CLAIMS

1. A method of *ex-vivo* engineering T-cells for immunotherapy comprising the genetic modification step of :

(a) introducing into said cells at least one rare-cutting endonuclease, such that said rare-cutting endonuclease specifically catalyzes cleavage in one targeted sequence of the genome of said cells in a gene selected from the group consisting of TCR alpha, TCR beta, CD52 and GR;

(b) introducing into said cells an exogenous nucleic acid successively comprising a first region of homology to sequences upstream of said cleavage, a sequence to be inserted in the genome of said cell and a second region of homology to sequences downstream of said cleavage;

wherein said introduced exogenous nucleic acid integrates at least one exogenous polynucleotide sequence encoding at least one recombinant protein of interest by homologous recombination into said gene selected from the group consisting of TCR alpha, TCR beta, CD52 and GR, thereby inactivating said gene.

2. The method according to claim 1, wherein said protein of interest is pTalpha or a functional variant thereof, a Chimeric Antigen Receptor (CAR), a multi-chain CAR, a bispecific antibody or a rare-cutting endonuclease targeting PDCD1 or CTLA-4.

3. The method according to claim 2, wherein said exogenous recombinant polynucleotide encodes a CAR for specific cell recognition.

4. The method according to claim 3, wherein said CAR is directed against CD19 antigen.

5. The method according to claim 3 or 4, wherein said CAR comprises an amino acid sequence having at least 70% identity with SEQ ID NO: 73.

6. The method according to any one of claims 1 to 5, wherein said cells are primary cells.

7. The method according to any one of claims 1 to 6, wherein said cells are T-cells, which are available from donors or from culture of primary cells or which are derived from stem cells, such as induced pluripotent stem cells.

8. The method according to claim 7, further comprising a step of expanding said T-cells in vitro.

9. The method according to any one of claims 1 to 8, wherein said rare-cutting endonuclease recognizes and cleaves a target sequence in the group consisting of:
- SEQ ID NO: 1 to 6 (GR),
- SEQ ID NO: 37, 57 to 60 (TCRalpha),
- SEQ ID NO: 38 or 39 (TCRbeta), and
- SEQ ID NO: 40, 61 to 65 (CD52).

10. The method according to any one of claims 1 to 9, wherein said rare-cutting endonuclease is a TALE-nuclease.

11. An isolated cell or cell line obtainable by the method according to any one of claims 1 to 10.

12. The isolated cell according to claim 11, wherein two genes selected from the group consisting of CD52 and GR, CD52 and TCR alpha, CDR52 and TCR beta, GR and TCR alpha, GR and TCR beta, TCR alpha and TCR beta have been inactivated.

13. The isolated cell according to claim 11 or 12, wherein said cell is a T-cell further genetically modified by inactivating at least one protein involved in the immune check-point, such as PDCD1 and/or CTLA-4.

14. Use of an isolated cell according to any one of claims 11 to 13 in the manufacture of a medicament for the treatment of cancer, viral infection, an autoimmune disorder or Graft versus Host Disease (GvHD) in a subject in need thereof.

15. Use of an isolated cell according to any one of claims 11 to 13 in the manufacture of a medicament for the treatment of leukemia and lymphoma in a subject in need thereof.

16. A pharmaceutical composition comprising the isolated cell according to any one of claims 11 to 13.