Summary

[0004] Provided are methods for cell processing, such as for transfer of viral vectors and/or immunoaffinity-based selection of cells. In some ~~embodiment~~examples, the cells are for use in cell therapy, such primary cells prepared for autologous or allogeneic transfer, e.g., in adoptive cell therapy. The methods may include additional cell processing steps, such as cell washing, isolation, separation.

[0005] In some ~~embodiment~~examples, the methods are carried out by incubating, in a vessel, such as an internal cavity of a centrifugal chamber, a composition (deemed an input composition), which contains cells and viral vector particles, the viral particles containing a recombinant viral vector, thereby generating an output composition that contains a plurality of the cells transduced with the viral vector. The centrifugal chamber typically is rotatable around an axis of rotation. The axis of rotation in some ~~embodiment~~examples is vertical. The chamber typically includes an end wall, a side wall extending from the end wall, such as a substantially rigid side wall, and at least one opening, such as an inlet/outlet or an inlet and an outlet. At least a portion of the side wall generally surrounds the internal cavity. The at least one opening (e.g., the inlet/outlet or the inlet and the outlet) is capable of permitting intake of liquid into the internal cavity and expression of liquid from the cavity. The at least one opening in some ~~embodiment~~examples is coaxial with the chamber and in some ~~embodiment~~examples is in an end wall of the chamber. The side wall may be a curvilinear, e.g., cylindrical or generally cylindrical.

[0006] In some ~~embodiment~~examples, the methods include incubating, in an internal cavity of a centrifugal chamber, an input composition containing cells and viral particles containing a recombinant viral vector, wherein said centrifugal chamber is rotatable around an axis of rotation and includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, at least a portion of said side wall surrounding said internal cavity and said at least one opening being capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity, wherein the centrifugal chamber is rotating around said axis of rotation during at least a portion of the incubation and the method generates an output composition containing a plurality of the cells transduced with the viral vector.

[0007] In some ~~embodiment~~examples, the centrifugal chamber further includes a movable member, such as a piston. In such ~~embodiment~~examples, the internal cavity is generally one of variable volume, e.g., a cavity of variable volume defined by the end wall, the side wall, and the movable member, e.g., the piston, such that the movable member is capable of moving within the chamber (such as axially within the chamber) to vary the internal volume of the cavity. In some ~~embodiment~~examples,

liquid is moved in and out of the chamber alternatively by way of a pump, syringe, and/or motor, or other device for intake and expressing liquid or gas, which for example pulls liquid from the cavity and/or pushes liquid in, while the volume of the cavity itself remains constant.

[0008] In some ~~embodiment~~examples, the methods include incubating, in an internal cavity of a centrifugal chamber, an input composition containing cells and a viral particle containing a recombinant viral vector, said centrifugal chamber being rotatable around an axis of rotation and comprising an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity, wherein the centrifugal chamber is rotating around the axis of rotation during at least a portion of the incubation, the total liquid volume of said input composition present in said cavity during rotation of said centrifugal chamber is no more than about 5 mL per square inch of the internal surface area of the cavity and the method generates an output composition comprising a plurality of the cells transduced with the viral vector.

[0009] The chamber may comprise two end walls. In some such ~~embodiment~~examples, one end wall together with other features defines the internal cavity, while the other is outside of the cavity. In some ~~embodiment~~examples, the cavity is bound by both end walls.

[0010] The at least one opening may comprise: an inlet and an outlet, respectively capable of permitting said intake and expression, or a single inlet/outlet, capable of permitting said intake and said expression.

[0011] Typically, the incubation is carried out at least in part under rotation of the chamber, such as under centrifugal force or acceleration. Thus, the methods in some ~~embodiment~~examples further include effecting rotation of the centrifugal chamber, such as around its axis of rotation, during at least a portion of the incubation.

[0012] In some of any such ~~embodiment~~examples, said rotating includes rotation at a relative centrifugal force (RCF) at an internal surface of the side wall of the cavity and/or at a surface layer of the cells of greater than at or about 200 g, greater than at or about 300 g, or greater than at or about 500 g. In some of any such ~~embodiment~~examples, said rotating includes rotation at a relative centrifugal force at an internal surface of the side wall of the cavity and/or at a surface layer of the cells that is: at or about 1000 g, 1500 g, 2000 g, 2100 g, 2200 g, 2500 g or 3000 g; or at least at or about 1000 g, 1500 g, 2000 g, 2100 g, 2200 g, 2500 g, or 3000 g. In some of any such ~~embodiment~~examples, said rotating includes rotation at a relative centrifugal force at an internal surface of the side wall of the cavity and/or at a surface layer of the cells that is: between or between

about 1000 and 3600, 1000 and 3200, 1000 and 2800, 1000 and 2000, 1000 and 1600, 1600 and 3600, 1600 and 3200, 1600 and 2800, 1600 and 2000, 2000 and 3600, 2000 and 3200, 2000 and 2800, 2800 and 3600, 2800 and 3200, 3200 and 3600, each inclusive; or at least at or about 2000 g, 2100, 2200 g, 2400 g, 2600g, 2800 g, 3000 g, 3200 g or 3600 g; or at or about 2000 g, 2100g, 2200 g, 2400 g, 2600g, 2800 g, 3000 g, 3200 g or 3600 g.

[0013] In some of any such ~~embodiment~~examples, the at least a portion of the incubation during which the chamber is rotating is for a time that is: greater than or about 5 minutes, such as greater than or about 10 minutes, greater than or about 15 minutes, greater than or about 20 minutes, greater than or about 30 minutes, greater than or about 45 minutes, greater than or about 60 minutes, greater than or about 90 minutes or greater than or about 120 minutes; or between or between about 5 minutes and 60 minutes, 10 minutes and 60 minutes, 15 minutes and 60 minutes, 15 minutes and 45 minutes, 30 minutes and 60 minutes or 45 minutes and 60 minutes, each inclusive.

[0014] In some ~~embodiment~~examples, the input composition (or the number of cells) in the cavity during the incubation, e.g., at any one time or during the entire incubation, and/or processed by the methods, includes at or about or at least about 1×10^6, 5×10^6, 1×10^7, 5×10^7, 1×10^8 or 5×10^8 of the cells.

[0015] In some of any such ~~embodiment~~examples, said input composition in the cavity contains at least at or about 1×10^7 of said cells, at least at or about 2×10^7 of said cells, 3×10^7 of said cells, at least at or about 4×10^7 of said cells, at least at or about 5×10^7 of said cells, at least at or about 6×10^7 of said cells, at least at or about 7×10^7 of said cells, at least at or about 8×10^7 of said cells, at least at or about 9×10^7 of said cells, at least at or about 1×10^8 of said cells, at least at or about 2×10^8 of said cells, at least at or about 3×10^8 of said cells or at least at or about 4×10^8 of said cells.

[0016] In some ~~embodiment~~examples, the internal surface area of the cavity is at least at or about 1×10^9 μm^2 or 1×10^{10} μm^2, and/or the length of the side wall in the direction extending from the end wall is at least about 5 cm and/or at least about 8 cm; and/or the internal cavity has a radius of at least about 2 cm at at least one cross-section.

[0017] In some ~~embodiment~~examples, the input composition includes at least or about 1 infectious unit (IU) per one of the cells, at least or about 2 IU per one of the cells, at least or about 3 IU per one of the cells, at least or about 4 IU per one of the cells, at least or about 5 IU per one of the cells, at least or about 10 IU per one of the cells, at least or about 20 IU per one of the cells, at least or about 30 IU per one of the cells, at least or about 40 IU per one of the cells, at least or

about 50 IU per one of the cells, or at least or about 60 IU per one of the cells. In some ~~embodiment~~examples, the input composition includes at or about 1 infectious unit (IU) per one of the cells, at or about 2 IU per one of the cells, at or about 3 IU per one of the cells, at or about 4 IU per one of the cells, at or about 5 IU per one of the cells, at or about 10 IU per one of the cells, at or about 20 IU per one of the cells, at or about 30 IU per one of the cells, at or about 40 IU per one of the cells, at or about 50 IU per one of the cells, or at or about 60 IU per one of the cells.

[0018] In some ~~embodiment~~examples, the average liquid volume or maximum liquid volume of the input composition, composition with viral vector particles and cells, and/or any liquid composition present in the cavity during the incubation is no more than about 10, 5, or 2.5 milliliters (mL) per square inch of the internal surface area of the cavity during the incubation. In some ~~embodiment~~examples, the maximum total volume of such liquid composition present in the cavity at any one time during the incubation is no more than 2 times, no more than 10 times, no more than 100 times, no more than 500 times or no more than 1000 times the total volume of the cells. In some ~~embodiment~~examples, the total volume of cells is the total volume of a pellet of the cells. In some ~~embodiment~~examples, the total volume of cells is the volume of a monolayer of the cells, such as a monolayer of cells present on the internal surface in the cavity during rotation of the centrifugal chamber.

[0019] In some ~~embodiment~~examples, the liquid volume of the input composition occupies all or substantially all of the volume of the internal cavity during at least a portion of the incubation. In other ~~embodiment~~examples, during at least a portion of the incubation, the liquid volume of the input composition occupies only a portion of the volume of the internal cavity, the volume of the cavity during this at least a portion further comprising a gas, which is taken into the cavity, e.g., via said at least one opening or another opening, prior to or during the incubation.

[0020] In some of any such ~~embodiment~~examples, the liquid volume of said input composition present in said cavity during said rotation is between or between about 0.5 mL per square inch of the internal surface area of the cavity (mL/sq.in) and 5 mL/sq.in, 0.5 mL/sq.in. and 2.5 mL/sq.in., 0.5 mL/sq.in. and 1 mL/sq.in., 1 mL/sq.in. and 5 mL/sq.in., 1 mL/sq.in. and 2.5 mL/sq.in. or 2.5 mL/sq.in. and 5 mL/sq.in.

[0021] In some of any such ~~embodiment~~examples, the maximum total liquid volume of said input composition present in said cavity at any one time during said incubation is no more than 2 times, no more than 10 times, or no more than 100 times, the total volume of said cells in said cavity or the average volume of the input composition over the course of the incubation is no more than 2, 10, or 100 times the total volume of cells in the cavity.

[0022] In some of any such ~~embodiment~~examples, the maximum volume of said input composition present in said cavity at any one time during said incubation or the average volume over the course of the incubation is no more than at or about 2 times, 10 times, 25 times, 50 times, 100 times, 500 times, or 1000 times the volume of a monolayer of said cells formed on the inner surface of said cavity during rotation of said chamber at a force of at or about 2000 g at an internal surface of the side wall.

[0023] In some of any such ~~embodiment~~examples, the liquid volume of the input composition is no more than 20 mL, no more than 40 mL, no more than 50 mL, no more than 70 mL, no more than 100 mL, no more than 120 mL, no more than 150 mL or no more than 200 mL.

[0024] In some of any such ~~embodiment~~examples, during at least a portion of the incubation in the chamber or during the rotation of the chamber, the liquid volume of the input composition occupies only a portion of the volume of the internal cavity of the chamber, the volume of the cavity during said at least a portion or during said rotation further comprising a gas, said gas taken into said cavity via said at least one opening, prior to or during said incubation.

[0025] In some ~~embodiment~~examples, the centrifugal chamber includes a movable member, whereby intake of gas into the centrifugal chamber effects movement of the movable member to increase the volume of the internal cavity of the chamber, thereby decreasing the total liquid volume of said input composition present in said cavity during rotation of said centrifugal chamber per square inch of the internal surface area of the cavity compared to the absence of gas in the chamber.

[0026] In some ~~embodiment~~examples, the number of cells in the cavity during the incubation is at or about the number of the cells sufficient to form a monolayer on the internal surface of the cavity during rotation of the centrifugal chamber at a force of at or about 2000 g and/or is no more than 1.5 times or 2 times such a number of the cells.

[0027] In some of any such ~~embodiment~~examples, the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 2000 g at an internal surface of the side wall; and/or the number of said cells in said input composition is no more than 1.5 times or 2 times the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 2000 g at an internal surface of the side wall.

