvant.~~

~~8. The plurality of modified PBMCs according to any one of claims 4-7, wherein the process further comprises:~~

~~a) incubating the plurality of input PBMCs with an adjuvant or a second adjuvant for a sufficient time for the input PBMCs to condition, thereby generating a conditioned plurality of input PBMCs; and/or~~

~~b) incubating the plurality of modified PBMCs comprising the antigen with an adjuvant or incubating the plurality of modified PBMCs comprising the antigen and the adjuvant with a second adjuvant for a sufficient time for the modified PBMCs comprising the antigen or the antigen and the adjuvant to condition, thereby generating a conditioned plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant.~~

5~~9~~. A composition comprising the conditioned plurality of modified PBMCs of any one of claims 1 to ~~-4~~8.

6~~10~~. A composition comprising the conditioned plurality of modified PBMCs of any one of claims 1 to ~~-4~~8 for use as a medicament.

~~11. A composition comprising the plurality of modified PBMCs of any one of claims 1-8 for use in the treatment of the human or animal body by surgery, therapy or diagnosis.~~

7~~12~~. Use of the~~a~~ composition comprising the conditioned plurality of modified PBMCs of claim 5 or 6~~any one of claims 9-11~~ in the manufacture of a medicament for ~~the treatment of~~treating a cancer~~,~~ or an infectious disease in an individual in need thereof~~or a viral-associated disease~~.

8. The use of claim 7, wherein: (i) the cancer comprises a head and neck cancer, cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer, perianal cancer, anogenital cancer, oral cancer, or salivary cancer; or (ii) the infectious disease comprises a human immunodeficiency virus (HIV), human papillomavirus (HPV), Epstein-Barr virus (EBV), molluscum contagiosum virus (MCV), hepatitis B virus (HBV), or hepatitis C virus (HCV).

~~13. Use of a conditioned plurality of PBMCs comprising an antigen in the manufacture of a medicament for stimulating an immune response in an individual, wherein the conditioned plurality of PBMCs is generated by a process comprising the step of: incubating a plurality of PBMCs with an adjuvant for a sufficient time for the PBMCs to condition, wherein the plurality of PBMCs comprises an antigen before the conditioning or wherein an antigen is introduced to the plurality of PBMCs after the conditioning, thereby generating the conditioned plurality of PBMCs comprising the antigen.~~

~~14. Use of a plurality of modified PBMCs comprising an antigen or an antigen and an adjuvant in the manufacture of a medicament for stimulating an immune response in an individual, wherein the plurality of PBMCs is generated by a process comprising the steps of:~~

~~a) passing a cell suspension comprising a plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the antigen or the antigen and the adjuvant to pass through to form a plurality of perturbed input PBMCs; and~~

~~b) incubating the plurality of perturbed input PBMCs with the antigen or the antigen and the adjuvant for a sufficient time to allow the antigen or the antigen and the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant.~~

~~15. The use of claim 14, wherein the input PBMCs comprise an adjuvant.~~

~~16. Use of a plurality of modified PBMCs comprising an antigen and an adjuvant in the manufacture of a medicament for stimulating an immune response in an individual, wherein the~~

plurality of modified PBMCs comprising the antigen and the adjuvant is generated by a process comprising the steps of:

a) passing a cell suspension comprising an input PBMCs comprising the antigen through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough for the adjuvant to pass through to form a plurality of perturbed input PBMCs; and

b) incubating the plurality of perturbed input PBMCs with the adjuvant for a sufficient time to allow the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen and the adjuvant.

17. The use of any one of claim 14-16, wherein the PBMCs are conditioned by a process comprising the steps of:

a) incubating a plurality of input PBMCs with an adjuvant or a second adjuvant for a sufficient time for the input PBMCs to condition, thereby generating a conditioned plurality of input PBMCs; and/or

b) incubating the plurality of modified PBMCs comprising the antigen with an adjuvant or incubating the plurality of modified PBMCs comprising the antigen and the adjuvant with a second adjuvant for a sufficient time for the modified PBMCs comprising the antigen or the antigen and the adjuvant to condition, thereby generating a conditioned plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant.

18. The use of any one of claim 14-17, wherein the plurality of modified PBMCs is formulated for administration in combination with an adjuvant.

19. Use of a plurality of modified PBMCs associated with an antigen in the manufacture of a medicament for stimulating an immune response in an individual, wherein the plurality of modified PBMCs is prepared by a process comprising the step of: incubating a plurality of input PBMCs with an antigen for a sufficient time to allow the antigen to associate with the cell surface of the input PBMCs, thereby generating the plurality of modified PBMCs associated with the antigen.

