CLAIMS

1. Use of an engineered regulatory T cell (Treg) comprising an antigen-specific chimeric antigen receptor (CAR) directed against a predetermined antigen in the manufacture of a medicament for induction of tolerance to a transplant in a subject; treating and/or preventing graft-versus-host disease (GvHD), an autoimmune or allergic disease; to promote tissue repair and/or tissue regeneration; or to ameliorate chronic inflammation secondary to metabolic disorders; wherein the CAR comprises an endodomain which comprises a STAT5 association motif and a JAK1- and/or a JAK2-binding motif.

2. The use according to claim 1 wherein the Treg is a Foxp3+ Treg.

3. The use according to claim 1 or claim 2 wherein:

(a) the CAR endodomain does not comprise a STAT3 association motif; or

(b) the CAR endodomain does not comprise the amino acid sequence YXXQ (SEQ ID NO: 52).

4. The use according to any one of claims 1 to 3, wherein:

(a) the CAR endodomain comprises two or more STAT5 association motifs;

(b) the one or more STAT5 association motifs is from an interleukin receptor (IL) receptor endodomain;

(c) the one or more STAT5 association motifs is from IL2Rβ, IL7Rα, IL-3Rβ (CSF2RB), IL-9R, IL-17Rβ, erythropoietin receptor, thrombopoietin receptor, growth hormone receptor and prolactin receptor;

(d) the STAT5 association motif comprises the amino acid motif YXXF/L (SEQ ID NO: 8); wherein X is any amino acid;

(e) the STAT5 association motif comprises one or more of the amino acid motifs YCTF (SEQ ID NO: 9), YFFF (SEQ ID NO: 10), YLSL (SEQ ID NO: 11), and/or YLSLQ (SEQ ID NO: 12);

(f) the STAT5 association motif comprises the amino acid motif YLSLQ (SEQ ID NO: 12);

(g) the endodomain comprises a first STAT5 association motif comprising the amino acid motif YLSLQ (SEQ ID NO: 12) and a second STAT5 association motif

comprising the amino acid motif YCTF (SEQ ID NO: 9) or YFFF (SEQ ID NO: 10); and/or

(h) the endodomain comprises the following STAT5 association motifs: YLSLQ (SEQ ID NO: 12), YCTF (SEQ ID NO: 9) and YFFF (SEQ ID NO: 10).

5. The use according to any one of claims 1 to 4, wherein:

(a) the JAK-binding motif is a JAK-1 binding motif;

(b) the JAK-binding motif is a JAK1-binding motif from an interleukin receptor (IL) receptor endodomain;

(c) the JAK-binding motif is a JAK1-binding motif comprising an amino acid motif shown as any one of SEQ ID NO: 13-19 or a variant which has at least 80% identity to SEQ ID NO: 13-19;

(d) the JAK-binding motif is a JAK1-binding motif being the amino acid motif shown as SEQ ID NO: 13; or a variant which has at least 80% identity to SEQ ID NO: 13;

(e) the CAR endodomain comprises an IL2Rβ endodomain shown as SEQ ID NO: 1; or a variant which has at least 80% sequence identity to SEQ ID NO: 1; and/or

(f) the CAR endodomain comprises a truncated IL2Rβ endodomain shown as any one of SEQ ID NO: 23 or 24; or a variant of SEQ ID NO: 23 or 24 which has at least 80% sequence identity thereto.

6. The use according to any one of claims 1 to 5, wherein the CAR endodomain further comprises a JAK3-binding motif, optionally:

(a) wherein the JAK3-binding motif comprises SEQ ID NO: 25 or 26 or a variant which has at least 80% sequence identity to SEQ ID NO: 25 or 26; and/or

(b) wherein the CAR endodomain comprises SEQ ID NO: 45 or 53; or a variant which has at least 80% sequence identity to SEQ ID NO: 45 or 53.

