Currently, effective treatments for autoimmune and inflammatory CNS diseases do not exist. Treatment is focused on merely reducing its symptoms, usually by general suppression of the immune system. There is a need for a therapy which specifically targets local immune responses associated with onset and progression of CNS disease.

SUMMARY ~~OF THE INVENTION~~

The present inventors have surprisingly found that exogenous FOXP3 expression in regulatory T cell (Tregs) (which already express endogenous FOXP3) enhances their regulatory function.

In one aspect, the present disclosure refers to a method for enhancing the suppressive ability of a regulatory T cell (Treg) comprising introducing a polynucleotide encoding a FOXP3 polypeptide into the Treg, wherein the ability of the regulatory T cell (Treg) to suppress immune responses is enhanced.

In another aspect, the present disclosure refers to an engineered Treg obtainable or obtained by the method as disclosed herein, wherein the engineered Treg is a $CD4^+CD25^+CD127^{-/low}CD45RA^+$ Treg, wherein the polynucleotide encoding a FOXP3 polypeptide and the polynucleotide encoding the exogenous TCR or the CAR are provided by a single expression vector and wherein the vector comprises a nucleic acid with the orientation: 5' FOXP3 – X – TCR/CAR; wherein X is an internal self-cleaving sequence.

In another aspect, the present disclosure refers to an engineered $CD4^+CD25^+CD127^{-/low}CD45RA^+$ Treg comprising an exogenous polynucleotide encoding a FOXP3 polypeptide and a polynucleotide encoding an exogenous T cell receptor (TCR) or a polynucleotide encoding a chimeric antigen receptor (CAR), and having higher FOXP3 expression than a corresponding non-engineered Treg, wherein the polynucleotide encoding a FOXP3 polypeptide and the polynucleotide encoding the exogenous TCR or the CAR are provided by a single expression vector, and wherein the vector comprises a nucleic acid with the orientation: 5' FOXP3 – X – TCR/CAR; wherein X is an internal self-cleaving sequence.

In another aspect, the present disclosure refers to a pharmaceutical composition comprising an engineered Treg as disclosed herein.

In another aspect, the present disclosure refers to an engineered Treg as disclosed herein, or a pharmaceutical composition as disclosed herein, for use in prevention and/or treatment of a disease.

In another aspect, the present disclosure refers to the use of an engineered Treg which is produced according to a method as disclosed herein, or which is defined as disclosed herein, in the manufacture of a medicament for treating or preventing a disease associated with a pathological immune response by suppressing said response

In another aspect, the present disclosure refers to the in vitro use of a polynucleotide encoding a FOXP3 polypeptide to enhance the ability of a regulatory T cell (Treg) to suppress immune responses.

Accordingly, the ~~invention~~disclosure provides a method for enhancing the ability of regulatory T cells (Tregs) to suppress immune responses.

The Tregs of the present ~~invention~~disclosure are natural Tregs or induced Tregs which developed from conventional T cells. For example, the Tregs of the present ~~invention~~disclosure are natural Tregs or induced Tregs which developed from conventional T cells *in vivo*. Suitable Treg cells include thymus-derived, natural Treg (nTreg) cells and peripherally generated, induced Treg (iTreg) cells. In other words, the Tregs of the present ~~invention~~disclosure express endogenous FOXP3. Surprisingly, the present inventors have determined that increasing FOXP3 expression in Tregs which already express endogenous FOXP3 (e.g. by introducing exogenous FOXP3) enhances the regulatory function of the Tregs to a greater degree than the regulatory function provided by expressing exogenous FOXP3 in conventional T cells which do not express endogenous FOXP3.

The present inventors have further determined that increasing FOXP3 expression in Tregs which already express endogenous FOXP3 enables improved retention of a Treg functional profile *in vivo* following administration to a subject. For example, it has been determined that natural Tregs which do not express exogenous FOXP3 may lose their Treg profile following administration to a subject – for example natural Tregs which do not express exogenous FOXP may have reduced levels of FOXP3 expression and be capable of producing pro-inflammatory, effector cytokines after a period following administration to a subject. Tregs provided by the present ~~invention~~disclosure may retain FOXP3 expression and have reduced capability to produce pro-inflammatory, effector cytokines after a period following administration to a subject.

In a preferred ~~embodiment~~example, the Tregs of the present ~~invention~~disclosure are natural Tregs.

In one aspect, the ~~invention~~disclosure provides a method for generating a population of regulatory T cells (Tregs) comprising providing a first population of Tregs and increasing FOXP3 expression in the first population of Tregs to generate a second population of Tregs.