920. Use of the composition comprising the conditioned plurality of modified PBMCs of claim 5 or 6 in the manufacture of a medicament for stimulating anThe use of any one of claims 13-19, wherein the stimulation of the immune response is for treating cancer, an infectious disease, or a viral associated disease in an individual in need thereof.

1021. The use of any one of claims 713 to -920, wherein the conditioned plurality of PBMCs is formulated for administration prior to, concurrently with, or following administration of:

a) a cytokine;

b) an immune checkpoint inhibitor; and/or

c) a therapeutic agent.

1122. The use of claim 1021, wherein the plurality of PBMCs is formulated for administration prior to, concurrently with, or following administration of:

a) a cytokine, wherein the cytokine is comprises IL-2;

b) an immune checkpoint inhibitor; wherein the immune checkpoint inhibitor is targeted to any one of PD-1, PD-L1, CTLA-4, LAG3, VISTA, and TIM-3; and/or

c) a therapeutic agent, wherein the therapeutic agent is comprises a chemotherapeutic agent.

1223. A method for generating a conditioned plurality of modified PBMCs comprising an antigen, wherein the method comprises comprising incubating a plurality of PBMCs comprising the antigen with a first an adjuvant for a sufficient time for the PBMCs to condition before or after introducing the antigen to the plurality of PBMCs, wherein the plurality of PBMCs comprises two or more of T cells, B cells, NK cells, monocytes, or combinations thereof, thereby generating the conditioned plurality of modified PBMCs comprising the antigen.

1324. TheA method of claim 12,for generating a plurality of modified PBMCs comprising an antigen, or an antigen and an adjuvant, comprising: wherein the antigen is intracellularly delivered to the plurality of PBMCs by

a) passing a cell suspension comprising thea plurality of input PBMCs through a cell-deforming constriction, wherein a diameter of the constriction is a function of a diameter of the input PBMCs in the suspension, thereby causing perturbations of the input PBMCs large enough forsuch that the antigen enters the PBMCs through the perturbations when contacted with the

PBMCsor the antigen and the adjuvant to pass through to form a plurality of perturbed input PBMCs; and

b) incubating the plurality of perturbed input PBMCs with the antigen or the antigen and the adjuvant for a sufficient time to allow the antigen or the antigen and the adjuvant to enter the perturbed input PBMCs, thereby generating the plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant.

1425. The method of claim 1324, wherein the method further comprises:

a) incubating the plurality of input PBMCs comprising the antigen with an adjuvant or a second adjuvant for a sufficient time for the input PBMCs to condition the plurality of PBMCs., thereby generating a conditioned plurality of input PBMCs; and/or

b) incubating the plurality of modified PBMCs comprising the antigen with an adjuvant or incubating the plurality of modified PBMCs comprising the antigen and the adjuvant with a second adjuvant for a sufficient time for the modified PBMCs comprising the antigen or the antigen and the adjuvant to condition, thereby generating the conditioned plurality of modified PBMCs comprising the antigen or the antigen and the adjuvant.

26. The plurality of PBMCs, composition, use or method according to any one of claims 4-25 wherein the process further comprises a step of incubating the input PBMCs and/or the modified PBMCs with an agent that enhances the viability and/or function of the modified PBMCs as compared to corresponding modified PBMCs prepared without the further incubation step.

1527. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 2, 4 to 11, 13, and 144-12 and 14-26, wherein the diameter of the cell-deforming constriction is about 10% to about 99% of the mean diameter of the plurality of input PBMCs.

1628. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 2, 4 to 11, 13, and 144-12 and 14-26, wherein the diameter of the cell-deforming constriction is: (a) about 4.2 µm to about 6 µm; or (b) about 4.2 µm to about 4.8 µm; or (c) about 4.5 µm.

17~~29~~. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 2, 4 to 11, and 13 to 16~~4-12 and 14-28~~, wherein the cell suspension comprising the plurality of input PBMCs is passed through multiple constrictions, wherein the multiple constrictions are arranged in series and/or in parallel.

18~~30~~. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 2, 4 to 11, and 13 to 17~~4-8 and 13-29~~, wherein the sufficient time comprises~~plurality of modified PBMCs is incubated with the adjuvant for about~~ 1 hour to less than ~~about~~ 24 hours; ~~or for about~~ 2 hours to ~~about~~ 10 hours; ~~or about~~ 3 hours to ~~about~~ 6 hours; or ~~for about~~ 4 hours~~, for the modified PBMCs to condition~~.