[0028] In some ~~embodiment~~examples, the centrifugation is for a duration of between 120 and 7200 seconds, such as between 120 and 3600 seconds, including values inclusive or within the range, such as whole-minute values inclusive or within the range.

[0029] In some ~~embodiment~~examples, the methods include a) providing to an internal cavity of a centrifugal chamber that has an internal surface area of at least at or about 1 x 10^9 μm^2 or at least at or about 1 x 10^{10} μm^2: i) an input composition including cells and viral particles containing a recombinant viral vector, wherein: the number of cells in the input composition is at least 1 x 10^7 cells, and the viral particles are present in the input composition at at least at or about 1 infectious unit (IU) per one of said cells, and the input composition contains a liquid volume that is less than the maximum volume of the internal cavity of the centrifugal chamber; and ii) gas, at a volume that is up to the remainder of the maximum volume of the internal cavity of the centrifugal chamber; and b) incubating the input composition, wherein at least a portion of the incubation is carried out in said internal cavity of said centrifugal chamber while effecting rotation of said centrifugal chamber; and wherein the method generates an output composition containing a plurality of the cells transduced with the viral vector.

[0030] In some ~~embodiment~~examples, the number of cells is at least or about 50 x 10^6 cells; 100 x 10^6 cells; or 200 x 10^6 cells; and/or the viral particles are present at at least 1.6 IU/cell, 1.8 IU/cell, 2.0 IU/cell, 2.4 IU/cell, 2.8 IU/cell, 3.2 IU/cell or 3.6 IU/cell, 4.0 IU/cell, 5.0 IU/cell, 6.0 IU/cell, 7.0 IU/cell, 8.0 IU/cell, 9.0 IU/cell or 10.0 IU/cell.

[0031] In some of any such ~~embodiment~~examples, the liquid volume of the input composition is less than or equal to 200 mL, less than or equal to 100 mL or less than or equal to 50 mL or less than or equal to 20 mL. In some of any such ~~embodiment~~examples, the volume of gas is up to 200 mL, up to 180 mL, up to 140 mL or up to 100 mL.

[0032] In some of any such ~~embodiment~~examples, said rotation is at a relative centrifugal force at an internal surface of the side wall of the cavity or at a surface layer of the cells of at least at or about 1000 g, 1500 g, 2000 g, 2400 g, 2600g, 2800 g, 3000 g, 3200 g or 3600 g.

[0033] In some ~~embodiment~~examples, the methods are for large-scale processing.

[0034] In some ~~embodiment~~examples, the composition in the cavity (e.g., input composition) includes at least 50 mL, at least 100 mL, or at least 200 mL, liquid volume, and/or at least or about 1 million cells per cm^2 of the internal surface area of the cavity during at least a portion of said incubation.

[0035] In some ~~embodiment~~examples, the maximum liquid volume of the input composition present in the cavity at any one time during said incubation is no more than at or about 2 times, 10 times,

25 times, 50 times, 100 times, 500 times, or 1000 times the volume of a monolayer of said cells formed on the inner surface of said cavity during rotation of said chamber, e.g., at a force, e.g., effective force, of at or about 2000 g.

[0036] In some ~~embodiment~~examples, the rotation of the chamber during at least a portion of the incubation is at a force of greater than at or about 200 g, greater than at or about 300 g, or greater than at or about 500 g, such as greater than at or about 1000 g, 1500 g, 2000 g, 2500 g, 3000 g, or 3200 g, at an internal wall of the cavity of the centrifugal chamber and/or a layer, e.g., surface layer, of the cells. In some ~~embodiment~~examples, the force is at least at or about 1000 g, 1500 g, 2000 g, or 2500 g, 3000 g or 3200 g. In some ~~embodiment~~examples, the force is at or about 2100 g, 2200 g or 3000 g.

[0037] In some ~~embodiment~~examples, the methods include incubating an input composition containing cells and viral particles containing a recombinant viral vector, at least a portion of said incubating being carried out under rotating conditions, thereby generating an output composition containing a plurality of the cells transduced with the viral vector, wherein said input composition contains greater than or about 20 mL, 50 mL, at least 100 mL, or at least 150 mL in volume, and/or said input composition comprises at least 1 x 10^8 cells; and said rotating conditions comprise a relative centrifugal force on a surface layer of the cells of greater than about 1500 g.

[0038] In some ~~embodiment~~examples of the methods, at least 25 % or at least 50 % of said cells in the output composition are transduced with said viral vector; and/or at least 25 % or at least 50 % of said cells in the output composition express a product of a heterologous nucleic acid contained within said viral vector.

[0039] In some of any such ~~embodiment~~examples, said incubation is carried out in a cavity of a centrifugal chamber and the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer or a bilayer on the inner surface of said cavity during said rotation.

[0040] In some ~~embodiment~~examples, said centrifugal chamber includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity,

[0041] In some ~~embodiment~~examples, said centrifugal chamber further includes a movable member and said internal cavity is a cavity of variable volume defined by said end wall, said

substantially rigid side wall, and said movable member, said movable member being capable of moving within the chamber to vary the internal volume of the cavity.

[0042] In some of any such ~~embodiment~~examples, the input composition in said cavity contains a liquid volume of at least 20 mL or at least 50 mL and at or about 1 million cells per cm^2 of the internal surface area of the cavity during at least a portion of said incubation.

[0043] In some of any such ~~embodiment~~examples, a further portion of the incubation is carried out outside of the centrifugal chamber and/or without rotation, said further portion carried out subsequent to the at least a portion carried out in the chamber and/or with rotation.

[0044] In some of any such ~~embodiment~~examples, the at least a portion of the incubation carried out in the cavity of the centrifugal chamber and/or the further portion of the incubation is effected at or at about 37 ºC ± 2 ºC.

[0045] In some of any such ~~embodiment~~examples, the incubation further includes transferring at least a plurality of the cells to a container during said incubation and said further portion of the incubation is effected in the container. In some ~~embodiment~~examples, the transferring is performed within a closed system, wherein the centrifugal chamber and container are integral to the closed system.

[0046] In some of any such ~~embodiment~~examples, the incubation is carried out for a time between at or about 1 hour and at or about 96 hours, between at or about 4 hours and at or about 72 hours, between at or about 8 hours and at or about 48 hours, between at or about 12 hours and at or about 36 hours, between at or about 6 hours and at or about 24 hours, between at or about 36 hours and at or about 96 hours, inclusive; or the further portion of the incubation is carried out for a time between at or about 1 hour and at or about 96 hours, between at or about 4 hours and at or about 72 hours, between at or about 8 hours and at or about 48 hours, between at or about 12 hours and at or about 36 hours, between at or about 6 hours and at or about 24 hours, between at or about 36 hours and at or about 96 hours, inclusive.

[0047] In some of any such ~~embodiment~~examples, the incubation or further portion of the incubation is carried out for a time that is no more than 48 hours, no more than 36 hours or no more than 24 hours; or the further portion of the incubation is carried out for a time that is no more than 48 hours, no more than 36 hours or no more than 24 hours.

[0048] In some of any such ~~embodiment~~examples, the incubation is performed in the presence of a stimulating agent; and/or the further portion of the incubation is performed in the presence of a stimulating agent.

[0049] In some of any such ~~embodiment~~examples, the incubation is carried out for a time that is no more than 24 hours; the cells in the composition have not been subjected to a temperature of greater than 30 ºC for more than 24 hours; and/or the incubation is not performed in the presence of a stimulating agent.

[0050] In some of any such ~~embodiment~~examples, the stimulating agent is an agent capable of inducing proliferation of T cells, CD4+ T cells and/or CD8+ T cells.

[0051] In some of any such ~~embodiment~~examples, the stimulating agent is a cytokine selected from among IL-2, IL-15 and IL-7.

[0052] In some of any such ~~embodiment~~examples, the output composition containing transduced cells contains at least 1 x 10^7 cells or at least 5 x 10^7 cells.

[0053] In some of any such ~~embodiment~~examples, the output composition containing transduced cells contains at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8, 8 x 10^8 cells or 1 x 10^9 cells.

[0054] In some of any such ~~embodiment~~examples, the cells are T cells. In some ~~embodiment~~examples, the T cells are unfractionated T cells, isolated CD4+ T cells and/or isolated CD8+ T cells.

[0055] In some of any such ~~embodiment~~examples, the method results in integration of the viral vector into a host genome of one or more of the at least a plurality of cells and/or into a host genome of at least at or about 20 % or at least at or about 30 % or at least at or about 40 % of the cells in the output composition.

[0056] In some of any such ~~embodiment~~examples, at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said input composition are transduced with said viral vector by the method; and/or at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said output composition are transduced with said viral vector; and/or at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said output composition express a product of a heterologous nucleic acid contained within said viral vector.

[0057] Particular ~~embodiment~~examples include methods of transduction carried out by incubating an input composition comprising cells and viral vector particles under rotating conditions, whereby a plurality of the cells are inoculated for transduction with the viral vector, wherein the input composition includes a total volume greater than 50 mL, such as at least 100 mL, or at least 150 mL in volume, and/or said input composition comprises at least 1 x 10^8 cells; and the rotating

conditions comprise centrifugal force of greater than about 1500 g. In some such ~~embodiment~~examples, the incubation is carried out in a cavity of a centrifugal chamber and the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer on the inner surface of the cavity during the rotation. In some such ~~embodiment~~examples, at least 25 % or at least 50 % of said cells are transduced with the viral vector.

[0058] In some ~~embodiment~~examples, the methods result in at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said input composition being transduced with the viral vector, and/or produce an output composition in which at least 10 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of the cells are transduced with the vector and/or express a recombinant product encoded by the vector. In some ~~embodiment~~examples, transduction efficiency is expressed for a particular input amount or relative amount of virus. For example, in some ~~embodiment~~examples, such efficiencies are achieved by the methods for an input composition comprising a virus at a ratio of about 1 or about 2 IU per cells.

[0059] In some ~~embodiment~~examples, among all the cells in said output composition produced by the methods, the average copy number of the recombinant viral vector is no more than about 10, no more than about 5, no more than about 2.5, or no more than about 1.5. In some ~~embodiment~~examples, among the cells in the output composition that contain the recombinant viral vector, the average copy number of the vector is no more than about 5, no more than about 2, no more than about 1.5, or no more than about 1.

[0060] In some of any such ~~embodiment~~examples, among all the cells in said output composition that contain the recombinant viral vector or into which the viral vector is integrated, the average copy number of said recombinant viral vector is no more than about 10, no more than about 5, no more than about 2.5, or no more than about 1.5; or among the cells in the output composition, the average copy number of said vector is no more than about 2, no more than about 1.5, or no more than about 1.

[0061] In some ~~embodiment~~examples, the centrifugal chamber is integral to a closed system, for example, where the closed system includes the chamber and at least one tubing line operably linked to the at least one opening via at least one connector, such that liquid and gas are permitted to move between said cavity and said at least one tubing line in at least one configuration of the system. The at least one tubing line typically includes a series of tubing lines. The at least one connector typically includes a plurality of connectors. The closed system may further include at least one container operably linked to the series of tubing lines, such that

the at least one connection permits liquid and/or gas to pass between the at least one container and the at least one opening via the series of tubing lines.

[0062] The at least one connector may include one or more connectors selected from the group consisting of valves, luer ports, and spikes, e.g., a rotational valve, such as a stopcock or multirotational port, and/or an aseptic connector.

[0063] The at least one container may include one or more bags, vials, and/or syringes, and may include container(s) designated as a diluent container, a waste container, a product collection container, output container, and/or an input container.

[0064] In some ~~embodiment~~examples, the at least one container includes at least one input container including the virus and/or cells (which may be a single input container comprising the virus and cells or two input containers comprising the virus and cells, respectively), a waste container, a product container, and at least one diluent or wash solution-containing container, each connected to said cavity via said series of tubing lines and the at least one opening.

[0065] In some of any such ~~embodiment~~examples, at least one container further includes a container that contains a gas prior to and/or during at least a point during said incubation and/or the closed system further includes a microbial filter capable of taking in gas to the internal cavity of the centrifugal chamber and/or the closed system contains a syringe port for effecting intake of gas.