The ~~invention~~disclosure provides a method for enhancing the ability of a Tregs to suppress immune responses comprising increasing FOXP3 expression in the Treg.

In some ~~embodiment~~examples of the ~~invention~~disclosure, FOXP3 expression is increased by introducing into the Tregs a polynucleotide encoding a FOXP3 protein.

In some ~~embodiment~~examples of the ~~invention~~disclosure, the method for enhancing the ability of regulatory T cells (Tregs) to suppress immune responses comprises:

(a) isolating a Treg from a cell population; and

(b) increasing FOXP3 expression in Tregs.

Suitably, the Treg may refer to a population of Tregs (i.e. a plurality of Tregs).

The ~~invention~~disclosure also provides an engineered Treg obtainable or obtained by the method of the ~~invention~~disclosure.

The ~~invention~~disclosure also provides an engineered Treg having higher FOXP3 expression than a non-engineered Treg.

In some ~~embodiment~~examples the engineered Treg comprises an exogenous polynucleotide encoding a FOXP3 protein.

The ~~invention~~disclosure also provides a pharmaceutical composition comprising an engineered Treg of the ~~invention~~disclosure.

The ~~invention~~disclosure also provides an engineered Treg of the ~~invention~~disclosure or a pharmaceutical composition of the ~~invention~~disclosure for use in prevention and/or treatment of a disease.

The ~~invention~~disclosure also provides the use of an engineered Treg of the ~~invention~~disclosure in the manufacture of a medicament.

The ~~invention~~disclosure also provides a method for prevention and/or treatment of a disease comprising administering to a subject an engineered Treg or a composition of the ~~invention~~disclosure.

DESCRIPTION OF THE FIGURES

Figure 1 shows the proliferation of Tconv cells transduced with a TCR construct, with and without peptide (shown with "*"), and the proliferation of the same cells in the presence of

CLAIMS

1. A method for enhancing the suppressive ability of a regulatory T cell (Treg) ~~to suppress immune responses~~ comprising introducing a polynucleotide encoding a FOXP3 polypeptide into the Treg, wherein the ability of the regulatory T cell (Treg) to suppress immune responses is enhanced.

2. A method according to claim 1 wherein:

(i) the FOXP3 polypeptide comprises an amino acid sequence which is at least 90~~80~~ % identical to SEQ ID NO: 3 or 4 ~~or a functional fragment thereof~~; or

(ii) the polynucleotide encoding the FOXP3 polypeptide comprises a polynucleotide sequence which is at least 90~~80~~ % identical to SEQ ID NO: 1 or 2 ~~or a functional fragment thereof~~,

wherein the FOXP3 polypeptide is capable of enhancing the ability of a Treg to suppress immune responses.

3. A method according to claim 1 or 2 wherein the polynucleotide encoding FOXP3 is a contiguous portion of an expression vector.

4. A method according to any preceding claim which further comprises introducing a polynucleotide encoding an exogenous T cell receptor (TCR) or a polynucleotide encoding a chimeric antigen receptor (CAR) into the Treg.

5. A method according to claim 4 wherein the polynucleotide encoding a FOXP3 polypeptide and the polynucleotide encoding the exogenous TCR or the CAR are provided by a single expression vector.

6. A method according to claim 5 wherein the vector comprises a nucleic acid with the orientation: 5' FOXP3 – X – TCR/CAR; wherein X is an internal self-cleaving sequence.

7. A method according to any preceding claim wherein the polynucleotide encoding FOXP3 is introduced into the ~~isolated~~ Treg by viral transduction; optionally wherein the polynucleotide encoding FOXP3 is introduced into the ~~isolated~~ Treg by retroviral transduction.

8. A method according to any preceding claim comprising:

(a) isolating the ~~a~~ Treg from a cell population; and

(b) increasing FOXP3 expression in the Treg by introducing a polynucleotide encoding a FOXP3 polypeptide into the Treg.

9. A method according to claim 8 wherein the cell population comprises or consists of peripheral blood mononuclear cells (PBMCs).

10. A method according to claim 8 or 9 wherein isolating the Treg comprises

isolating $CD4^+$ T cells; and

isolating the Treg from the $CD4^+$ T cells.

11. A method according to any of claims 8-10 wherein the isolation of the Treg comprises selection using immuno-magnetic beads or fluorescence-activated cell sorting (FACS).