7. Use of a pharmaceutical composition comprising an engineered Treg as defined in any one of claims 1 to 6 in the manufacture of a medicament for induction of tolerance to a transplant; treating and/or preventing graft-versus-host disease (GvHD), an autoimmune or allergic disease; to promote tissue repair and/or tissue regeneration; or to ameliorate chronic inflammation secondary to metabolic disorders.

8. The use according to any of claims 1 to 7, wherein:

(a) the subject is a transplant recipient undergoing immunosuppression therapy; or

(b) the autoimmune or allergic disease is selected from inflammatory skin diseases including psoriasis and dermatitis (e.g. atopic dermatitis); responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); dermatitis; allergic conditions such as food allergy, eczema and asthma; rheumatoid arthritis; systemic lupus erythematosus (SLE) (including lupus nephritis, cutaneous lupus); diabetes mellitus (e.g. type 1 diabetes mellitus or insulin dependent diabetes mellitus); multiple sclerosis and juvenile onset diabetes..

9. The use according to claim 8, wherein:

(a) the transplant is selected from a liver, kidney, heart, lung, pancreas, intestine, stomach, bone marrow, vascularized composite tissue graft, and skin transplant;

(b) the transplant is a liver transplant and the CAR comprises an antigen binding domain which is capable of specifically binding to an antigen selected from: a HLA antigen present in the transplanted liver but not in the recipient, or a liver-specific antigen such as NTCP;

(c) the CAR comprises an antigen binding domain which is capable of specifically binding to an antigen whose expression is up-regulated during rejection or tissue inflammation such as CCL19, MMP9, SLC1A3, MMP7, HMMR, TOP2A, GPNMB, PLA2G7, CXCL9, FABP5, GBP2, CD74, CXCL10, UBD, CD27, CD48, CXCL11;

(d) the CAR comprises an antigen binding domain which is capable of specifically binding to a HLA antigen that is present in the graft donor but not in the graft recipient; and/or

(e) the CAR comprises an antigen binding domain which is capable of specifically binding to the HLA antigen HLA-A2 that is present in the graft donor but not in the graft recipient.

10. The use according to any of claims 1 to 9, wherein:

(i) the CAR comprises a CD8α transmembrane domain, preferably comprising the amino acid sequence of SEQ ID NO:87, or a variant which is at least 80% identical to SEQ ID NO: 87; and/or

(ii) the CAR comprises an antigen binding domain comprising SEQ ID NOs: 93-95 and SEQ ID NOs: 105-107.

~~11. A chimeric antigen receptor (CAR) comprising an endodomain which comprises a STAT5 association motif and a JAK1- and/or a JAK2-binding motif, wherein the CAR:~~

~~(a) comprises a CD8α transmembrane domain, preferably comprising the amino acid sequence of SEQ ID NO:87, or a variant which is at least 80% identical to SEQ ID NO: 87; and/or~~

~~(b) comprises an antigen binding domain which comprises SEQ ID NOs: 93-95 and 105-107; and/or~~

~~(c) comprises a JAK3-binding motif.~~

~~12. A CAR according to claim 11, wherein the JAK3-binding motif comprises SEQ ID NO: 25 or 26 or a variant which has at least 80% sequence identity to SEQ ID NO: 25 or 26; and/or the CAR endodomain comprises SEQ ID NO: 45 or 53; or a variant which has at least 80% sequence identity to SEQ ID NO: 45 or 53.~~

~~13. A polynucleotide encoding a CAR according to any of claims 11 or 12.~~

~~14. An engineered Foxp3+ Treg comprising a chimeric antigen receptor (CAR) according to any of claims 11 or 12 or a polynucleotide according to claim 13.~~

~~15. A method of producing an engineered Treg according to claim 14, comprising the following steps:~~

~~(i) isolation of a cell-containing sample from a subject or provision of a cell-containing sample; and~~

~~(ii) transduction or transfection of the cell-containing sample with a polynucleotide, a nucleic acid, or a vector encoding the CAR, to provide a population of engineered cells;~~

~~wherein the cell-containing sample comprises Tregs and/or Tregs are enriched and/or generated from the cell-containing sample prior to or after step (ii).~~