[0066] The methods in some ~~embodiment~~examples further include, prior to and/or during the incubation, effecting intake of the input composition into said cavity. The intake may include flow of liquid from the at least one input container into the cavity through said at least one opening. The intake may include intake of virus from one input container and input of cells from another, to produce the input composition for incubation.

[0067] In some ~~embodiment~~examples, the method includes, prior to and/or during said incubation, providing or effecting intake of gas into said cavity under sterile conditions, said intake being effected by (a) flow of gas from the container that includes gas, (b) flow of gas from an environment external to the closed system, via the microbial filter, or (c) flow of gas from a syringe connected to the system at the syringe port.

[0068] In some ~~embodiment~~examples, the effecting intake of the gas into the internal cavity of the centrifugal chamber is carried out simultaneously or together with the effecting intake of the input composition to the internal cavity of the centrifugal chamber.

[0069] In some of any such ~~embodiment~~examples, the input composition and gas are combined in a single container under sterile conditions outside of the chamber prior to said intake of said input composition and gas into the internal cavity of the centrifugal chamber.

[0070] In some ~~embodiment~~examples, the effecting of the intake of the gas is carried out separately, either simultaneously or sequentially, from the effecting of the intake of the input composition into said cavity.

[0071] In some of any such ~~embodiment~~examples, the intake of gas is effected by permitting or causing flow of the gas from a sterile closed container containing the gas, an external environment through a microbial filter, or a syringe containing said gas.

[0072] In some of any such ~~embodiment~~examples, the gas is air.

[0073] In some ~~embodiment~~examples of the provided process methods, the incubation is part of a continuous process, where the method further includes, during at least a portion of the incubation, effecting continuous intake of said input composition into the cavity, typically during rotation of the chamber, and during a portion of the incubation, effecting continuous expression (i.e. outtake) of liquid from said cavity through said at least one opening, typically during rotation of the chamber. The continuous intake and outtake in some ~~embodiment~~examples occur simultaneously.

[0074] In some ~~embodiment~~examples, the method includes during a portion of said incubation, effecting continuous intake of gas into said cavity during rotation of the chamber; and/or during a portion of said incubation, effecting continuous expression of gas from said cavity.

[0075] In some ~~embodiment~~examples, the method includes the expression of liquid and the expression of gas from said cavity, where each is expressed, simultaneously or sequentially, into a different container.

[0076] In some of any such ~~embodiment~~examples, at least a portion of the continuous intake and the continuous expression occur simultaneously.

[0077] In some ~~embodiment~~examples, the incubation is part of a semi-continuous process, such as one in which the method further includes effecting intake of the input composition into the cavity through the at least one opening, conducting all or part of the incubation, such as the centrifugation, and then effecting expression of liquid from the cavity, and then repeating the process, whereby another input composition is taken in to the cavity, followed by centrifugation, followed by expression. The process can be iterative and include several more rounds of intake, processing, and expression.

[0078] In some of any such ~~embodiment~~examples, the incubation is part of a semi-continuous process, the method further including prior to said incubation, effecting intake of said input composition, and optionally gas, into said cavity through said at least one opening; subsequent to said incubation, effecting expression of liquid and/or optionally gas from said cavity;

effecting intake of another input composition including cells and said viral particles containing a recombinant viral vector, and optionally gas, into said internal cavity; and incubating said another input composition in said internal cavity, wherein the method generates another output composition containing a plurality of cells of the another input composition that are transduced with said viral vector.

[0079] In some of any such ~~embodiment~~examples, said providing or said intake of the input composition into the cavity includes intake of a single composition including the cells and the viral particles containing the recombinant viral vector; or intake of a composition including the cells and a separate composition containing the viral particles containing the recombinant viral vector, whereby the compositions are mixed, effecting intake of the input composition.

[0080] The intake may include intake of a single composition containing the cells and the virus; or intake of a composition containing the cells and a separate composition containing the virus, whereby the compositions are mixed, effecting intake of the input composition. In some ~~embodiment~~examples of the continuous or semi-continuous process, at least 1 x 10^8 cells or at least 1x10^9 cells or at least 1x10^{10} cells or more are processed in total, over the multiple rounds or continuous process.

[0081] In some ~~embodiment~~examples, the method includes effecting rotation of the centrifugal chamber prior to and/or during said incubation and effecting expression of liquid from the cavity into said waste container following the incubation; effecting expression of liquid from the at least one diluent container into said cavity via the at least one opening and effecting mixing of the contents of the cavity; and effecting expression of liquid from said cavity into the product container, thereby transferring cells transduced with the viral vector into the product bag.

[0082] In some ~~embodiment~~examples, the method further includes carrying out other processing steps, or at least a portion of one or more other processing steps, within the same chamber and/or closed system. In some ~~embodiment~~examples, the one or more processing steps can includes processes in which the cells are isolated, such as separated or selected, stimulated, and formulated within the same chamber and/or closed system. In some cases, the one or more further processing steps also can include washing cells, suspending cells and/or diluting or concentrating cells, which can be carried out prior to or subsequent to any one or more of the processing steps for isolating, such as separating or selecting, stimulating, transducing and/or formulating the cells. In some ~~embodiment~~examples, the one or more other processing steps can be carried out prior to, simultaneously with or subsequent to the incubation of cells with the viral vector particles in the methods of transduction. In some ~~embodiment~~examples, the one or more further processing steps, or a portion of

the one or more further processing steps, can be carried out in a cavity of a centrifugal chamber that is the same or different as a cavity of a centrifugal chamber employed in the incubation of cells with the viral vector particles.

[0083] Among the provided processing methods, including isolation, e.g. selection, methods, stimulation methods, formulation methods and other processing methods, are those carried out according to any of the ~~embodiment~~examples as described above.

[0084] For example, in some ~~embodiment~~examples, the method further includes (a) washing a biological sample (e.g., a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cells (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product) containing cells in a cavity of a chamber, prior to the incubation for isolating, e.g. selecting cells, and/or prior to the incubation for incubating cells with viral vector particles, (b) isolating, e.g. selecting, the cells from a sample (e.g., a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cells (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product) in a cavity prior to the incubation of such cells with viral vector particles and/or (c) stimulating cells in a cavity prior to and/or during the incubation of such cells with viral vector particles, e.g., by exposing cells to stimulating conditions, thereby inducing cells of the input composition to proliferate. In some ~~embodiment~~examples, the isolating includes immunoaffinity-based selection.

[0085] In some of any such ~~embodiment~~examples, the method includes (a) washing a biological sample containing said cells in an internal cavity of a centrifugal chamber prior to said incubation; and/or (b) isolating said cells from a biological sample, wherein at least a portion of the isolation step is performed in an internal cavity of a centrifugal chamber prior to said incubation; and/or (c) stimulating cells prior to and/or during said incubation, said stimulating including exposing said cells to stimulating conditions, thereby inducing cells of the input composition to proliferate, wherein at least a portion of the step of stimulating cells is performed in an internal cavity of a centrifugal chamber.

[0086] In some ~~embodiment~~examples, the methods may further include isolation, e.g., selection, of the cells in the chamber, e.g., by immunoaffinity based selection. In some ~~embodiment~~examples, the isolation, e.g. selection, of cells is carried out prior to the incubation of cells with the viral vector particles in the methods of transduction, whereby the isolated, such as selected, cells are the cells present in the input composition and/or incubated with the viral vector particles. In some ~~embodiment~~examples, the isolation, e.g., selection, includes incubation of cells with a selection reagent,

such as an immunoaffinity reagent. In some ~~embodiment~~examples, at least a portion of the isolation, e.g. selection, step, such as incubation of cells with a selection reagent, e.g. an immunoaffinity reagent, is carried out in the cavity of a chamber, which, in some cases, can include rotation of the chamber, for example, for mixing of the reagent and cells.

[0087] In some ~~embodiment~~examples, the methods may further include stimulating cells prior to, during and/or subsequent to the incubation of cells with the viral vector particles, in which at least all or a portion of the stimulation can be carried out in a cavity of a centrifugal chamber. In some ~~embodiment~~examples, the stimulating conditions may include incubation of cells in the presence of an agent capable of activating one or more intracellular signaling domains of one or more components of a TCR complex, such as a primary agent that specifically binds to a member of a TCR complex, e.g., CD3, and a secondary agent that specifically binds to a T cell costimulatory molecule, e.g., CD28, CD137 (4-1-BB), OX40, or ICOS, including antibodies such as those present on the surface of a solid support, such as a bead. In some ~~embodiment~~examples, at least a portion of the stimulation, such as incubation of cells in the presence of a stimulating condition, is carried out in the cavity of a chamber, which, in some cases, can include rotation of the chamber, for example, for mixing of the reagent and cells.

[0088] In some of any such ~~embodiment~~examples, the method includes formulating cells, such as cells produced or generated in accord with the provided methods, including cell transduced by the method, in a pharmaceutically acceptable buffer in an internal cavity of a centrifugal chamber, thereby producing a formulated composition. In some ~~embodiment~~examples, the methods further include effecting expression of the formulated composition to one or a plurality of containers. In some ~~embodiment~~examples, the methods include the effecting of expression of the formulated composition includes effecting expression of a number of the cells present in a single unit dose to one or each of said one or a plurality of containers.

[0089] In some of any such ~~embodiment~~examples, each of said a cavity of a centrifugal chamber is the same or different as a cavity of a centrifugal employed in one or more of the other steps and/or in the process of incubating and/or rotating an input composition containing cells and viral particles.

[0090] In some of any such ~~embodiment~~examples, each of said centrifugal chambers is integral to a closed system, said closed system including said chamber and at least one tubing line operably linked to the at least one opening via at least one connector, whereby liquid and gas are permitted to move between said cavity and said at least one tubing line in at least one configuration of said system.

[0091] The cells processed by the methods typically are primary cells, such as cells obtained from a subject, typically a human. The cells may be derived from a subject to which the therapy is to be administered, such as one having a disease or condition targeted by a recombinant molecule expressed by a vector transduced, e.g., a recombinant antigen receptor such as a chimeric antigen receptor or transgenic TCR. Alternatively, the cells may be from a different subject. Thus, the methods encompass processing for autologous and allogeneic transfer. The cells may include suspension cells, e.g., white blood cells, e.g., T cells, such as isolated $CD8^+$ T cells, or isolated $CD4^+$ T cells or subsets thereof, or NK cells.

[0092] In some ~~embodiment~~examples, during the incubation, the centrifugal chamber is associated with a sensor, for example, a sensor capable of monitoring the position of the movable member, and control circuitry, such as circuitry capable of receiving and transmitting information to and from the sensor, causing movement of said movable member, and/or that is further associated with a centrifuge and thus is capable of causing rotation of the chamber during said incubation.

[0093] In some ~~embodiment~~examples, the chamber contains the movable member and during the incubation is located within a centrifuge and associated with a sensor capable of monitoring the position of the movable member, and control circuitry capable of receiving and transmitting information from the sensor and causing movement of the movable member, intake and expression of liquid to and from said cavity via said one or more tubing lines, and rotation of the chamber via the centrifuge.

[0094] In some ~~embodiment~~examples, the chamber, control circuitry, centrifuge, and/or sensor are housed within a cabinet, e.g., during the incubation.

[0095] In some ~~embodiment~~examples of any of the viral transfer, e.g., transduction methods, the recombinant viral vector encodes a recombinant receptor, which is thereby expressed by cells of the output composition. In some ~~embodiment~~examples, the recombinant receptor is a recombinant antigen receptor, such as a functional non-T cell receptor, e.g., a chimeric antigen receptor (CAR), or a transgenic T cell receptor (TCR). In some ~~embodiment~~examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain.

