transplantation, treatment with therapeutic protein and gene therapy, to avoid degradation of self or therapeutic molecules/tissues by the immune system.

SUMMARY:

The present disclosure relates to an isolated population of $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ T regulatory (Treg) cells.

The present disclosure also relates to a method for detecting and/or isolating $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes comprising the following steps of: (a) contacting said population of human PBMCs or lymphocytes with means useful for isolating a population of human $CD8^{+}CD45RC^{low}$ Treg cells; (b) contacting the isolated population of human $CD8^{+}CD45RC^{low}$ Treg cells obtained at step (a) with means useful for isolating $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells.

The present disclosure also relates to a method for expanding $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells comprising a step of contacting an isolated population of $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells with a culture medium suitable for expanding Treg cells. The present disclosure also relates to a method for producing a population of IFNγ+IL-10+IL-34+ secreting human CD8+CD45RClow Treg cells comprising (i) isolating $CD8^{+}CD45RC^{low}$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes, optionally isolating $CD8^{+}CD45RC^{low}GITR^{+}$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes, (ii) optionally freezing and subsequently thawing the isolated Treg cells, and (iii) expanding said cells in culture.

The present disclosure also relates to an isolated population of expanded $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells.

The present disclosure also relates to a pharmaceutical composition comprising an isolated population of $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells (expanded or not) according to the present disclosure.

The present disclosure also relates to an isolated population of $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells (expanded or not) according to the present disclosure for use as a medicament.

The present disclosure also relates to an isolated population of $IFN\gamma^{+}IL\text{-}10^{+}IL\text{-}34^{+}$ secreting human $CD8^{+}CD45RC^{low}$ Treg cells (expanded or not) according to the present disclosure for use in a method for preventing or treating transplant rejection, GVHD (graft versus host

disease), chronic inflammatory diseases, autoimmune diseases, unwanted immune response against therapeutic proteins or allergies in a patient in need thereof.

In one aspect of the present disclosure, there is provided an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ T regulatory (Treg) cells, wherein said Treg cells are preferably $GITR^+$ and/or $Foxp3^+$.

In another aspect of the present disclosure, there is provided a method for detecting and/or isolating $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes, wherein said Treg cells optionally are $GITR^+$ and/or $Foxp3^+$, comprising the following steps of: (a) contacting said population of human PBMCs or lymphocytes with means useful for isolating a population of human $CD8^+CD45RC^{low}$ Treg cells, optionally with means useful for isolating a population of $GITR^+$ and/or $Foxp3^+$ $CD8^+CD45RC^{low}$ Treg cells; (b) contacting the isolated population of human $CD8^+CD45RC^{low}$ Treg cells obtained at step (a) with means useful for isolating $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells.

In yet another aspect of the present disclosure, there is provided a method for expanding $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells, said Treg cells optionally being $GITR^+$ and/or $Foxp3^+$, comprising a step of contacting an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells with a culture medium suitable for expanding Treg cells.

In a further aspect of the present disclosure, there is provided a method for producing a population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells comprising:

- isolating $CD8^+GITR^+$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes, optionally isolating $CD8^+CD45RC^{low}GITR^+$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes,
- optionally freezing and subsequently thawing the isolated Treg cells, and
- expanding said cells in culture.

In another aspect of the present disclosure, there is provided an isolated population of expanded $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells, wherein preferably said Treg cells are $GITR^+$ and/or $Foxp3^+$.

In yet another aspect of the present disclosure, there is provided a pharmaceutical composition comprising an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells as defined herein or an isolated population of expanded $IFN\gamma^+IL$-

10^+IL-34^+ secreting human $CD8^+CD45RC^{low}$ Treg cells as defined herein and at least one pharmaceutically acceptable carrier.

In a further aspect of the present disclosure, there is provided an isolated population of IFNγ$^+$IL-10^+IL-34^+ secreting human $CD8^+CD45RC^{low}$ Treg cells as defined herein or an isolated population of expanded IFNγ$^+$IL-10^+IL-34^+ secreting human $CD8^+CD45RC^{low}$ Treg cells as defined herein for use as a medicament.

In another aspect of the present disclosure, there is provided a use of an isolated population of IFNγ$^+$IL-10^+IL-34^+ secreting human $CD8^+CD45RC^{low}$ Treg cells as defined herein or of an isolated population of expanded IFNγ$^+$IL-10^+IL-34^+ secreting human $CD8^+CD45RC^{low}$ Treg cells as defined herein for the manufacture of a medicament for preventing or treating transplant rejection, GVHD, chronic inflammatory diseases, autoimmune diseases, unwanted immune response against therapeutic proteins or allergies in a patient in need thereof.

In yet another aspect, the present disclosure also relates to an *in vitro* method for determining whether a patient is at risk of transplant rejection, GVHD, chronic inflammatory diseases, autoimmune diseases, unwanted immune response against therapeutic proteins or allergies, comprising a step of determining the presence of IFNγ$^+$IL-10^+IL-34^+ secreting human $CD8^+CD45RC^{low}$ Treg cells in a biological sample obtained from said patient by a method of the present disclosure, wherein the presence of IFNγ$^+$IL-10^+IL-34^+ secreting human $CD8^+CD45RC^{low}$ Treg cells is indicative of a reduced risk of transplant rejection, GVHD, chronic inflammatory diseases, autoimmune diseases, unwanted immune response against therapeutic proteins or allergies.

