into a patient. Once the cells are prepared for infusion, the cells are quickly transferred to the point of care site and administered to the patient. The major drawback of this process is that cGMP facilities need to be present near every point of care site. The cGMP facilities require considerable monetary capital to staff and run under the required rules and regulations. The need to establish a multiplicity of these centers at or near every point of care is cost prohibitive and a severe limitation to the commercial potential of this class of drug. This leads to a difficult choice of incurring great expense by building a large number of cGMP facilities in order to increase accessibility to patients or to providing limited accessibility for patients by building only a limited number of cGMP facilities to minimize the capital expenditures. Thus, there is a need in the field of live cell therapeutics for methods that enable cells in non-nutritive buffer to have a more extended shelf-life. Furthermore, a method is needed that would enable the packaging, shipping and mass distribution of formulated cell products suspended in non-nutrient containing infusion buffer.

[0006] Problems with maintaining the identity and function of cells used in adoptive immunotherapy after formulation are described, for example, in U.S. Patent Publication No. 2003/0175272 to Gruenberg. This publication teaches that T-cells must be reactivated just prior to patient administration (no more than 4 hours prior to infusion) to maintain functional characteristics of cytokine production. The function of the cells can be maintained up to 48 hours only if the formulation includes autologous plasma. However, collection of plasma from every intended patient is not conducive to mass distribution and commercialization.

SUMMARY

[0007] In a first embodiment, disclosed is a biologic drug composition. The drug composition comprises living cells formulated in non-nutritive buffer. The living cells, after being stored for greater than about 6 hours in the non-nutritive buffer, maintain their identity and at least one functional characteristic that defined the living cells prior to formulation in the non-nutritive buffer. These living cells are useful in immunotherapy after storage in the non-nutritive buffer. They maintain their identity and at least one functional characteristic that defined the living cells prior to formulation in the non-nutrient buffer for at least 72 hours.

[0007a] In a first aspect, there is provided a method of handling a biological drug composition with living cells for use in immunotherapy comprising: formulating the living cells in a non-nutritive buffer, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized anti-CD3 and anti-CD28 monoclonal antibodies in the non-nutritive buffer; and maintaining the living cells in the non-nutritive buffer at a storage temperature below 20°C, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful for immunotherapy after being stored for greater than 24 hours in the non-nutritive buffer, wherein the living cells in the composition express CD40L after storage in an amount of at least 80% relative to the expression of CD40L at the time of formulation.

[0008] In another embodiment, disclosed is a method of handling a biological drug composition with living cells. The method comprises formulating the living cells in a non-nutritive buffer and maintaining the living cells in the non-nutritive buffer at a storage temperature below about 20°C. The living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer. The living cells are useful for immunotherapy after being stored for greater than about 72 hours in the non-nutritive buffer. Preferably, the storage temperature is in a range between about 4°C and about 8°C and the concentration of the cells in the non-nutritive buffer is about 10^7 cells/ml or greater. In compositions of T-cells, the living cells are preferably formulated in an activated state. In order to activate the T-cells, it is preferable to use immobilized monoclonal antibodies reactive to cell surface molecules. Preferably, the cell surface molecules are a combination of first one of the following: CD3, MHCI, MHCII, CD2 and second a co-stimulatory molecule. Preferably the co-stimulatory molecule is CD28. The living cells are placed in a flexible container or syringe, wherein the flexible container or syringe is packaged in a temperature controlled device that maintains the living cells at the storage temperature. The method also includes shipping and distributing the package in the temperature controlled device to the point of care.

[0008a] In another aspect, there is provided a method of handling a biological drug composition with living cells comprising: formulating the living cells in a non-nutritive

buffer, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized anti-CD3 and anti-CD28 monoclonal antibodies in the non-nutritive buffer; and maintaining the living cells in the non-nutritive buffer at a storage temperature below 20°C, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful for immunotherapy after being stored for greater than 24 hours in the non-nutritive buffer, wherein the functional characteristic is expression of CD40L, FasL, perforin and granzyme B, expression of costimulatory molecules, expression of adhesion molecules, secretion of cytokines, chemokines or combinations thereof, wherein the inflammatory cytokine secreted is IFN-γ and the level of secretion is in an amount of at least 80% compared to the levels at the time of formulation.