[0096] In some of any such ~~embodiment~~examples, the cells include primary human T cells obtained from a human subject and prior to the incubation with viral vector particles and/or prior to completion of the transduction and/or, where the method includes formulation, prior to the formulation, the primary human T cells have not been present externally to the subject at a temperature of greater than 30°C for greater than 1 hour, greater than 6 hours, greater than 24

hours, or greater than 48 hours or prior to the incubation and/or prior to the completion of the transduction, and/or where the method includes formulation, prior to the formulation, the primary human T cells have not been incubated in the presence of an antibody specific for CD3 and/or an antibody specific for CD28 and/or a cytokine, for greater than 1 hour, greater than 6 hours, greater than 24 hours, or greater than 48 hours.

[0097] Provided herein are methods for isolation, e.g. selection, of cells including (a) incubating a selection reagent and primary cells in an internal cavity of a centrifugal chamber under mixing conditions, whereby a plurality of the primary cells bind to said selection reagent and (b) separating the plurality of the primary cells from another one or more of the primary cells based on binding to the selection reagent, thereby enriching the primary cells based on binding to the selection reagent, wherein the centrifugal chamber is rotatable around an axis of rotation and the internal cavity has a maximum volume of at least 50 mL, at least 100 mL, or at least 200 mL. In some ~~embodiment~~examples, the methods for isolation, e.g. selection, occur in a closed system. In some ~~embodiment~~examples, prior to the step of separating the plurality of cells, the cells incubated with the selection reagent, are expressed from or transferred out of the chamber, but maintained in the closed system. In some ~~embodiment~~examples, optionally, subsequent to incubation with the selection reagent and prior to separating the cells, the method further includes one or more washing steps, which in some cases, can be performed in the cavity of the chamber in accord with the provided methods. In some ~~embodiment~~examples, the step of separating the cells can be effecting using a solid support, such as using an immunoaffinity-column, including those for magnetic separation, which can be contained in the closed system.

[0098] Provided herein are methods for stimulation of cells, including incubating a stimulation agent and primary cells under conditions whereby the stimulation agent binds to a molecule expressed by a plurality of the primary cells and the plurality of the cells are activated or stimulated, wherein at least a portion of the incubation is carried out in an internal cavity of a centrifugal chamber under mixing conditions, where the centrifugal chamber is rotatable around an axis of rotation and the internal cavity has a maximum volume of at least 50 mL, at least 100 mL, or at least 200 mL.

[0099] In some ~~embodiment~~examples, the methods of stimulation are performed as part of a process that includes transducing cells, whereby all or a part of such process is performed in a centrifugal chamber and/or as part of the same closed system. In some ~~embodiment~~examples, the primary cells that are stimulated with a stimulation agent include or are cells obtained following isolation, e.g. selection, of cells from a biological sample, such as in accord with the provided

methods. In some ~~embodiment~~examples, at least a portion of the stimulation is carried out simultaneously or during the incubation of cells with the viral vector particles, such that the primary cells include or are cells present in the input composition and/or are cells in which transduction has occurred or is initiated. In some ~~embodiment~~examples, at least a portion of the stimulation is carried out prior to the incubation of cells with the viral vector particles, such that the cells incubated with the viral vector particles are stimulated cells, which, in some cases, includes proliferating cells.

[0100] In some ~~embodiment~~examples, at least a portion of the one of more other processing steps of the method, including isolation, e.g. selection, stimulation, washing and/or formulation, that is carried out in a chamber includes where the chamber includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of the side wall surrounds the internal cavity and the at least one opening is capable of permitting intake of liquid into the internal cavity and expression of liquid from the cavity.

[0101] Provided herein are compositions containing transduced cells produced by the methods of any of the above ~~embodiment~~examples. In some of any such ~~embodiment~~examples, the composition contains cells that are primary cells and/or human cells and/or include white blood cells, and/or T cells, and/or NK cells. In some of any such ~~embodiment~~examples, the composition contains at least 5 x 10^7 cells, 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8 cells, 8 x 10^8 cells or 1 x 10^9 cells. In some of any such ~~embodiment~~examples, the composition contains a therapeutically effective number of cells for use in adoptive T cell therapy. In some of any such ~~embodiment~~examples, the cells are T cells and subsequent to transduction, the cells in the composition are not subjected to cell expansion in the presence of a stimulating agent and/or the cells are not incubated at a temperature greater than 30 ºC for more than 24 hours or the composition does not contain a cytokine or the composition does not contain a stimulating agent that specifically binds to CD3 or a TCR complex.

[0102] Provided herein are compositions containing at least 1 x 10^7 or at least 5 x 10^7 T cells, at least a plurality of which are transduced with a recombinant viral vector, where subsequent to transduction, the cells in the composition have not been subjected to cell expansion in the presence of a stimulating agent and/or the cells have not been incubated at a temperature greater than 30 ºC for more than 24 hours and/or at least 30, 40, 50, 60, 70, or 80 % of the T cells in the composition contain high surface expression of CD69 or TGF-beta-II. In some ~~embodiment~~examples, the composition contains at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8, 8 x 10^8 cells or 1 x 10^9 cells.

[0102a] In one aspect, the present disclosure refers to a composition, comprising at least 1 x 10^7 T cells, at least a plurality of which are transduced with a recombinant viral vector or express a recombinant antigen receptor, wherein subsequent to transduction, the cells in the composition have not been subjected to cell expansion in the presence of a stimulating agent.

[0102b] In one aspect, the present disclosure refers to a composition, comprising at least 1 x 10^7 T cells, at least a plurality of which are transduced with a recombinant viral vector or express a recombinant antigen receptor, wherein subsequent to transduction, the cells in the composition have not been incubated at a temperature greater than 30ºC for more than 24 hours.

[0103] In some of any such ~~embodiment~~examples, the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.

[0104] In some of any such ~~embodiment~~examples, at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said composition are transduced with the viral vector.

[0105] In some of any such ~~embodiment~~examples, the viral vector encodes a recombinant receptor and transduced cells in the composition express the recombinant receptor. In some ~~embodiment~~examples, the recombinant receptor is a recombinant antigen receptor. In some ~~embodiment~~examples, the recombinant antigen receptor is a functional non-T cell receptor. In some ~~embodiment~~examples, the functional non-T cell receptor is a chimeric antigen receptor (CAR). In some ~~embodiment~~examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain. In some ~~embodiment~~examples, the recombinant antigen receptor is a transgenic T cell receptor (TCR).

[0106] In some of any such ~~embodiment~~examples, among all the cells in the composition, the average copy number of the recombinant viral vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2, or among the cells in the composition transduced with the recombinant viral vector, the average copy number of said vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2.

[0107] In some of any such ~~embodiment~~examples, the composition contains a pharmaceutically acceptable excipient.

[0108] Provided herein are methods of treatment, including administering to a subject having a disease or condition the composition of any of the above ~~embodiment~~examples. In some ~~embodiment~~examples, the transduced T cells in the composition exhibit increased or longer expansion and/or persistence in the subject than transduced T cells in a composition in which, subsequent to transduction, the cells in the composition have been subjected to cell expansion in the presence of a stimulating agent and/or the cells have been incubated at a temperature greater than 30 ºC for more than 24 hours.

[0109] In some of any such ~~embodiment~~examples, the recombinant receptor, chimeric antigen receptor or transgenic TCR specifically binds to an antigen associated with the disease or condition. In some ~~embodiment~~examples, the disease or condition is a cancer, an autoimmune disease or disorder, or an infectious disease.

[0110] Provided herein are compositions containing at least 1 x 10^7 cells and at least at or about 1 infectious unit (IU) per cell of viral particles containing a recombinant viral vector. In some ~~embodiment~~examples, the cells contain at least or about 50 x 10^6 cells, 100 x 10^6 cells, or 200 x 10^6 cells, and/or said viral particles are present in the composition in an amount that is at least 1.6 IU/cell, 1.8 IU/cell, 2.0 IU/cell, 2.4 IU/cell, 2.8 IU/cell, 3.2 IU/cell, 3.6 IU/cell, 4.0 IU/cell, 5.0 IU/cell, 6.0 IU/cell, 7.0 IU/cell, 8.0 IU/cell, 9.0 IU/cell or 10.0 IU/cell.

[0111] In any of such ~~embodiment~~examples, the liquid volume of the composition is less than or equal to 220 mL, less than or equal to 200 mL, less than or equal to 100 mL, less than or equal to 50 mL or less than or equal to 20 mL.

[0112] In some of any such ~~embodiment~~examples, the cells are primary cells. In some of any such ~~embodiment~~examples, the cells are human cells. In some of any such ~~embodiment~~examples, the cells include suspension cells, the cells include white blood cells and/or the cells include T cells or NK cells. In some ~~embodiment~~examples, the cells are T cells and the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.

[0113] In some of any such ~~embodiment~~examples, the viral vector encodes a recombinant receptor. In some ~~embodiment~~examples, the recombinant receptor is a recombinant antigen receptor. In some ~~embodiment~~examples, the recombinant antigen receptor is a functional non-T cell receptor. In some ~~embodiment~~examples, the functional non-T cell receptor is a chimeric antigen receptor (CAR). In some ~~embodiment~~examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain. In some ~~embodiment~~examples, the recombinant antigen receptor is a transgenic T cell receptor (TCR).

[0114] Provided herein are centrifugal chambers rotatable around an axis of rotation, including an internal cavity containing the composition of any of the above ~~embodiment~~examples.

[0115] Provided herein are centrifugal chambers rotatable around an axis of rotation, containing an internal cavity containing (a) a composition containing at least 5 x 10^7 primary T cells transduced with a recombinant viral vector and/or (b) a composition containing at least 5 x 10^7 primary T cells and viral particles containing a recombinant viral vector.

[0116] In some of any such ~~embodiment~~examples, the chamber further contains an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity.

[0117] In some of any such ~~embodiment~~examples, said composition in said cavity contains at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8 cells, 8 x 10^8 cells or 1 x 10^9 of the cells.

[0118] In some of any such ~~embodiment~~examples, the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.

[0119] In some of any such ~~embodiment~~examples of the chamber, at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said composition are transduced with a viral vector.

[0120] In some of any such ~~embodiment~~examples of the chamber, the viral vector encodes a recombinant receptor and cells in the composition express the recombinant receptor. In some ~~embodiment~~examples, the recombinant receptor is a recombinant antigen receptor. In some ~~embodiment~~examples, the recombinant antigen receptor is a functional non-T cell receptor. In some ~~embodiment~~examples, the functional non-T cell receptor is a chimeric antigen receptor (CAR). In some ~~embodiment~~examples, the recombinant receptor is a chimeric receptor containing an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain. In some ~~embodiment~~examples, the recombinant antigen receptor is a transgenic T cell receptor (TCR).

[0121] In some of any such ~~embodiment~~examples of the chamber, among all the cells in the composition, the average copy number of said recombinant viral vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2 or among the cells in the composition transduced with the recombinant viral vector, the average copy number of said vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2.

[0122] Provided herein are centrifugal chambers rotatable around an axis of rotation, including an internal cavity containing the composition of any of the above ~~embodiment~~examples. In some ~~embodiment~~examples, the chamber further contains a volume of gas up to the maximum volume of the internal cavity of the chamber. In some ~~embodiment~~examples, the gas is air.

[0123] In some of any such ~~embodiment~~examples of the chamber, the chamber is rotatable around an axis of rotation and includes an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity. In some ~~embodiment~~examples the side wall is curvilinear. In some ~~embodiment~~examples the side wall is generally cylindrical.

[0124] In some of any such ~~embodiment~~examples of the chamber, said at least one opening includes an inlet and an outlet, respectively capable of permitting said intake and expression or said at least one opening includes a single inlet/outlet, capable of permitting said intake and said expression. In some of any such ~~embodiment~~examples of the chamber, aid at least one opening is coaxial with the chamber and is located in the end wall.

[0125] In some of any such ~~embodiment~~examples, the chamber further includes a movable member and said internal cavity is a cavity of variable volume defined by said end wall, said substantially rigid side wall, and said movable member, said movable member being capable of moving within the chamber to vary the internal volume of the cavity. In some ~~embodiment~~examples, the movable member is a piston and/or the movable member is capable of axially moving within the chamber to vary the internal volume of the cavity.

