CLAIMS

1) A method of preparing T-cells for immunotherapy comprising

   (a) Modifying T-cells ex vivo by inactivating at least:

   - A first gene expressing a target for an immunosuppressive agent, thereby conferring immunosuppressive resistance to said T-cells, and

   - A second gene encoding a component of the T-cell receptor (TCR);

   (b) Expanding said cells in vitro, optionally in presence of said immunosuppressive agent.

2) The method according to claim 1 for preparing T-cells for immunotherapy, comprising the following steps:

   (a) Providing a T-cell;

   (b) Selecting a gene in said T-cell expressing a target for an immunosuppressive agent;

   (c) Introducing into said T-cell, ex vivo, rare-cutting endonucleases able to selectively inactivate by DNA cleavage respectively:

   - said gene encoding a target for said immunosuppressive agent and
   - at least one gene encoding one component of the T-cell receptor (TCR)

   (d) Expanding said cells in vitro, optionally in presence of said immunosuppressive agent.

3) The method according to claim 1 or 2, wherein said T-cells are primary cells.

4) The method according to any one of claims 1 to 3, wherein said T-cells are human cells.

5) The method according to any one of claims 1 to 4, wherein said target for an immunosuppressive agent is a receptor for an immunosuppressive agent.

6) The method according to any one of claims 1 to 5, wherein said transformed T-cells are expanded in presence of said immunosuppressive agent.

7) The method according to any one of claims 1 to 6, wherein said gene expresses a target for an immunosuppressive agent which is CD52, and said immunosuppressive agent is an antibody targeting CD52 antigen.

8) The method according to any one of claims 1 to 7, wherein said gene expresses a glucocorticoid receptor (GR) and said immunosuppressive agent is a corticosteroid.

9) The method according to claim 8, wherein said corticosteroid is dexamethasone.

10) The method according to any one of claims 1 to 9, wherein the at least two inactivated genes are selected from the group consisting of CD52 and TCR alpha, CD52 and TCR beta, GR and TCR alpha, GR and TCR beta.

11) The method according to any one of claims 2 to 10, wherein said rare-cutting endonucleases are co-transfected in step c).

12) The method according to any one of claims 2 to 11, wherein said rare-cutting endonucleases are encoded by mRNA.

13) The method according to any one of claims 2 to 12, wherein said rare-cutting endonucleases are introduced into said cell in step (c) by way of RNA electroporation.

14) The method according to any one of claims 2 to 13, wherein said rare-cutting endonucleases are TALE-nucleases.

15) The method according to claim 14, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of TCRalpha selected from SEQ ID NO: 37, SEQ ID NO: 57 to SEQ ID NO: 60.

16) The method according to claim 14, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of TCRbeta selected from SEQ ID NO: 38 and SEQ ID NO: 39.

17) The method according to claim 14, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of GR selected from SEQ ID NO:1 to SEQ ID NO:6

18) The method according to claim 14, wherein at least one of these TALE-nucleases is directed against one of the gene target sequences of CD52 selected from SEQ ID NO: 40, SEQ ID NO: 61 to SEQ ID NO: 65.

19) The method according to any one of claims 1 to 18, comprising introducing into said T-cells a Chimeric Antigen Receptor (CAR).

20) The method of claim 19 wherein said Chimeric Antigen Receptor sequence is SEQ ID NO: 73.

21) The method of claim 19 wherein said Chimeric Antigen Receptor is a multi-chain Chimeric Antigen Receptor.

22) The method according to any one of claims 1 to 21 comprising introducing into said T-cell a TALE-nuclease able to selectively inactivate by DNA cleavage PDCD1 or CTLA-4 gene.

23) The method according to any one of claims 1 to 22, wherein said T-cells in step a) are derived from inflammatory T-lymphocytes, cytotoxic T-lymphocytes, regulatory T-lymphocytes or helper T-lymphocytes.

24) The method according to any one of claims 1 to 23, wherein said T-cells in step a) are derived from CD4+ T-lymphocytes and/or CD8+ T-lymphocytes.

25) An isolated human T-cell or cell line obtainable from the method of any one of claims 1 to 24.

26) An isolated T-cell, in which at least two genes selected from the group consisting of: CD52 and TCR alpha, CD52 and TCR beta, GR and TCR alpha, GR and TCR beta, have been inactivated.

27) An isolated T-cell according to claim 25 or 26, wherein said T-cell is a primary cell.

28) An isolated T-cell according to any one of claims 25 to 27, further comprising an exogenous polynucleotide sequence encoding a Chimeric Antigen Receptor.

29) An isolated T-cell of claim 28, wherein said Chimeric Antigen Receptor is a multi-chain Chimeric Antigen Receptor.

30) An isolated T-cell according to any one of claims 25 to 29 for its use as a medicament.

31) An isolated T-cell according to any one of claims 25 to 29 for treating a cancer.

32) An isolated T-cell according to any one of claims 25 to 29 for treating lymphoma.

33) A pharmaceutical composition comprising at least one isolated T-cell according to any one of claims 25 to 29.

34) An isolated T-cell comprising at least two polynucleotides, said polynucleotides encoding at least a first and second TALENs, the first TALEN being directed against a gene encoding TCR and the second being directed against a gene encoding a target for an immunosuppressive agent such as to confer immunosuppressive resistance to said T-cell by inactivating said gene encoding a target for an immunosuppressive agent.

35) An isolated T-cell according to claim 34, wherein the first TALEN is directed against one of the selected target sequences of the TCR alpha gene selected from SEQ ID NO: 37 and SEQ ID NO: 57 to SEQ ID NO: 60.

36) An isolated T-cell according to claim 34, wherein the first TALEN is directed against one of the selected target sequences of the TCR beta gene SEQ ID NO: 38 and SEQ ID NO: 39.

37) An isolated T-cell according to claim 34, wherein said second TALEN is directed against a gene encoding CD52 or GR.

38) An isolated T-cell according to claim 37, wherein the second TALEN is directed against one of the selected target sequences of the CD52 gene selected from: SEQ ID NO: 40 and SEQ ID NO: 61 to SEQ ID NO: 65.

39) An isolated T-cell according to claim 37, wherein the second TALEN is directed against one of the selected target sequences of the GR gene selected from: SEQ ID NO:1 to SEQ ID NO:6.

40) Use of an isolated T-cell according to claim 25 to 29 for the manufacture of a medicament for treating a cancer.

41) Use of an isolated T-cell according to any one of claim 25 to 29 for the manufacture of a medicament for treating lymphoma.