cytokines, preferably GM-CSF and IL-4, combined with further maturation of the DCs thus obtained by additional exposure to a combination of maturation factors, preferably clinical-grade IFN-g and the detoxified derivative of endotoxin, MPLA.

The obtained product comprising mature dendritic cells produced/generated within about 4 days of total in vitro culture time (preferably within about 3 days to about 5 days culture), are thereafter loaded with one or more antigens, in particular tumor-associated antigens (TAA). This fast method according to the disclosure is able to generate large number of DC from leukapheresis products preferably in a closed system using serum-free medium.

In one aspect, the present disclosure refers to an in vitro method for manufacturing an autologous dendritic cell vaccine, said method comprising the following steps:

~~- providing isolated monocytic dendritic cell precursors;~~

- culturing ~~said~~ dendritic cell precursors for ~~about~~ 48 to 96 hours in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) to obtain immature dendritic cells;
- contacting the immature DCs for the last 24 hours with Interferon gamma (IFN-g) and monophosphoryl lipid A (MPLA), under culture conditions suitable for maturation of the immature DCs to form a mature DC population; and
- transfecting the mature DC's with antigen-encoding mRNA.

In one example, the method according to the disclosure comprises the following steps:

- obtaining from a patient a mononuclear cell leukapheresis product,
- isolation of monocytes from the leukapheresis,
- incubating the monocytes with clinical grade cytokines, preferably suitable amounts of GM-CSF and IL-4 for the differentiation into dendritic cells,
- addition of maturation factors MPLA and IFN-g for final maturation of the monocyte-derived dendritic cells, and
- recovering the obtained cells and transfecting them with a nucleic acid sequence, in particular mRNA, that encodes for one or several antigens or epitopes.

In one example, the monocytes are contacted with GM-CSF and IL-4 for about 1 to 4, preferably 1 to 3, more preferably 2 to 3 days (with 1 day counting for 24 hours), during which time the DC precursors differentiate into immature dendritic cells. In a further example, the maturation time in the presence of IFN-g and MPLA takes 1 to 3, preferably 1 to 2, more preferably about 2 days (24 hours). The culture conditions are suitable for maturation of the immature DCs to form a mature DC population.

In another aspect, the present disclosure refers to transfected dendritic cells obtainable by the method as disclosed herein. In a further example, the disclosure provides the mature

(and transfected) dendritic cells or population of mature (and transfected) dendritic cells obtainable by the method provided herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition or vaccine comprising the mature, transfected, and optionally cryopreserved, dendritic cells obtained by the method as disclosed herein. The disclosure also provides a composition, kit, clinical grade bag or cryovial comprising the dendritic cells obtained by the method of the disclosure.

In yet another aspect, the present disclosure refers to the transfected dendritic cells obtained by the method as disclosed herein, or the pharmaceutical composition as disclosed herein for use as a medicament, in particular in active immunotherapy. The transfected dendritic cells are especially useful for preparing a composition for immunotherapy, in particular their use in immunotherapy, more in particular in treating cancer. Hence the disclosure also provides a method for immunotherapy, tumor therapy or a method for activating T cells, which comprises administering transfected dendritic cells obtained by the method provided herein, to a subject.

In yet another aspect, the present disclosure refers to a mature, mRNA electroporated, cryopreserved and thawed dendritic cell population, said dendritic cells being maturated in the presence of IFN-g and MPLA and being characterized by a cell surface level of the T-cell co-inhibitory ligand PD-L1 below 400, as expressed by relative mean fluorescence intensity (relMFI).

In yet another aspect, the present disclosure refers to a use of the dendritic cells as disclosed herein for the manufacture of a medicament for use as a vaccine.

In yet another aspect, the present disclosure refers to the use of the transfected dendritic cells obtained by the method as disclosed herein, or the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating or preventing malignant disorders, non-malignant disorders or infectious diseases.

CLAIMS

1. An in vitro method for manufacturing an autologous dendritic cell vaccine, said method comprising the following steps:

~~- providing isolated monocytic dendritic cell precursors;~~

- culturing ~~said~~ dendritic cell precursors for ~~about~~ 48 to 96 hours in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4) to obtain immature dendritic cells;

- contacting the immature DCs for the last 24 hours with Interferon gamma (IFN-g) and monophosphoryl lipid A (MPLA), under culture conditions suitable for maturation of the immature DCs to form a mature DC population; and

- transfecting the mature DC's with antigen-encoding mRNA.

2. Method according to claim 1, wherein the dendritic cells are differentiated and matured in a clinical-grade fully closed system.

3. Method according to claims 1 or 2, wherein the monocytic dendritic cell precursors are provided in culture bags, in particular wherein the cell density in said culture bags at initiation of cell culture ranges from 0.5 to 2 x 10E6 cells/mL.

4. Method according to any one of claims 1 to 3, wherein the concentration of said GM-CSF, IL-4 and IFN-g is between 500 and 2500U/ml.

5. Method according to any one of claims 1 to 3, wherein the concentration of said MPLA is between 1-10µg/ml.

6. Method according to any one of claims 1 to 5, wherein the transfection is performed by electroporation, in particular using a square wave pulse.

7. Method according to any one of claims 1 to 6, wherein the transfected DCs are further resuspended in a cryopreservation medium and are stored in the vapour phase of a liquid nitrogen container.

8. Method according to any one of claims 1 to 7, wherein said antigen is selected from the group consisting of a tumor antigen, a tumor-associated antigen, a cancer-testis antigen, a mutanome-derived antigen, a ~~(oncogenic)~~ viral antigen, a bacterial antigen, a yeast antigen, a parasitic antigen and a fungal antigen.

9. Transfected d~~D~~endritic cells obtainable by the method of any one of claims 1 to 8.

10. A pharmaceutical composition or vaccine comprising the mature, transfected, and optionally cryopreserved, dendritic cells obtained by the method of any one of claims 1 to 8.

11. The transfected dendritic cells obtained by the method of claims 1 to 8, or the pharmaceutical composition according to claim 10, for use as a medicament, in particular in active immunotherapy.

12. A mature, mRNA electroporated, cryopreserved and thawed dendritic cell population, said dendritic cells being maturated in the presence of IFN-g and MPLA and being characterized by a cell surface level of the T-cell co-inhibitory ligand PD-L1 below 400, as expressed by relative mean fluorescence intensity (relMFI).

13. Use of the dendritic cells according to claim 9, for the manufacture of a medicament for use as a vaccine.

14. Use of the transfected dendritic cells obtained by the method of claims 1 to 8, or the pharmaceutical composition according to claim 10, in the manufacture of a medicament for treating or preventing malignant disorders, non-malignant disorders or infectious diseases.

15. The method according to claim 1, wherein the dendritic cell precursors had been isolated by collecting heparinized blood; apheresis or leukapheresis; preparation of buffy coats; resetting; centrifugation; density gradient centrifugation; differential lysis of cells; filtration; elutriation; fluorescence-activated cell sorting; or immunomagnetic isolation.

16. The method according to claim 1, wherein the dendritic cell precursors are monocytes.