[0126] In some of any such ~~embodiment~~examples, the internal surface area of said cavity is at least at or about 1 x 10^9 μm^2, the internal surface area of said cavity is at least at or about 1 x 10^{10} μm^2, the length of said rigid wall in the direction extending from said end wall is at least about 5 cm, the length of said rigid wall in the direction extending from said end wall is at least about 8 cm and/or the cavity contains a radius of at least about 2 cm at at least one cross-section.

[0127] In some of any such ~~embodiment~~examples of the chamber, the liquid volume of said composition present in said cavity is between or between about 0.5 mL per square inch of the internal surface area of the cavity (mL/sq.in) and 5 mL/sq.in, 0.5 mL/sq.in. and 2.5 mL/sq.in., 0.5 mL/sq.in. and 1 mL/sq.in., 1 mL/sq.in. and 5 mL/sq.in., 1 mL/sq.in. and 2.5 mL/sq.in. or 2.5 mL/sq.in. and 5 mL/sq.in. In some of any such ~~embodiment~~examples, the liquid volume of said composition present in said cavity is at least 0.5 mL/sq.in., 1 mL/sq.in., 2.5 mL/sq.in., or 5 mL/sq.in.

[0128] Provided herein are closed systems containing the centrifugal chamber of any of the above ~~embodiment~~examples. In some of any such ~~embodiment~~examples of the closed system, the centrifugal chamber is capable of rotation at a speed up to 8000 g, wherein the centrifugal chamber is capable of withstanding a force of 500, 1000, 1500, 2000, 2500, 3000 or 3200 g, without substantially yielding, bending, or breaking or otherwise resulting in damage of the chamber and/or while substantially holding a generally cylindrical shape under such force.

Brief Description of the Drawings

[0129] **FIG. 1A** shows transduction efficiency calculated as percentage of $CD3^+$ T cells with surface expression of a chimeric antigen receptor (CAR) encoded by a viral vector, following

CLAIMS

1. A transduction method, comprising incubating, in an internal cavity of a centrifugal chamber, an input composition comprising cells and viral particles containing a recombinant viral vector, wherein

said centrifugal chamber is rotatable around an axis of rotation and comprises an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, at least a portion of said side wall surrounding said internal cavity and said at least one opening being capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity;

the centrifugal chamber is rotating around said axis of rotation during at least a portion of the incubation; and

the method generates an output composition comprising a plurality of the cells transduced with the viral vector.

2. A transduction method, comprising incubating, in an internal cavity of a centrifugal chamber, an input composition comprising cells and a viral particle containing a recombinant viral vector,

said centrifugal chamber being rotatable around an axis of rotation and comprising an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity, wherein:

the centrifugal chamber is rotating around the axis of rotation during at least a portion of the incubation;

the total liquid volume of said input composition present in said cavity during rotation of said centrifugal chamber is no more than about 5 mL per square inch of the internal surface area of the cavity; and

the method generates an output composition comprising a plurality of the cells transduced with the viral vector.

3. The method of claim 1 or claim 2, wherein said rotating comprises rotation at a relative centrifugal force (RCF) at an internal surface of the side wall of the cavity and/or at a

surface layer of the cells of greater than at or about 200 g, greater than at or about 300 g, or greater than at or about 500 g.

4. The method of any of claims 1-3, wherein said rotating comprises rotation at a relative centrifugal force at an internal surface of the side wall of the cavity and/or at a surface layer of the cells that is:

at or about 600 g, 800 g, 1000 g, 1100 g, 1600 g, 2000 g, 2100 g, 2200 g, 2500 g or 3000 g; or

at least at or about 600 g, 800 g, 1000 g, 1100 g, 1600 g, 2000 g, 2100 g, 2200 g, 2500 g or 3000 g.

5. The method of any of claims 1-4, wherein said rotating comprises rotation at a relative centrifugal force at an internal surface of the side wall of the cavity and/or at a surface layer of the cells that is between or between about 500 g and 2500 g, 500 g and 2000 g 500 g and 1600 g, 500 g an 1000 g, 600 g and 1600 g, 600 g and 1000 g, 1000 g and 2000 g or 1000 g and 1600 g, each inclusive.

6. The method of any of claims 1-5, wherein the at least a portion of the incubation during which the chamber is rotating is for a time that is:

greater than or about 5 minutes, greater than or about 10 minutes, greater than or about 15 minutes, greater than or about 20 minutes, greater than or about 30 minutes, greater than or about 45 minutes, greater than or about 60 minutes, greater than or about 90 minutes or greater than or about 120 minutes; or

between or between about 5 minutes and 60 minutes, 10 minutes and 60 minutes, 15 minutes and 60 minutes, 15 minutes and 45 minutes, 30 minutes and 60 minutes or 45 minutes and 60 minutes, each inclusive.

7. The transduction method of any of claims 1-6, wherein said centrifugal chamber further comprises a movable member and said internal cavity is a cavity of variable volume defined by said end wall, said substantially rigid side wall, and said movable member, said movable member being capable of moving within the chamber to vary the internal volume of the cavity.

8. The method of any of claims 1-7, wherein said side wall is curvilinear.

9. The method of claim 8, wherein said side wall is generally cylindrical.

10. The method of any of claims 7-9, wherein:

the movable member is a piston; and/or

the movable member is capable of axially moving within the chamber to vary the internal volume of the cavity.

11. The method of any of claims 1-10, wherein

said at least one opening comprises an inlet and an outlet, respectively capable of permitting said intake and expression; or

said at least one opening comprises a single inlet/outlet, capable of permitting said intake and said expression.

12. The method of any of claims 1-11, wherein said at least one opening is coaxial with the chamber and is located in the end wall.

13. The method of any of claims 1-12, wherein:

the internal surface area of said cavity is at least at or about 1×10^9 μm^2;

the internal surface area of said cavity is at least at or about 1×10^{10} μm^2;

the length of said rigid wall in the direction extending from said end wall is at least about 5 cm;

the length of said rigid wall in the direction extending from said end wall is at least about 8 cm; and/or

the cavity comprises a radius of at least about 2 cm at at least one cross-section.

14. The method of any of claims 1-13, wherein:

the average liquid volume of said input composition present in said cavity during said incubation is no more than about 5 milliliters (mL) per square inch of the internal surface area of the cavity during said incubation;

~~the maximum liquid volume of said input composition present in said cavity at any one time during said incubation is no more than about 5 mL per square inch of the maximum internal surface area of the cavity;~~

~~the average liquid volume of said input composition present in said cavity during said incubation is no more than about 2.5 milliliters (mL) per square inch of the internal surface area of the cavity during said incubation; or~~

~~the maximum liquid volume of said input composition present in said cavity at any one time during said incubation is no more than about 2.5 mL per square inch of the maximum internal surface area of the cavity.~~

~~15. The method of any of claims 1-14, wherein the liquid volume of said input composition present in said cavity during said rotation is between or between about 0.5 mL per square inch of the internal surface area of the cavity (mL/sq.in) and 5 mL/sq.in, 0.5 mL/sq.in. and 2.5 mL/sq.in., 0.5 mL/sq.in. and 1 mL/sq.in., 1 mL/sq.in. and 5 mL/sq.in., 1 mL/sq.in. and 2.5 mL/sq.in. or 2.5 mL/sq.in. and 5 mL/sq.in.~~

~~16. The method of any of claims 1-15, wherein:~~

~~the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 1000 g or at or about 2000 g at an internal surface of the side wall and/or at a surface layer of the cells; and/or~~

~~the number of said cells in said input composition is no more than 1.5 times or 2 times the number of said cells sufficient to form a monolayer on the surface of said cavity during rotation of said centrifugal chamber at a force of at or about 1000 g or at or about 2000 g at an internal surface of the side wall and/or at a surface layer of the cells.~~

~~17. The method of any of claims 1-16, wherein~~

~~said input composition in the cavity comprises at least at or about 1 x 10^6 of said cells; or~~

~~said input composition in the cavity comprises at least at or about 5 x 10^6 of said cells; or~~

~~said input composition in the cavity comprises at least at or about 1 x 10^7 of said cells; or~~

~~said input composition in the cavity comprises at least at or about 1 x 10^8 of said cells.~~

~~18. The method of any of claims 1-17, wherein said input composition in the cavity comprises at least at or about 1 x 10^7 of said cells, at least at or about 2 x 10^7 of said cells, at least at or about 3 x 10^7 of said cells, at least at or about 4 x 10^7 of said cells, at least at or about 5 x 10^7 of said cells, at least at or about 6 x 10^7 of said cells, at least at or about 7 x 10^7 of said cells, at least at or about 8 x 10^7 of said cells, at least at or about 9 x 10^7 of said cells, at least at or about 1 x 10^8 of said cells, at least at or about 2 x 10^8 of said cells, at least at or about 3 x 10^8 of said cells or at least at or about 4 x 10^8 of said cells.~~

~~19. The method of any of claims 1-18, wherein:~~

~~said input composition comprises at least at or about 1 infectious unit (IU) of viral particles per one of said cells, at least at or about 2 IU per one of said cells, at least at or about 3 IU per one of said cells, at least at or about 4 IU per one of said cells, at least at or about 5 IU per one of said cells, at least at or about 10 IU per one of said cells, at least at or about 20 IU per one of said cells, at least at or about 30 IU per one of said cells, at least at or about 40 IU per one of said cells, at least at or about 50 IU per one of said cells, or at least at or about 60 IU per one of said cells; or~~

~~said input composition comprises at or about 1 infectious unit (IU) of viral particles per one of said cells, at or about 2 IU per one of said cells, at or about 3 IU per one of said cells, at or about 4 IU per one of said cells, at or about 5 IU per one of said cells, at or about 10 IU per one of said cells, at or about 20 IU per one of said cells, at or about 30 IU per one of said cells, at or about 40 IU per one of said cells, at or about 50 IU per one of said cells, or at or about 60 IU per one of said cells.~~

~~20. The method of any of claims 1-19, wherein:~~

~~the maximum total liquid volume of said input composition present in said cavity at any one time during said incubation is no more than 2 times, no more than 10 times, or no more than 100 times, the total volume of said cells in said cavity or the average volume of the input composition over the course of the incubation is no more than 2, 10, or 100 times the total volume of cells in the cavity.~~

~~21. The method of any of claims 1-20, wherein the maximum volume of said input composition present in said cavity at any one time during said incubation or the average volume over the course of the incubation is no more than at or about 2 times, 10 times, 25 times, 50~~

~~times, 100 times, 500 times, or 1000 times the volume of a monolayer of said cells formed on the inner surface of said cavity during rotation of said chamber at a force of at or about 1000 g or at or about 2000 g at an internal surface of the side wall and/or at a surface layer of the cells.~~

~~22. The method of any of claims 1-21, wherein the liquid volume of the input composition is no more than 20 mL, no more than 40 mL, no more than 50 mL, no more than 70 mL, no more than 100 mL, no more than 120 mL, no more than 150 mL or no more than 200 mL.~~

~~23. The method of any of claims 1-22, wherein the input composition occupies all or substantially all of the volume of the internal cavity during at least a portion of said incubation.~~

~~24. The method of any of claims 1-23, wherein, during at least a portion of the incubation in the chamber or during the rotation of the chamber, the liquid volume of the input composition occupies only a portion of the volume of the internal cavity of the chamber, the volume of the cavity during said at least a portion or during said rotation further comprising a gas, said gas taken into said cavity via said at least one opening, prior to or during said incubation.~~

~~25. The method of claim 24, wherein the centrifugal chamber comprises a movable member, whereby intake of gas into the centrifugal chamber effects movement of the movable member to increase the volume of the internal cavity of the chamber, thereby decreasing the total liquid volume of said input composition present in said cavity during rotation of said centrifugal chamber per square inch of the internal surface area of the cavity compared to the absence of gas in the chamber.~~

~~26. A method of transduction, comprising:~~

~~a) providing to an internal cavity of a centrifugal chamber that has an internal surface area of at least at or about 1 x 10^9 μm^2 or at least at or about 1 x 10^{10} μm^2:~~