19. The conditioned plurality of modified PBMCs, composition, use, or method according to claim 18, wherein the sufficient time comprises 1 hour, 2 hours, 3 hours, 3.5 hours, 4 hours, 4.5 hours, 5 hours, 5.5 hours, 6 hours, 8 hours, 12 hours, 16 hours, or 20 hours.

~~31. The plurality of PBMCs, composition, use or method according to any one of claims 1-30, wherein the antigen and/or the adjuvant are present in at least about 70% of the cells in the plurality of PBMCs.~~

20~~32~~. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 1 to 19~~2-4, 6-18 and 20-31~~, wherein the adjuvant, the first adjuvant, and/or the second adjuvant comprises ~~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.

21~~33~~. The conditioned plurality of modified PBMCs, composition, use, or method according to claim 20~~any one of claims 2-4, 6-18 and 20-32~~, wherein the adjuvant is ~~a~~ CpG oligodeoxynucleotide (ODN).

22. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 3-20, wherein the first adjuvant and the second adjuvant are the same.

23. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 3-20, wherein the first adjuvant and the second adjuvant are not the same.

2434. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 1 to -2333, wherein the antigen comprises ~~is~~ a disease-associated antigen, an infectious disease antigen, or a cancer antigen.

2535. The conditioned plurality of modified PBMCs, composition, use, or method according to claim 2434, wherein the disease-associated antigen is a human papillomavirus (HPV) antigen.

26. The conditioned plurality of modified PBMCs, composition, use, or method according to claim 24, wherein the infectious disease antigen is a hepatitis B virus (HBV) antigen.

27. The conditioned plurality of modified PBMCs, composition, use, or method according to claim 24, wherein the cancer antigen is from a head and neck cancer, cervical cancer, vulvar cancer, vaginal cancer, penile cancer, anal cancer, perianal cancer, anogenital cancer, oral cancer or salivary cancer.

~~36. The plurality of PBMCs, composition, use or method according to any one of claims 1-35, wherein the cells are further modified to increase expression of:~~
~~a) one or more of co-stimulatory molecules; and/or~~
~~b) one or more cytokines.~~

~~37. The plurality of PBMCs, composition, use or method according to claim 36, wherein:~~
~~a) the cells are further modified to increase expression of one or more of co-stimulatory molecules 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, CD112, or scFv anti-CD28; and/or~~
~~b) the cells are further modified to increase expression of one or more cytokines, wherein the cytokine is IL-15, IL-12, IL-2, IFN-α, or IL-21.~~

2838. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 1 to 273, 8-13, 17, 18, 20-23 and 25-37, wherein:
a) one or more co-stimulatory molecules is upregulated in the B cells of the conditioned plurality of modified PBMCs exhibit increased expression of: (a) one or more co-stimulatory moleculescompared to the B cells in a plurality of nonconditioned PBMCs, wherein the co-stimulatory molecule is CD80 or CD86; and/or
(b) one or more cytokines; as compared to a corresponding non-conditioned plurality of PBMCsthe conditioned PBMCs have increased expression of one or more of IFN-γ, IL-6, MCP-1, MIP-1β, IP-10, or TNF-α compared to unconditioned PBMCs.

29. The conditioned plurality of modified PBMCs, composition, use, or method according to claim 28, wherein: (a) the one or more co-stimulatory molecules comprise B7-H2 (ICOSL), B7-1 (CD80), B7-2 (CD86), CD70, LIGHT, HVEM, CD40, 4-1BBL, OX40L, TL1A, GITRL, CD30L, TIM4, SLAM, CD48, CD58, CD155, CD112, or scFv anti-CD28 ; and/or (b) the one or more cytokines comprise IL-15, IL-12, IL-2, IFN-α, IFN-γ, IL-6, MCP-1, MIP-1β, IP-10, TNF-α, or IL-21.

3039. The conditioned plurality of modified PBMCs, composition, use, or method according to claim 28 or 2938, wherein expression of (a) the one or more co-stimulatory molecules, (b) the one or more cytokines, or (c) both (a) and (b) are increased by at least:
a) the CD80 and/or CD86 is upregulated in the B cells of the conditioned plurality of modified PBMCs 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 as compared to the corresponding non-conditionedB cells in a plurality of nonconditioned PBMCs; and/or
b) the expression of one or more of IFN-γ, IL-6, MCP-1, MIP-1β, IP-10, or TNF-α is increased by 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.

31. The conditioned plurality of modified PBMCs, composition, use, or method according to any one of claims 1 to 29, wherein the conditioned plurality of modified PBMCs are capable of inducing an in vivo immune response, which is at least 2-fold greater than that induced by the corresponding non-conditioned plurality of modified PBMCs.