Interleukin-34 (IL34) was identified in 2008 (9). Studies showed that IL34 shares homology with M-CSF and they act through a common receptor, CD115, also called CSF-1R, (9) expressed on the cell surface of monocytes, and in the brain through a newly described receptor, Receptor-type Protein-tyrosine Phosphatase ζ (PTP-ζ) (10). However, studies have demonstrated that IL34 and M-CSF display distinct biological activity and signal activation (11), in part due to their differing spatial and temporal expression (12). Up to now IL34 function has been mainly linked with the survival and function of monocytes and macrophages (osteoclasts, microglia) (12). IL34 protein expression in resting cells has been observed in keratinocytes, hair follicles, neurons, proximal renal tubule cells and seminiferous tubule germ cells (12), and also in heart, brain, lung, liver, kidney, spleen, thymus, testicles, ovaries, prostate, colon, small intestine, spleen red pulp and osteoclasts (9). So far, IL34 has not been linked to the effects on immune function of DCs or T cells (12).

More recently, IL34 was shown to induce the differentiation of human monocytes into immunosuppressive macrophages (also referred as IL34-Mφ) since said IL34-differenciated macrophages suppressed TCR-dependent T cell proliferation (13), to induce transplantation tolerance (13) and also to induce IL-17-producing effector T helper cells, called Th17 cells (14). However, IL34-differenciated macrophages have never been shown to be useful for generating and expanding Treg from peripheral blood mononuclear cells (PBMCs).

SUMMARY:

The present disclosure relates to methods for obtaining a population of regulatory T cells. In particular, the present disclosure is defined by the claims.

In a first aspect of the present disclosure, there is provided a method for obtaining a population of human Treg cells, wherein said Treg cells are $CD8^+$ $Foxp3^+$ Treg cells and/or $CD4^+$ $Foxp3^+$ Treg cells, comprising the steps of: (a) culturing a population of human monocytes in presence of interleukin-34 (IL-34) polypeptide in order to obtain a population of immunosuppressive macrophages; (b) co-culturing a population of human peripheral blood mononuclear cells (PBMCs) and the population of immunosuppressive macrophages obtained at step (a); and (c) optionally isolating the population of human Treg cells obtained at step (b).

In a second aspect of the present disclosure, there is provided a use of IL-34 in the method as disclosed herein, for obtaining an enriched population of Tregs from a population of PBMCs, wherein said Treg cells are $CD8^+$ $Foxp3^+$ Treg cells and/or $CD4^+$ $Foxp3^+$ Treg cells.

DETAILED DESCRIPTION OF THE INVENTION:

The invention is based on results about a new efficient method for generating and expanding Treg from PBMCs without prior step of isolation of Tregs (usually 5% for Foxp3+ Tregs) contained in a biological sample obtained from a patient such as a blood sample. The inventors have indeed shown that human IL34-differentiated macrophages expand and potentiate human Tregs including human CD4+ and CD8+ $Foxp3^+$ Tregs (above 60%) and CD4+ and CD8+ $CD45RC^{low}$ Tregs (near 100%). Moreover, the inventors have demonstrated that the IL34-expanded Tregs obtained by a method of the present invention displayed a highly potent suppressive capacity up to a 16:1 effector: suppressor ratio, comparatively to unstimulated and polyclonaly stimulated Tregs.

CLAIMS

1. A method for obtaining a population of human Treg cells, wherein said Treg cells are $CD8^+$ $Foxp3^+$ Treg cells and/or $CD4^+$ $Foxp3^+$ Treg cells, comprising the steps of: (a) culturing a population of human monocytes in presence of interleukin-34 (IL-34) polypeptide in order to obtain a population of immunosuppressive macrophages; (b) co-culturing a population of human peripheral blood mononuclear cells (PBMCs) and the population of immunosuppressive macrophages obtained at step (a); and (c) optionally isolating the population of human Treg cells obtained at step (b).

2. The method according to claim **1** wherein the population of immunosuppressive macrophages are allogeneic to the population of human PBMCs.

3. The method according to claim **1** or claim **2,** wherein the population of human monocytes is a population of $CD14^+$ human monocytes.

4. The method according to any one of claims **1** to **3,** wherein the IL-34 polypeptide is human IL-34 polypeptide.

5. The method according to any one of claims **1** to **4,** wherein the IL-34 polypeptide comprises or consists of SEQ ID NO: 1 or has a polypeptide sequence having at least 80% identity to SEQ ID NO: 1.

6. The method according to any one of claims **1** to **5**, wherein the IL-34 polypeptide is added to the culture medium at a concentration ranging from 1 to 500 ng/mL.

7. The method according to claim **6**, wherein the IL-34 polypeptide is added to the culture medium at a concentration of 50 ng/mL.

8. The method according to any one of claims **1** to **7**, wherein the co-culture of PBMCs with the population of immunosuppressive macrophages obtained at step (a) is performed in a medium comprising at least one cytokine selected from the group consisting of IL-15, IL-12, IL-4, IL-7, IL-2, IFN-γ, IL-34 and pro-inflammatory cytokines selected from the group consisting of IL-1, IL-6 and TNFα.

9. The method according to claim **8**, wherein the cytokines are IL-15 and/or IL-2.

10. The method according to claim **8** or claim **9**, wherein the cytokines are human IL-15 and/or human IL-2.

11. The method according to any one of claims **1** to **10,** wherein the Treg cells are $CD8^+Foxp3^+CD45RC^{low}$ Treg cells and/or $CD4^+Foxp3^+CD45RC^{low}$ Treg cells.

12. Use of IL-34 in the method of any one of claims **1** to **11**, for obtaining an enriched population of Tregs from a population of PBMCs, wherein said Treg cells are $CD8^+$ $Foxp3^+$ Treg cells and/or $CD4^+$ $Foxp3^+$ Treg cells.