~~i) an input composition comprising cells and viral particles comprising a recombinant viral vector, wherein:~~

~~the number of cells in the input composition is at least 1 x 10^7 cells, and~~

~~the viral particles are present in the input composition at at least at or about 1 infectious unit (IU) per one of said cells, and~~

~~the input composition comprises a liquid volume that is less than the maximum volume of the internal cavity of the centrifugal chamber; and~~

~~ii) gas at a volume that is up to the remainder of the maximum volume of the internal cavity of the centrifugal chamber; and~~

~~b) incubating the input composition, wherein at least a portion of the incubation is carried out in said internal cavity of said centrifugal chamber while effecting rotation of said centrifugal chamber; and~~

~~wherein the method generates an output composition comprising a plurality of the cells transduced with the viral vector.~~

~~27. The method of claim 26, wherein:~~

~~the number of cells is at least at or about 50 x 10^6 cells; 100 x 10^6 cells; or 200 x 10^6 cells; and/or~~

~~the viral particles are present at at least 1.6 IU/cell, 1.8 IU/cell, 2.0 IU/cell, 2.4 IU/cell, 2.8 IU/cell, 3.2 IU/cell or 3.6 IU/cell, 4.0 IU/cell, 5.0 IU/cell, 6.0 IU/cell, 7.0 IU/cell, 8.0 IU/cell, 9.0 IU/cell or 10.0 IU/cell .~~

~~28. The method of claim 26 or claim 27, wherein:~~

~~the liquid volume of the input composition is less than or equal to 200 mL, less than or equal to 100 mL, less than or equal to 50 mL or less than or equal to 20 mL; and/or~~

~~the liquid volume of the input composition is no more than 50%, no more than 40%, no more than 30%, no more than 20%, or no more than 10% of the volume of the internal surface area of the cavity during rotation or the maximum internal surface area of the cavity.~~

~~29. The method of any of claims 26-28, wherein the volume of gas is up to 200 mL, up to 180 mL, up to 140 mL or up to 100 mL.~~

~~30. The method of any of claims 26-29, wherein said rotation is at a relative centrifugal force at an internal surface of the side wall of the cavity or at a surface layer of the cells of at least at or about 600 g, 800 g, 1000 g, 1100 g, 1500 g, 1600 g, 2000 g, 2400 g, 2600g, 2800 g, 3000 g, 3200 g or 3600 g.~~

~~31. A method of transduction, comprising incubating an input composition comprising cells and viral particles comprising a recombinant viral vector, at least a portion of said incubating being carried out under rotating conditions, thereby generating an output composition comprising a plurality of the cells transduced with the viral vector, wherein:~~

~~said input composition comprises greater than or about 20 mL, 50 mL, at least 100 mL, or at least 150 mL in volume, and/or said input composition comprises at least 1 x 10^8 cells; and~~

~~said rotating conditions comprise a relative centrifugal force on a surface layer of the cells of greater than about 800 g or greater than about 1000 g or greater than about 1500 g.~~

~~32. The method of claim 31, wherein:~~

~~at least 25 % or at least 50 % of said cells in the output composition are transduced with said viral vector; and/or~~

~~at least 25 % or at least 50 % of said cells in the output composition express a product of a heterologous nucleic acid comprised within said viral vector.~~

~~33. The method of claim 31 or claim 32, wherein said incubation is carried out in a cavity of a centrifugal chamber and the number of said cells in said input composition is at or about the number of said cells sufficient to form a monolayer or a bilayer on the inner surface of said cavity during said rotation.~~

~~34. The method of claim 33, wherein said centrifugal chamber comprises an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of fluid into said internal cavity and expression of fluid from said cavity.~~

~~35. The method of claim 34, wherein said centrifugal chamber further comprises a movable member and said internal cavity is a cavity of variable volume defined by said end wall, said substantially rigid side wall, and said movable member, said movable member being capable of moving within the chamber to vary the internal volume of the cavity.~~

~~36. The method of any of claims 1-30 or 33-35, wherein the input composition in said cavity comprises a liquid volume of at least 20 mL or at least 50 mL and at or about 1 million cells per cm^2 of the internal surface area of the cavity during at least a portion of said incubation.~~

~~37. The method of any of claims 1-36, wherein a further portion of the incubation is carried out outside of the centrifugal chamber and/or without rotation, said further portion carried out subsequent to the at least a portion carried out in the chamber and/or with rotation.~~

~~38. The method of any of claims 1-37, wherein the at least a portion of the incubation carried out in the cavity of the centrifugal chamber and/or the further portion of the incubation is effected at or at about 37 ºC ± 2 ºC.~~

~~39. The method of claim 37 or claim 38, wherein the incubation further comprises transferring at least a plurality of the cells to a container during said incubation and said further portion of the incubation is effected in the container.~~

~~40. The method of claim 39, wherein the transferring is performed within a closed system, wherein the centrifugal chamber and container are integral to the closed system.~~

~~41. The method of any of claims 37-40, wherein:~~

~~the incubation is carried out for a time between at or about 1 hour and at or about 96 hours, between at or about 4 hours and at or about 72 hours, between at or about 8 hours and at or about 48 hours, between at or about 12 hours and at or about 36 hours, between at or about 6 hours and at or about 24 hours, between at or about 36 hours and at or about 96 hours, inclusive; or~~

~~the further portion of the incubation is carried out for a time between at or about 1 hour and at or about 96 hours, between at or about 4 hours and at or about 72 hours, between at or about 8 hours and at or about 48 hours, between at or about 12 hours and at or about 36 hours, between at or about 6 hours and at or about 24 hours, between at or about 36 hours and at or about 96 hours, inclusive.~~

~~42. The method of any of claims 37-41, wherein:~~

~~the incubation is carried out for a time that is no more than 48 hours, no more than 36 hours or no more than 24 hours; or~~

~~the further portion of the incubation is carried out for a time that is no more than 48 hours, no more than 36 hours or no more than 24 hours.~~

~~43. The method of any of claims 37-41, wherein:~~

~~the incubation is performed in the presence of a stimulating agent; and/or~~

~~the further portion of the incubation is performed in the presence of a stimulating agent.~~

~~44. The method of any of claims 37-41, wherein:~~

~~the incubation is carried out for a time that is no more than 24 hours;~~

~~the cells in the composition have not been subjected to a temperature of greater than 30 ºC for more than 24 hours; and/or~~

~~the incubation is not performed in the presence of a stimulating agent.~~

~~45. The method of claim 43 or claim 44, wherein the stimulating agent is an agent capable of inducing proliferation of T cells, CD4+ T cells and/or CD8+ T cells.~~

~~46. The method of any of claims 43-45, wherein the stimulating agent is a cytokine selected from among IL-2, IL-15 and IL-7.~~

~~47. The method of any of claims 1-46, wherein the output composition containing transduced cells comprises at least at or about 1 x 10^7 cells or at least at or about 5 x 10^7 cells.~~

~~48. The method of claim 47, wherein the output composition containing transduced cells comprises at least at or about 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8, 8 x 10^8 cells or 1 x 10^9 cells.~~

~~49. The method of claim 47 or claim 48, wherein the cells are T cells.~~

~~50. The method of claim 49, wherein the T cells are unfractionated T cells, isolated CD4+ T cells and/or isolated CD8+ T cells.~~

51. The method of any of claims 1-50, wherein the method results in integration of the viral vector into a host genome of one or more of the at least a plurality of cells and/or into a host genome of at least at or about 20 % or at least at or about 30 % or at least at or about 40 % of the cells in the output composition.

52. The method of any of claims 1-51, wherein:

at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said input composition are transduced with said viral vector by the method; and/or

at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said output composition are transduced with said viral vector; and/or

at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said output composition express a product of a heterologous nucleic acid comprised within said viral vector.

53. The method of any of claims 1-52, wherein, for an input composition comprising a virus at a ratio of about 1 or about 2 IU per cells, said method is capable of producing an output composition in which at least 10 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of the cells in said output composition generated by the method comprise said recombinant viral vector and/or express a product of a recombinant nucleic acid comprised within said vector.

54. The method of any of claims 1-53, wherein:

among all the cells in said output composition that contain the recombinant viral vector or into which the viral vector is integrated, the average copy number of said recombinant viral vector is no more than about 10, no more than about 5, no more than about 2.5, or no more than about 1.5; or

among the cells in the output composition, the average copy number of said vector is no more than about 2, no more than about 1.5, or no more than about 1.

55. The method of any of claims 1-30 and 33-54, wherein the centrifugal chamber is integral to a closed system, said closed system comprising said chamber and at least one tubing

line operably linked to the at least one opening via at least one connector, whereby liquid and gas are permitted to move between said cavity and said at least one tubing line in at least one configuration of said system.

56. The method of claim 55, wherein:

said at least one tubing line comprises a series of tubing lines;

said at least one connector comprises a plurality of connectors; and

said closed system further comprises at least one container operably linked to said series of tubing lines, the connection permitting liquid and/or gas to pass between said at least one container and said at least one opening via the series of tubing lines.

57. The method of claim 55 or 56, wherein said at least one connector comprises a connector selected from the group consisting of valves, luer ports, and spikes.

58. The method of any of claims 55-57, wherein said at least one connector comprises a rotational valve.

59. The method of claim 58, wherein said rotational valve is a stopcock or multirotational port.

60. The method of any of claims 55-59, wherein said at least one connector comprises an aseptic connector.

61. The method of any of claims 56-60, wherein said at least one container comprises a container selected from the group consisting of bags, vials, and syringes.

62. The method of any of claims 56-61, wherein said at least one container comprises a diluent container, a waste container, a product collection container, and/or an input product container.

63. The method of any of claims 56-62, wherein:

said at least one container comprises at least one input container comprising said viral vector particles and said cells, a waste container, a product container, and at least one diluent

~~container, each connected to said cavity via said series of tubing lines and said at least one opening.~~

~~64. The method of claim 63, wherein said method further comprises, prior to and/or during said incubation, effecting intake of said input composition into said cavity, said intake comprising flowing of liquid from said at least one input container into said cavity through said at least one opening.~~

~~65. The method of any of claims 56-64, wherein at least one container further comprises a container that comprises a gas prior to and/or during at least a point during said incubation and/or the closed system further comprises a microbial filter capable of taking in gas to the internal cavity of the centrifugal chamber and/or the closed system contains a syringe port for effecting intake of gas.~~

~~66. The method of claim 65, wherein the method comprises, prior to and/or during said incubation, providing or effecting intake of gas into said cavity under sterile conditions, said intake being effected by (a) flow of gas from the container that comprises gas, (b) flow of gas from an environment external to the closed system, via the microbial filter, or (c) flow of gas from a syringe connected to the system at the syringe port.~~

~~67. The method of claim 66, wherein the effecting intake of the gas into the internal cavity of the centrifugal chamber is carried out simultaneously or together with the effecting intake of the input composition to the internal cavity of the centrifugal chamber.~~

~~68. The method of claim 66 or claim 67, wherein the input composition and gas are combined in a single container under sterile conditions outside of the chamber prior to said intake of said input composition and gas into the internal cavity of the centrifugal chamber.~~

~~69. The method of claim 68, wherein the effecting of the intake of the gas is carried out separately, either simultaneously or sequentially, from the effecting of the intake of the input composition into said cavity.~~

~~70. The method of any of claims 66-69, wherein the intake of gas is effected by permitting or causing flow of the gas from a sterile closed container comprising the gas, an external environment through a microbial filter, or a syringe comprising said gas.~~

~~71. The method of any of claims 24-70, wherein the gas is air.~~

~~72. The method of any of claims 1-71, wherein the incubation is part of a continuous process, the method further comprising:~~

~~during at least a portion of said incubation, effecting continuous intake of said input composition into said cavity during rotation of the chamber; and~~

~~during a portion of said incubation, effecting continuous expression of liquid from said cavity through said at least one opening during rotation of the chamber.~~

~~73. The method of claim 72, further comprising:~~

~~during a portion of said incubation, effecting continuous intake of gas into said cavity during rotation of the chamber; and/or~~

~~during a portion of said incubation, effecting continuous expression of gas from said cavity.~~

~~74. The method of claim 73, wherein the method comprises the expression of liquid and the expression of gas from said cavity, where each is expressed, simultaneously or sequentially, into a different container.~~

~~75. The method of any of claims 72-74, wherein at least a portion of the continuous intake and the continuous expression occur simultaneously.~~

~~76. The method of any of claims 1-75, wherein the incubation is part of a semi-continuous process, the method further comprising:~~

~~prior to said incubation, effecting intake of said input composition, and optionally gas, into said cavity through said at least one opening;~~

~~subsequent to said incubation, effecting expression of liquid and/or optionally gas from said cavity;~~

effecting intake of another input composition comprising cells and said viral particles containing a recombinant viral vector, and optionally gas, into said internal cavity; and

incubating said another input composition in said internal cavity,

wherein the method generates another output composition comprising a plurality of cells of the another input composition that are transduced with said viral vector.