12. A method according to any of claims 8-11 wherein the Treg is isolated by selecting for (i) $CD4^+CD25^+CD127^-$ and/or $CD4^+CD25^+CD127^{low}$ cells; (ii) $CD4^+CD25^{hi}CD127^-$ and/or $CD4^+CD25^+CD127^{low}$ cells; (iii) $CD4^+CD25^+CD127^-CD45RA^+$ and/or $CD4^+CD25^+CD127^{low}CD45RA^+$.

13. A method according to any of claims 8-12 wherein the Treg is isolated by selecting for $FOXP3^+$ cells; preferablye wherein the Treg is isolated by selecting for $CD4^+CD25^+FOXP3^+Helios^+Neuropilin1^+$ cells.

14. An engineered Treg obtainable or obtained by the method of any of claims 1-13, wherein the engineered Treg is a $CD4^+CD25^+CD127^{-/low}CD45RA^+$ Treg, wherein the polynucleotide encoding a FOXP3 polypeptide and the polynucleotide encoding the exogenous TCR or the CAR are provided by a single expression vector and wherein the vector comprises a nucleic acid with the orientation: 5' FOXP3 – X – TCR/CAR; wherein X is an internal self-cleaving sequence.

15. An engineered $CD4^+CD25^+CD127^{-/low}CD45RA^+$ Treg comprising an exogenous polynucleotide encoding a FOXP3 polypeptide and a polynucleotide encoding an exogenous T cell receptor (TCR) or a polynucleotide encoding a chimeric antigen receptor (CAR), and having higher FOXP3 expression than a corresponding non-engineered Treg, wherein the polynucleotide encoding a FOXP3 polypeptide and the polynucleotide encoding the exogenous TCR or the CAR are provided by a single expression vector, and wherein the vector comprises a nucleic acid with the orientation: 5' FOXP3 – X – TCR/CAR; wherein X is an internal self-cleaving sequence.

16. An engineered Treg according to claim 15 wherein the FOXP3 polypeptide (i) comprises an amino acid sequence which is at least 9080 % identical to SEQ ID NO: 3 or 4 ~~or a functional fragment thereof~~ or (ii) is encoded by a polynucleotide sequence which is at least 9080 % identical to SEQ ID NO: 1 or 2 ~~or a functional fragment thereof~~, wherein the FOXP3 polypeptide is capable of enhancing the ability of a Treg to suppress immune responses.

~~17. An engineered Treg according to claim 16 wherein the exogenous polynucleotide encoding FOXP3 is a contiguous portion of an expression vector.~~

~~18. An engineered Treg according to any of claims 14 to 17 further comprising a polynucleotide encoding an exogenous T cell receptor (TCR) or a polynucleotide encoding a chimeric antigen receptor (CAR).~~

~~19. An engineered Treg according to claim 18 wherein the polynucleotide encoding a FOXP3 polypeptide and the polynucleotide encoding the exogenous TCR or the CAR are provided by a single expression vector.~~

~~20. An engineered Treg according to claim 19 wherein the vector comprises a nucleic acid with the orientation: 5' FOXP3 – X – TCR/CAR; wherein X is an internal self-cleaving sequence~~

17~~21~~. A pharmaceutical composition comprising an engineered Treg according to any of claims 14-16~~20~~.

18~~22~~. An engineered Treg according to any of claims 14-16~~20~~, or a pharmaceutical composition according to claim 17~~21~~, for use in prevention and/or treatment of a disease.

19~~23~~. Use of an engineered Treg which is produced according to a method of any of claims 1-15, or which is defined according to any of claims 14-16~~20~~, in the manufacture of a medicament for treating or preventing a disease associated with a pathological immune response by suppressing said response~~prevention and/or treatment of a disease~~.

~~24. A method of prevention and/or treatment of a disease comprising administering to a subject an engineered Treg or a pharmaceutical composition according to any of claims 14-21.~~

20~~25~~. The~~An engineered Treg for use, or a pharmaceutical composition for use, according to claim 22,~~ use ~~of an engineered Treg~~ according to claim 19~~23, or a method according to claim 24~~, wherein the disease is an autoimmune disease; preferably wherein the disease is multiple sclerosis.

21~~26~~. The use according to claim 19,~~An engineered Treg according to any of claims 13 to 17 for use in prevention and/or treatment of a disease~~ wherein the disease is transplant rejection or graft-vs-host disease.

22~~27~~.– In vitro ~~U~~use of a polynucleotide encoding a FOXP3 polypeptide to enhance the ability of a regulatory T cell (Treg) to suppress immune responses.