[0008b] In yet another aspect, there is provided a method of handling a biological drug composition with living cells comprising: formulating the living cells in a non-nutritive buffer, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized anti-CD3 and anti-CD28 monoclonal antibodies in the non-nutritive buffer; and maintaining the living cells in the non-nutritive buffer at a storage temperature below 20°C, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful for immunotherapy after being stored for greater than 24 hours in the non-nutritive buffer, wherein the living cells in the non-nutritive buffer are stable for at least 72 hours.

[0009] In yet another embodiment, disclosed is a method of providing living cell compositions to a point of care facility. The method comprises formulating the living cells in a non-nutritive buffer at a processing facility and transporting the cells to a point of care facility in a package equipped to maintain a storage temperature below about 20°C. The living cells are at the storage temperature for up to about 72 hours while maintaining their identity and at least one of the functional characteristics of the living cells useful in immunotherapy.

[0009a] In yet another aspect, there is provided a method of providing living cell compositions to a point of care facility comprising: formulating the living cells in a non-

nutritive buffer at a processing facility, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized monoclonal antibodies in the non-nutritive buffer; and transporting the cells to a point of care facility in a package equipped to maintain a storage temperature below 20oC, wherein the living cells are at the storage temperature for greater than 24 hours while maintaining their identity and at least one of the functional characteristics of the living cells useful in immunotherapy, wherein the live cells in the composition express CD40L after transportation and removal from storage temperature in an amount of at least 80% relative to the expression of CD40L at the time of formulation.

[0009b] In a further aspect, there is provided a method of providing living cell compositions to a point of care facility comprising: formulating the living cells in a non-nutritive buffer at a processing facility, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized monoclonal antibodies in the non-nutritive buffer; and transporting the cells to a point of care facility in a package equipped to maintain a storage temperature below 20oC, wherein the living cells are at the storage temperature for greater than 24 hours while maintaining their identity and at least one of the functional characteristics of the living cells useful in immunotherapy, wherein the cells in the composition secrete IFN-γ after transportation and removal from storage temperature in an amount of at least 80% compared to the levels at the time of formulation.

[0010] In a further embodiment, disclosed is a method of administering immunotherapy to a patient. The method comprises administering a composition that includes living cells formulated in non-nutritive buffer, wherein the composition has been stored for up to about 72 hours in the non-nutritive buffer, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that

defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful in immunotherapy.

[0011] In yet a further embodiment, disclosed is another method of administering immunotherapy to a patient. The method comprises administering a composition that includes living cells formulated in non-nutritive buffer, wherein the composition was previously stored in a frozen state, for example in liquid nitrogen, for up to 2 years or more and has been thawed and formulated and then stored for up to about 72 hours in the non-nutritive buffer, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful in immunotherapy.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Fig. 1A is a plot of the temperature change in the container during transportation where the container was not preconditioned.

[0013] Fig. 1B is a plot of the temperature recorded inside the preconditioned aerogel insulated box.

[0014] Fig. 1C is a plot of the air temperature during transportation.

[0015] Figs. 2A-2C show the expression of CD40L for HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping.

[0016] Figs. 3A-3C show the cell viability of HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping.

[0017] Figs. 4A-4C show the secretion of IFN-γ of HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping.

[0018] Figs. 5A-5C show the secretion of IFN-γ of HTC273, HTC245, and HTC264, respectively, cells before and after packaging and shipping followed by incubation at 37°C for 6 hours.

[0019] Figs 6A-6C show the expression of CD40L of HTC273, HTC264, and HTC245, respectively, cells and formulated for ID, IT and IV administration.

[0020] Figs. 7A-7C show the cell viability for HTC273, HTC264, and HTC245, respectively, cells and formulated for ID, IT and IV administration.

[0021] Figs. 8A-8C show the secretion of IFN-γ of HTC273, HTC264, and HTC245, respectively, cells and formulated for ID, IT and IV administration.

WHAT IS CLAIMED IS:

1. A method of handling a biological drug composition with living cells for use in immunotherapy comprising:

formulating the living cells in a non-nutritive buffer, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized anti-CD3 and anti-CD28 monoclonal antibodies in the non-nutritive buffer; and

maintaining the living cells in the non-nutritive buffer at a storage temperature below 20°C, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful for immunotherapy after being stored for greater than 24 hours in the non-nutritive buffer, wherein the living cells in the composition express CD40L after storage in an amount of at least 80% relative to the expression of CD40L at the time of formulation.