77. The method of any of claims 64-76, wherein said providing or said intake of the input composition into the cavity comprises:

intake of a single composition comprising the cells and the viral particles containing the recombinant viral vector; or

intake of a composition comprising the cells and a separate composition comprising the viral particles containing the recombinant viral vector, whereby the compositions are mixed, effecting intake of the input composition.

78. The method of claim 64-77, wherein the method further comprises:

effecting rotation of said centrifugal chamber prior to and/or during said incubation;

effecting expression of liquid from said cavity into said waste container following said incubation;

effecting expression of liquid from said at least one diluent container into said cavity via said at least one opening and effecting mixing of the contents of said cavity; and

effecting expression of liquid from said cavity into said product container, thereby transferring cells transduced with the viral vector into said product container.

79. The method of any of claims 1-78, further comprising:

(a) washing a biological sample comprising said cells in an internal cavity of a centrifugal chamber prior to said incubation; and/or

(b) isolating said cells from a biological sample, wherein at least a portion of the isolation step is performed in an internal cavity of a centrifugal chamber prior to said incubation; and/or

(c) stimulating cells prior to and/or during said incubation, said stimulating comprising exposing said cells to stimulating conditions, thereby inducing cells of the input composition to proliferate, wherein at least a portion of the step of stimulating cells is performed in an internal cavity of a centrifugal chamber.

~~80. The method of claim 79, wherein said isolating comprises carrying out immunoaffinity-based selection.~~

~~81. The method of claim 79 or 80, wherein said stimulating conditions comprise the presence of an agent capable of activating one or more intracellular signaling domains of one or more components of a TCR complex.~~

~~82. The method of claim 81, wherein said agent comprises a primary agent that specifically binds to a member of a TCR complex and a secondary agent that specifically binds to a T cell costimulatory molecule.~~

~~83. The method of claim 82, wherein the primary agent specifically binds to CD3; and/or~~

~~the costimulatory molecule is selected from the group consisting of CD28, CD137 (4-1-BB), OX40, or ICOS.~~

~~84. The method of claim 83, wherein said primary and secondary agents comprise antibodies and/or are present on the surface of a solid support.~~

~~85. The method of any of claims 79-84, wherein said biological sample in (a) and/or in (b) is or comprises a whole blood sample, a buffy coat sample, a peripheral blood mononuclear cells (PBMC) sample, an unfractionated T cell sample, a lymphocyte sample, a white blood cell sample, an apheresis product, or a leukapheresis product.~~

~~86. The method of any of claims 1-85, further comprising formulating cells transduced by the method in a pharmaceutically acceptable buffer in an internal cavity of a centrifugal chamber, thereby producing a formulated composition.~~

~~87. The method of claim 86, further comprising effecting expression of the formulated composition to one or a plurality of containers.~~

~~88. The method of claim 87, wherein the effecting of expression of the formulated composition comprises effecting expression of a number of the cells present in a single unit dose to one or each of said one or a plurality of containers.~~

~~89. The method of any of claims 79-88, wherein each of said a cavity of a centrifugal chamber is the same or different as a cavity of a centrifugal employed in one or more of the other steps and/or in the process of incubating and/or rotating an input composition containing cells and viral particles.~~

~~90. The method of any of claims 79-89, wherein each of said centrifugal chambers is integral to a closed system, said closed system comprising said chamber and at least one tubing line operably linked to the at least one opening via at least one connector, whereby liquid and gas are permitted to move between said cavity and said at least one tubing line in at least one configuration of said system.~~

~~91. The method of any of claims 1-90, wherein said cells in said input composition are primary cells.~~

~~92. The method of any of claims 1-91, wherein:~~
~~said cells in said input composition comprise suspension cells;~~
~~said cells in said input composition comprise white blood cells; and/or~~
~~said cells in said input composition comprise T cells or NK cells.~~

~~93. The method of any of claims 1-92, wherein said cells in said input composition are unfractionated T cells, isolated $CD8^+$ T cells, or isolated $CD4^+$ T cells.~~

~~94. The method of any of claims 1-93, wherein said cells in said input composition are human cells.~~

~~95. The method of any of claims 7-94, wherein, during said incubation, said centrifugal chamber is associated with a sensor, said sensor capable of monitoring the position of said movable member, and control circuitry, said circuitry capable of receiving and transmitting information to and from said sensor and causing movement of said movable~~

member, said control circuitry further associated with a centrifuge capable of causing rotation of said chamber during said incubation.

96. The method of any of claims 7-95, wherein said chamber comprises said movable member and during said incubation, said centrifugal chamber is located within a centrifuge and associated with a sensor, said sensor capable of monitoring the position of said movable member, and control circuitry capable of receiving and transmitting information from said sensor and causing movement of said movable member, intake and expression of liquid and/or gas to and from said cavity via said one or more tubing lines, and rotation of said chamber via said centrifuge.

97. The method of claim 95 or claim 96, wherein said chamber, said control circuitry, said centrifuge, and said sensor are housed within a cabinet during said incubation.

98. The method of any of claims 1-97, wherein said recombinant viral vector encodes a recombinant receptor, which is thereby expressed by cells of the output composition.

99. The method of claim 98, wherein said recombinant receptor is a recombinant antigen receptor.

100. The method of claim 99, wherein said recombinant antigen receptor is a functional non-T cell receptor.

101. The method of claim 100, wherein said functional non-T cell receptor is a chimeric antigen receptor (CAR).

102. The method of claim 99, wherein said recombinant antigen receptor is a transgenic T cell receptor (TCR).

103. The method of claim 99, wherein said recombinant receptor is a chimeric receptor comprising an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain.

~~104. The method of any of claims 1-103, wherein:~~

~~the cells comprise primary human T cells obtained from a human subject; and~~

~~prior to said incubation and/or prior to completion of said transduction and/or, where the method includes formulation, prior to the formulation, the primary human T cells have not been present externally to the subject at a temperature of greater than 30°C for greater than 1 hour, greater than 6 hours, greater than 24 hours, or greater than 48 hours; or~~

~~prior to said incubation and/or prior to the completion of the transduction, and/or where the method includes formulation, prior to the formulation, the primary human T cells have not been incubated in the presence of an antibody specific for CD3 and/or an antibody specific for CD28 and/or a cytokine, for greater than 1 hour, greater than 6 hours, greater than 24 hours, or greater than 48 hours.~~

~~105. A method for selection, the method comprising:~~

~~(a) incubating a selection reagent and primary cells in an internal cavity of a centrifugal chamber under mixing conditions, whereby a plurality of the primary cells bind to said selection reagent; and~~

~~(b) separating said plurality of said primary cells from another one or more of the primary cells based on binding to the selection reagent,~~

~~thereby enriching the primary cells based on binding to the selection reagent,~~

~~wherein said centrifugal chamber is rotatable around an axis of rotation and said internal cavity has a maximum volume of at least 50, at least 100, or at least 200 mL.~~

~~106. A method for stimulation of cells, the method comprising incubating a stimulation agent and primary cells under conditions whereby the stimulation agent binds to a molecule expressed by a plurality of the primary cells and said plurality of the cells are activated or stimulated, wherein~~

~~at least a portion of the incubation being carried out in an internal cavity of a centrifugal chamber under mixing conditions,~~

~~said centrifugal chamber is rotatable around an axis of rotation; and~~

~~said internal cavity has a maximum volume of at least 50, at least 100, or at least 200 mL.~~

~~107. The method of claim 105 or claim 106, wherein the chamber further comprises an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity.~~

~~108. A composition, comprising transduced cells produced by the method of any of claims 1-107.~~

~~109. The composition of claim 108, wherein said cells:~~
~~are primary cells; and/or~~
~~are human cells; and/or~~
~~comprise white blood cells; and/or~~
~~comprise T cells; and/or~~
~~comprise NK cells.~~

~~110. The composition of claim 108 or claim 109, wherein the composition comprises at least at or about 5 x 10^7 cells, 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8, 8 x 10^8 cells or 1 x 10^9 cells.~~

~~111. The composition of any of claims 106-110, wherein the composition comprises a therapeutically effective number of cells for use in adoptive T cell therapy.~~

~~112. The composition of any of claims 106-111, wherein:~~
~~the cells are T cells; and~~
~~subsequent to transduction, the cells in the composition are not subjected to cell expansion in the presence of a stimulating agent and/or the cells are not incubated at a temperature greater than 30 ºC for more than 24 hours or the composition does not contain a cytokine or the composition does not contain a stimulating agent that specifically binds to CD3 or a TCR complex.~~

1~~13~~. A composition, comprising at least 1 x 10^7 ~~or at least 5 x 10^7 cells~~ T cells, at least a plurality of which are transduced with a recombinant viral vector or express a recombinant ~~or engineered~~ antigen receptor, wherein~~:~~

subsequent to transduction, the cells in the composition have not been subjected to cell expansion in the presence of a stimulating agent.~~; and/or~~

2. The composition of claim 1, wherein subsequent to transduction, the cells in the composition have not been subjected to cell expansion in the presence of a cytokine.

3. The composition of claim 1 or claim 2, wherein subsequent to transduction, the cells in the composition have not been subjected to cell expansion in the presence of IL-2.

4. The composition of any one of claims 1-3, wherein subsequent to transduction, the cells in the composition have not been incubated at a temperature greater than 30~~-~~ºC for more than 24 hours.

5. A composition, comprising at least 1 x 10^7 T cells, at least a plurality of which are transduced with a recombinant viral vector or express a recombinant antigen receptor, wherein subsequent to transduction, the cells in the composition have not been incubated at a temperature greater than 30ºC for more than 24 hours.

6. The composition of any one of claims 1-5, wherein subsequent to transduction, the cells in the composition have not been incubated at a temperature greater than 37ºC or at a temperature of about 37ºC for more than 24 hours.

7. The composition of any one of claims 1-6, wherein the composition comprises at least 5 x 10^7 T cells, at least a plurality of which are transduced with the recombinant viral vector or express the recombinant antigen receptor.

8~~14~~. The composition of any one of claims 1-7~~A composition, comprising at least 1 x 10^7 or at least 5 x 10^7 primary human T cells, at least a plurality of which are transduced with a recombinant viral vector or express a recombinant or engineered antigen receptor~~, wherein at

least 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the T cells in the composition comprise high surface expression of CD69 and/or TGF-beta-II.

9115. The composition of any one of claims 1-8claim 114, wherein said at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the T cells in the composition comprise no surface expression of CD62L. and/or

10. The composition of any one of claims 1-9, wherein at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the T cells in the composition comprise high expression of CD25, ICAM, GM-CSF, IL-8, and/or IL-2.

11116. The composition of any one of claims 1-10of claims 113-115, wherein said the composition comprises at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8 cells, 8 x 10^8 cells, or 1 x 10^9 cells.

12117. The composition of any one of claims 1-11of claims 109-116, wherein said the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.

13118. The composition of any one of claims 1-12of claims 108-117, wherein at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said the cells in said the composition are transduced with the recombinant viral vector.

14. The composition of any one of claims 1-13, wherein at least 2.5%, at least 5%, at least 6%, at least 8%, at least 10%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, or at least 75% of the cells in the composition express the recombinant antigen receptor.

