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 IFNγ$^+$IL-10$^+$IL-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 IFNγ$^+$IL-10$^+$IL-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, wherein the means useful for isolating the population of human CD8$^+$CD45RC^{low} Treg cells are binding partners, preferably antibodies, to suitable cell surfaces molecules or markers selected from the group comprising CD3, CD8 and CD45RC; (b) contacting the isolated population of human CD8$^+$CD45RC^{low} Treg cells obtained at step (a) with means useful for isolating IFNγ$^+$IL-10$^+$IL-34$^+$ secreting human CD8$^+$CD45RC^{low} Treg cells, and wherein the means useful for isolating IFNγ$^+$IL-10$^+$IL-34$^+$ secreting human CD8$^+$CD45RC^{low} Treg cells are a bispecific antibody which binds to IFNγ and to a cell surface molecule selected from the group comprising CD3, CD8 and CD45RC, a bispecific antibody which binds to IL-10 and to a cell surface molecule selected from the group comprising CD3, CD8 and CD45RC and a bispecific antibody which binds to IL-34 and to a cell surface molecule selected from the group comprising CD3, CD8 and CD45RC.

In yet another aspect of the present disclosure, there is provided a method for expanding IFNγ$^+$IL-10$^+$IL-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 IFNγ$^+$IL-10$^+$IL-34$^+$ secreting human CD8$^+$CD45RC^{low} Treg cells with a culture medium suitable for expanding Treg cells.

In yet another aspect of the present disclosure, there is provided a pharmaceutical composition comprising an isolated population of IFNγ$^+$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 $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ 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 $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ 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 $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ secreting human $CD8^+CD45RC^{low}$ Treg cells in a biological sample obtained from said patient by a method of the present disclosure, wherein the presence of $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$ 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.

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 $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$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 $IFN\gamma^+IL\text{-}10^+IL\text{-}34^+$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.

CLAIMS:

1. An isolated population of IFNγ$^+$IL-10$^+$IL-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 antigen-specific and/or genetically modified Treg cells comprising a chimeric antigen receptor (CAR) or a chimeric autoantibody receptor (CAAR).

3. A method for detecting and/or isolating IFNγ$^+$IL-10$^+$IL-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, wherein the means useful for isolating the population of human CD8$^+$CD45RC^{low} Treg cells are binding partners, preferably antibodies, to suitable cell surfaces molecules or markers selected from the group comprising CD3, CD8 and CD45RC; (b) contacting the isolated population of human CD8$^+$CD45RC^{low} Treg cells obtained at step (a) with means useful for isolating IFNγ$^+$IL-10$^+$IL-34$^+$ secreting human CD8$^+$CD45RC^{low} Treg cells, and wherein the means useful for isolating IFNγ$^+$IL-10$^+$IL-34$^+$ secreting human CD8$^+$CD45RC^{low} Treg cells are a bispecific antibody which binds to IFNγ and to a cell surface molecule selected from the group comprising CD3, CD8 and CD45RC, a bispecific antibody which binds to IL-10 and to a cell surface molecule selected from the group comprising CD3, CD8 and CD45RC and a bispecific antibody which binds to IL-34 and to a cell surface molecule selected from the group comprising CD3, CD8 and CD45RC.

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.

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

10. An isolated population of IFNγ$^+$IL-10$^+$IL-34$^+$ secreting human CD8$^+$CD45RC^{low} Treg cells according to claim **1** or claim **2** for use as a medicament.

11. Use of an isolated population of IFNγ$^+$IL-10$^+$IL-34$^+$ secreting human CD8$^+$CD45RC^{low} Treg cells according to claim **1** or claim **2** 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.

12. 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 according to claim **3** or claim **4**, wherein the presence of a 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.