2. The method of claim 1 wherein the storage temperature is in a range between 0°C and 10°C.

3. The method of claim 1 wherein the concentration of the cells in the non-nutritive buffer is 10^6 cells/ml or greater and/or the living cells are placed in a flexible container or syringe, wherein the flexible container or syringe is packaged in a temperature controlled device that maintains the living cells at the storage temperature.

4. A method of handling a biological drug composition with living cells comprising:

formulating the living cells in a non-nutritive buffer, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized anti-CD3 and anti-CD28 monoclonal antibodies in the non-nutritive buffer; and

maintaining the living cells in the non-nutritive buffer at a storage temperature below 20°C, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful for immunotherapy after being stored for greater than 24 hours in the non-nutritive buffer,

wherein the functional characteristic is expression of CD40L, FasL, perforin and granzyme B, expression of costimulatory molecules, expression of adhesion molecules, secretion of cytokines, chemokines or combinations thereof,

wherein the inflammatory cytokine secreted is IFN-γ and the level of secretion is in an amount of at least 80% compared to the levels at the time of formulation.

5. The method of claim 4 wherein the storage temperature is in a range between 0°C and 10°C.

6. The method of claim 4 wherein the concentration of the cells in the non-nutritive buffer is 10^6 cells/ml or greater and/or the living cells are placed in a flexible container or syringe, wherein the flexible container or syringe is packaged in a temperature controlled device that maintains the living cells at the storage temperature.

7. The method of claim 4 wherein the secretion of IFN-γ after storage is recovered after incubation at 37°C for at least 24 hours.

8 A method of handling a biological drug composition with living cells comprising:

formulating the living cells in a non-nutritive buffer, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized anti-CD3 and anti-CD28 monoclonal antibodies in the non-nutritive buffer; and

maintaining the living cells in the non-nutritive buffer at a storage temperature below 20°C, wherein the living cells maintain their identity and at least one of the functional characteristics of the cells that defined the living cells prior to formulation in the non-nutritive buffer, the living cells useful for immunotherapy after being stored for greater than 24 hours in the non-nutritive buffer,

wherein the living cells in the non-nutritive buffer are stable for at least 72 hours.

9. The method of claim 8 wherein the storage temperature is in a range between 0°C and 10°C.

10. The method of claim 8 wherein the concentration of the cells in the non-nutritive buffer is 10^6 cells/ml or greater and/or the living cells are placed in a flexible container or syringe, wherein the flexible container or syringe is packaged in a temperature controlled device that maintains the living cells at the storage temperature.

11. A method of providing living cell compositions to a point of care facility comprising:

formulating the living cells in a non-nutritive buffer at a processing facility, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized monoclonal antibodies in the non-nutritive buffer; and

transporting the cells to a point of care facility in a package equipped to maintain

a storage temperature below 20°C, wherein the living cells are at the storage temperature for greater than 24 hours while maintaining their identity and at least one of the functional characteristics of the living cells useful in immunotherapy, wherein the live cells in the composition express CD40L after transportation and removal from storage temperature in an amount of at least 80% relative to the expression of CD40L at the time of formulation.

12. The method of claim 11 further comprising placing the formulated cells in a flexible container or syringe prior to transporting.

13. The method of claim 11 wherein the monoclonal antibodies are cross-linked.

14. The method of claim 11 wherein the live cells in the non-nutritive buffer are stored for at least 72 hours.

15. A method of providing living cell compositions to a point of care facility comprising:

formulating the living cells in a non-nutritive buffer at a processing facility, the living cells being CD4+ cells and having been cryopreserved, thawed and reactivated by immobilized monoclonal antibodies in the non-nutritive buffer; and

transporting the cells to a point of care facility in a package equipped to maintain a storage temperature below 20°C, wherein the living cells are at the storage temperature for greater than 24 hours while maintaining their identity and at least one of the functional characteristics of the living cells useful in immunotherapy,

wherein the cells in the composition secrete IFN-γ after transportation and removal from storage temperature in an amount of at least 80% compared to the levels at the time of formulation.

16. The method of claim 15 further comprising placing the formulated cells in a flexible container or syringe prior to transporting.

17. The method of claim 15 wherein the monoclonal antibodies are cross-linked.

18. The method of claim 15 wherein the secretion of IFN-γ after transportation and storage is recovered after incubation at 37°C for at least 24 hours.

19. The method of claim 15 wherein the live cells in the non-nutritive buffer are at the storage temperature for at least 72 hours.