15. The composition of any one of claims 1-14, wherein the cells in the composition are primary cells.

119. The composition of any of claims 108-118, wherein:

the viral vector encodes a recombinant receptor; and

transduced cells in the composition express the recombinant receptor.

~~120. The composition of claim 119, wherein said recombinant receptor is a recombinant antigen receptor.~~

~~121. The composition of claim 120, wherein said recombinant antigen receptor is a functional non-T cell receptor.~~

16~~122~~. The composition of any one of claims 1-15~~claim 121~~, wherein the recombinant antigen receptor~~said functional non-T cell receptor~~ is a chimeric antigen receptor (CAR).

17~~123~~. The composition of claim 16~~any of claims 119-122~~, wherein the CAR comprises~~said recombinant receptor is a chimeric receptor comprising~~ an extracellular portion that specifically binds to a ligand and an intracellular signaling portion comprising ~~containing~~ an activating domain and a costimulatory domain.

18~~124~~. The composition of any one of claims 1-15~~claim 120~~, wherein ~~said~~ the recombinant antigen receptor is a transgenic T cell receptor (TCR).

19~~125~~. The composition of any one of claims 1-18~~of claims 110-124~~, wherein~~:~~

among all the cells in the composition, the average copy number of ~~said~~ the recombinant viral vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2.~~; or~~

20. The composition of any one of claims 1-19, wherein among the cells in the composition transduced with the recombinant viral vector, the average copy number of the recombinant viral ~~said~~ vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2.

21~~126~~. The composition of any one of claims 1-20~~of claims 110-125~~, comprising a pharmaceutically acceptable excipient.

~~127. An article of manufacture comprising a container or plurality of containers, the container or the plurality of containers collectively containing a composition according to any of claims 113-126.~~

128. The article of manufacture of claim 127, wherein the container or plurality of containers comprises two or more or three or more bags and the composition further comprises a pharmaceutically acceptable excipient.

129. A method of treatment, the method comprising administering to a subject having a disease or condition the composition of any of claims 110-126.

130. The method of claim 129, wherein the transduced T cells in the composition exhibit increased or longer expansion and/or persistence in the subject than transduced T cells in a composition in which, subsequent to transduction, the cells in the composition have been subjected to cell expansion in the presence of a stimulating agent and/or the cells have been incubated at a temperature greater than 30 ºC for more than 24 hours.

131. The method of claim 129 or claim 130, wherein the recombinant receptor, chimeric antigen receptor or transgenic TCR specifically binds to an antigen associated with the disease or condition.

132. The method of any of claims 129-131, wherein the disease or condition is a cancer, and autoimmune disease or disorder, or an infectious disease.

133. A composition, comprising:

at least 1 x 10^7 cells; and

at least at or about 1 infectious unit (IU) per cell of viral particles comprising a recombinant viral vector.

134. The composition of claim 133, wherein:

said cells comprise at least or about 50 x 10^6 cells; 100 x 10^6 cells; or 200 x 10^6 cells; and/or

said viral particles are present in the composition in an amount that is at least 1.6 IU/cell, 1.8 IU/cell, 2.0 IU/cell, 2.4 IU/cell, 2.8 IU/cell, 3.2 IU/cell, 3.6 IU/cell, 4.0 IU/cell, 5.0 IU/cell, 6.0 IU/cell, 7.0 IU/cell, 8.0 IU/cell, 9.0 IU/cell or 10.0 IU/cell.

~~135. The composition of claim 133 or claim 134, wherein the liquid volume of the composition is less than or equal to 220 mL, less than or equal to 200 mL, less than or equal to 100 mL, less than or equal to 50 mL or less than or equal to 20 mL.~~

~~136. The composition of any of claims 133-135, wherein said cells are primary cells.~~

~~137. The composition of any of claims 133-136, wherein said cells are human cells.~~

~~138. The composition of any of claims 133-137, wherein:~~
~~said cells comprise suspension cells;~~
~~said cells comprise white blood cells; and/or~~
~~said cells comprise T cells or NK cells.~~

~~139. The composition of claim 138, wherein said cells are T cells and the T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.~~

~~140. The composition of any of claims 133-139, wherein the viral vector encodes a recombinant receptor.~~

~~141. The composition of claim 140, wherein said recombinant receptor is a recombinant antigen receptor.~~

~~142. The composition of claim 141, wherein said recombinant antigen receptor is a functional non-T cell receptor.~~

~~143. The composition of claim 142, wherein said functional non-T cell receptor is a chimeric antigen receptor (CAR).~~

~~144. The composition of any of claims 140-143, wherein said recombinant receptor is a chimeric receptor comprising an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain.~~

~~145. The composition of claim 141, wherein said recombinant antigen receptor is a transgenic T cell receptor (TCR).~~

~~146. A centrifugal chamber rotatable around an axis of rotation, said chamber comprising an internal cavity comprising the composition of any of claims 110-126.~~

~~147. A centrifugal chamber rotatable around an axis of rotation, said chamber comprising an internal cavity comprising: (a) a composition containing at least 5 x 10^7 primary T cells transduced with a recombinant viral vector and/or (b) a composition containing at least 5 x 10^7 primary T cells and viral particles containing a recombinant viral vector.~~

~~148. The centrifugal chamber of claim 146 or 147, said chamber further comprising an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity.~~

~~149. The centrifugal chamber of claim 147 or 148, wherein said composition in said cavity comprises at least 1 x 10^8 cells, 2 x 10^8 cells, 4 x 10^8 cells, 6 x 10^8, 8 x 10^8 cells or 1 x 10^9 of the cells.~~

~~150. The centrifugal chamber of claim 147 or claim 148, wherein said T cells are unfractionated T cells, isolated CD8+ T cells, or isolated CD4+ T cells.~~

~~151. The centrifugal chamber of any of claims 147-150, wherein at least 2.5 %, at least 5 %, at least 6 %, at least 8 %, at least 10 %, at least 20 %, at least 25 %, at least 30 %, at least 40 %, at least 50 %, or at least 75 % of said cells in said composition are transduced with a viral vector.~~

~~152. The centrifugal chamber of any of claims 147-151, wherein:~~
~~the viral vector encodes a recombinant receptor; and~~
~~cells in the composition express the recombinant receptor.~~

~~153. The centrifugal chamber of claim 151, wherein said recombinant receptor is a recombinant antigen receptor.~~

~~154. The centrifugal chamber of claim 153, wherein said recombinant antigen receptor is a functional non-T cell receptor.~~

~~155. The centrifugal chamber of claim 154, wherein said functional non-T cell receptor is a chimeric antigen receptor (CAR).~~

~~156. The centrifugal chamber of any of claims 151-155, wherein said recombinant receptor is a chimeric receptor comprising an extracellular portion that specifically binds to a ligand and an intracellular signaling portion containing an activating domain and a costimulatory domain.~~

~~157. The centrifugal chamber of claim 153, wherein said recombinant antigen receptor is a transgenic T cell receptor (TCR).~~

~~158. The centrifugal chamber of any of claims 147-157, wherein:~~

~~among all the cells in the composition, the average copy number of said recombinant viral vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2; or~~

~~among the cells in the composition transduced with the recombinant viral vector, the average copy number of said vector is no more than about 10, no more than 8, no more than 6, no more than 4, or no more than about 2.~~

~~159. A centrifugal chamber rotatable around an axis of rotation, said chamber comprising an internal cavity comprising the composition of any of claims 133-145.~~

~~160. The centrifugal chamber of claim 159, further comprising a volume of gas up to the maximum volume of the internal cavity of the chamber.~~

~~161. The centrifugal chamber of claim 160, wherein said gas is air.~~

~~162. The centrifugal chamber of any of claims 146-161, said chamber being rotatable around an axis of rotation and comprising an end wall, a substantially rigid side wall extending from said end wall, and at least one opening, wherein at least a portion of said side wall surrounds said internal cavity and said at least one opening is capable of permitting intake of liquid into said internal cavity and expression of liquid from said cavity.~~

~~163. The centrifugal chamber of any of claims 146-162, wherein said side wall is curvilinear.~~

~~164. The centrifugal chamber of claim 163, wherein said side wall is generally cylindrical.~~

~~165. The centrifugal chamber of any of claims 162-164, wherein~~

~~said at least one opening comprises an inlet and an outlet, respectively capable of permitting said intake and expression; or~~

~~said at least one opening comprises a single inlet/outlet, capable of permitting said intake and said expression.~~

~~166. The centrifugal chamber of any of claims 162-165, wherein said at least one opening is coaxial with the chamber and is located in the end wall.~~

~~167. The centrifugal chamber of any of claims 162-166, wherein said centrifugal chamber further comprises a movable member and said internal cavity is a cavity of variable volume defined by said end wall, said substantially rigid side wall, and said movable member, said movable member being capable of moving within the chamber to vary the internal volume of the cavity.~~

~~168. The centrifugal chamber of claim 167, wherein:~~

~~the movable member is a piston; and/or~~

~~the movable member is capable of axially moving within the chamber to vary the internal volume of the cavity.~~

~~169. The centrifugal chamber of any of claims 162-168, wherein:~~

~~the internal surface area of said cavity is at least at or about 1 x 10^9 μm^2;~~

~~the internal surface area of said cavity is at least at or about 1 x 10^{10} μm^2;~~

~~the length of said rigid wall in the direction extending from said end wall is at least about 5 cm;~~

~~the length of said rigid wall in the direction extending from said end wall is at least about 8 cm; and/or~~

~~the cavity comprises a radius of at least about 2 cm at at least one cross-section.~~

~~170. The centrifugal chamber of any of claims 159-169, wherein the liquid volume of said composition present in said cavity is between or between about 0.5 mL per square inch of the internal surface area of the cavity (mL/sq.in) and 5 mL/sq.in, 0.5 mL/sq.in. and 2.5 mL/sq.in., 0.5 mL/sq.in. and 1 mL/sq.in., 1 mL/sq.in. and 5 mL/sq.in., 1 mL/sq.in. and 2.5 mL/sq.in. or 2.5 mL/sq.in. and 5 mL/sq.in.~~

~~171. The centrifugal chamber of any of claims 159-169, wherein the liquid volume of said composition present in said cavity is at least 0.5 mL/sq.in., 1 mL/sq.in., 2.5 mL/sq.in., or 5 mL/sq.in.~~

~~172. A closed system, comprising the centrifugal chamber of any of claims 147-158 and 162-171.~~

~~173. The closed system of claim 172, further comprising a multi-way manifold operably connected to one or a plurality of containers.~~

~~174. The closed system, comprising the centrifugal chamber of any of claims 159-171.~~

~~175. The closed system of claim 174, further comprising a sterile filter.~~

~~176. The closed system of any of claims 172-175, wherein the centrifugal chamber is capable of rotation at a speed up to 8000 g, wherein the centrifugal chamber is capable of withstanding a force of up to 500 g, 600 g, 1000 g, 1100 g, 1200 g, 1400 g, 1500 g, 1600 g, 2000 g, 2500 g, 3000 g or 3200 g, without substantially yielding, bending, or breaking or otherwise~~

resulting in damage of the chamber and/or while substantially holding a generally cylindrical shape under such force.

177. The method of claim 49, wherein at least at or about 30, 40, 50, 60, 70, 80, or 80 % of the T cells in the output composition comprise high expression of CD69 and/or TGF-beta-II.

178. The method of claim 177, wherein said at least 30, 40, 50, 60, 70, 80, or 80 % of the T cells in the composition comprise no surface expression of CD62L and/or comprise high expression of CD25, ICAM, GM-CSF, IL-8 and/or IL-2.

179. A method comprising

(a) washing primary human cells; and

(b) incubating said cells with a selection reagent under agitation conditions whereby at least a plurality of the human cells are specifically bound by the selection reagents,

wherein said washing and incubating are carried out within a closed, sterile system and at least in part in an internal cavity of a centrifugal chamber integral to the closed, sterile system.

180. The method of claim 179, wherein the method steps are carried out in an automated fashion based on input from a user that the method should be initiated, resulting in completion of the method steps.