DETAILED DESCRIPTION OF THE INVENTION:

The invention results from the identification of highly suppressive subpopulations of $CD8^+$ Tregs. In particular, the inventors identified a highly suppressive subpopulation of IFNγ$^+$IL-10^+IL-34^+secreting subpopulation of $CD8^+CD45RC^{low}$ Tregs. The inventors demonstrated their superior potential compared to classical $CD4^+CD25^{high}CD127^{low}$ Tregs on the anti-donor $CD4^+CD25^-$ effector T cell response and the role of IFNγ, IL-10, and IL-34 in their suppressive activity. In addition, the inventors described the specific expansion of IFNγ$^+$IL-10^+IL-34^+secreting $CD8^+CD45RC^{low}$ Tregs and their potential as a cell-based therapy in transplantation to inhibit human skin graft rejection and GVHD using humanized mice.

Treg cells population of the invention

In a first aspect, the invention relates to isolated populations of $CD8^+$ Tregs and in particular to an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ T regulatory (Treg) cells. The invention also relates to an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ T regulatory (Treg) cells, wherein said Treg cells are $GITR^+$ and/or $Foxp3^+$.

In one embodiment, the invention relates to an isolated population of CD8+CD45RClow Tregs. In one embodiment, the invention relates to an isolated population of CD8+GITR+ Tregs. In one embodiment, invention relates to an isolated population of CD8+Foxp3+ Tregs. In one embodiment, the invention relates to an isolated population of CD8+CD45RClowGITR+ Tregs. In one embodiment, invention relates to an isolated population of CD8+CD45RClowFoxp3+ Tregs. In one embodiment, invention relates to an

CLAIMS:

1. An isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ T regulatory (Treg) cells, wherein said Treg cells are preferably $GITR^+$ and/or $Foxp3^+$.

2. The isolated population according to claim **1**, wherein said Treg cells are genetically modified Treg cells comprising a chimeric antigen receptor (CAR).

3. A method for detecting and/or isolating $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes, wherein said Treg cells optionally are $GITR^+$ and/or $Foxp3^+$, comprising the following steps of: (a) contacting said population of human PBMCs or lymphocytes with means useful for isolating a population of human $CD8^+CD45RC^{low}$ Treg cells, optionally with means useful for isolating a population of $GITR^+$ and/or $Foxp3^+$ $CD8^+CD45RC^{low}$ Treg cells; (b) contacting the isolated population of human $CD8^+CD45RC^{low}$ Treg cells obtained at step (a) with means useful for isolating $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells.

4. The method according to claim **3**, wherein the means useful for isolating the population of human $CD8^+CD45RC^{low}$ Treg cells are a combination of at least three antibodies consisting of a monoclonal anti-human CD3 antibody, a monoclonal anti-human CD8 antibody and a monoclonal anti-human CD45RC antibody, optionally further comprising an anti-human GITR antibody, and wherein the means useful for isolating $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells are a combination of at least two bispecific antibodies selected from the group consisting of a bispecific antibody which binds to IFNγ and to a cell surface molecule specific for T cells (e.g. CD3, CD8, CD45), a bispecific antibody which binds to IL-10 and to a cell surface molecule specific for T cells (e.g. CD3, CD8, CD45) and a bispecific antibody which binds to IL-34 and to a cell surface molecule specific for T cells (e.g. CD3, CD8, CD45).

5. A method for expanding $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells, said Treg cells optionally being $GITR^+$ and/or $Foxp3^+$, comprising a step of contacting an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells with a culture medium suitable for expanding Treg cells.

6. The method according to claim **5**, wherein the isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells is frozen and subsequently thawed before being contacted cells with a culture medium suitable for expanding Treg cells.

7. The method according to claim **5** or **6**, wherein said culture medium suitable for expanding Treg cells comprises at least an antigen-specific stimulating agent selected from the group consisting of antigens, cells, MHC polymers and antibodies.

8. The method according to any one of claims **5** to **7**, wherein said culture medium comprises at least one cytokine, preferably wherein said culture medium comprises an amount of interleukin-2 (IL-2) and/or an amount of interleukin-15 (IL-15).

9. A method for producing a population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells comprising:

- isolating $CD8^+GITR^+$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes, optionally isolating $CD8^+CD45RC^{low}GITR^+$ Treg cells from a biological sample containing human peripheral blood mononuclear cells (PBMCs) or lymphocytes,
- optionally freezing and subsequently thawing the isolated Treg cells, and
- expanding said cells in culture.

10. An isolated population of expanded $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells, wherein preferably said Treg cells are $GITR^+$ and/or $Foxp3^+$.

11. The isolated population according to claim **10**, wherein said Treg cells are antigen-specific.

12. A pharmaceutical composition comprising an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells according to claim **1** or claim **2** or an isolated population of expanded $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells according to claim **10** or claim **11** and at least one pharmaceutically acceptable carrier.

13. An isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells according to claim **1** or claim **2** or an isolated population of expanded $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells according to claim **10** or claim **11** for use as a medicament.

14. Use of an isolated population of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells according to claim **1** or claim **2** or of an isolated population of expanded $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells according to claim **10** or claim **11** for the manufacture of a medicament for preventing or treating transplant rejection, GVHD, chronic inflammatory diseases, autoimmune diseases, unwanted immune response against therapeutic proteins or allergies in a patient in need thereof.

15. An *in vitro* method for determining whether a patient is at risk of transplant rejection, GVHD, chronic inflammatory diseases, autoimmune diseases, unwanted immune response against therapeutic proteins or allergies, comprising a step of determining the presence of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells in a biological sample obtained from said patient by a method according to claim **3** or claim **4**, wherein the presence of a $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells is indicative of a reduced risk of transplant rejection, GVHD, chronic inflammatory diseases, autoimmune diseases, unwanted immune response against therapeutic proteins or allergies.