cells are called allogeneic and the immunologic rejection is called allogeneic rejection. The exceptions are transplantations between genetically identical twins. Thus, to prevent rejection of the allogeneic graft, the subject's immune system must be suppressed. Immunosuppressive agents that target different pathways of the immune system are widely used therapeutically for immunosuppression. One example is targeting calcineurin by Cyclosporin A (CsA) or FK506 (Tacrolimus), which prevents the activation of T cells. Another drug, CTLA-4-Ig prevents activation of CD28 costimulation and therefore similarly suppresses T cell activation. However, in the case of adoptive cell transfer (ACT) therapy, the use of immune suppressive agent may have a detrimental effect on the grafted immune cells. Therefore, it remains a need to develop immune cells resistant to the immunosuppressive agent treatment, to effectively use an adoptive immunotherapy approach in these conditions.

SUMMARY

Using CD4cre.miR1792tg.CD28ko mice, the inventors showed that transgenic expression of miR-17~92 can compensate CD28 signalling *in vitro* and *in vivo* and correct the transcriptome of CD28ko cells. Additionally, the inventors provide evidence that miR-17~92 promotes calcineurin/NFAT activity, NFAT nuclear translocation and drives an NFAT-dependent gene expression signature. Moreover, the inventors demonstrate that miR-17~92 cluster targets Rcan3, a repressor of the calcineurin-NFAT axis and they furthermore demonstrate that the decreased expression of this biological calcineurin inhibitor at the same time conveys the cells with a higher resistance to a chemical calcineurin inhibitor. The inventors also demonstrate that forced miR-17~92 expression not only restores costimulatory function in CD28-deficient T cells and imposes CNI resistance in those cells but also conveys CNI resistance to wildtype, i.e. CD28-sufficient T cells. They also showed that the inactivation of a miR-17~92 cluster target gene, Rcan3 gene conveys CNI resistance to immune cells.

The present disclosure describes a calcineurin inhibitor (CNI)-resistant immune cell in which regulatory activity, preferably expression of the miR-17~92 cluster or paralogs thereof is increased or miR-17~92 target genes decreased for use in adoptive cell transfer therapy in a subject in need thereof wherein said immune cell is administered in said subject in combination with a CNI.

In one aspect, the present disclosure refers to a use of a calcineurin inhibitor (CNI)-resistant immune cell in which regulatory activity of the miR-17-92 cluster or paralogs thereof is increased in the manufacture of a medicament for use in combinatorial therapy of adoptive cell transfer therapy in a subject in need thereof, wherein said medicament is to be administered in combination with a CNI in said subject.

In an example, said immune cell is engineered to overexpress at least one miRNA selected from the group consisting of miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, miR-

92a-1, miR-106a, miR-18b, miR-19b-2, miR-20b, miR-92a-2, miR363, miR-106b, miR-93, miR-25, preferably miR-17 or miR-19. In an example said immune cell is engineered by introducing into said immune cell a nucleic acid construct comprising at least one miRNA sequence selected from the group consisting of: SEQ ID NO : 17 to 46, more preferably at least one pre-miRNA sequence selected from the group consisting of: SEQ ID NO: 1 to 16. In an

example, said nucleic acid construct is introduced by electroporation.

In another example, said immune cell is engineered to inactivate or repress the expression of at least one miR-17~92 cluster target gene, preferably Rcan3 gene preferably said immune cell is engineered by introducing a Cas9/CRISPR complex able to target Rcan3 gene into said immune cell.

In an example, said calcineurin inhibitor is selected form the group consisting of: cyclosporine A, FK506 and CTLA-4 Ig.

In another example, the immune cell is selected from the group consisting of: T cell, B cell, tumor infiltrating lymphocytes, NK cell, macrophage and regulatory T cell and originate from said subject or a donor. In an example, said immune cell further express a recombinant antigen receptor, preferably a chimeric antigen receptor.

In an example, the adoptive cell transfer therapy is used for the treatment of a cancer, an autoimmune disease, an inflammatory disease, an infection, a disease requiring hematopoietic stem cells transplantation (HSCT) or the prevention of organ rejection, preferably a disease selected from the group consisting of: graft versus host disease, hematologic malignancy and posttransplant lymphoproliferative disease. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the CNI resistant immune cell as disclosed herein, a calcineurin inhibitor and a pharmaceutically acceptable carrier. In another aspect, the present disclosure refers to the use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for adoptive cell transfer therapy in a subject in need thereof. The present disclosure also describes a pharmaceutical composition comprising a CNI-resistant immune cell as previously described and a calcineurin inhibitor, in particular for use in adoptive cell transfer therapy in a subject in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. miR-17~92 deficiency phenocopies CD28-deficiency. miR1792lox (grey, left), wt (black), miR1792tg (grey, right A) Quantification of flow cytometry intracellular IL-2 staining in $CD4^+$ cells stimulated for 3h with PMA/Iono/BFA; B) IL-2 secretion measured by ELISA in culture supernatants at 48h; C) Proliferation of CFSE labelled $T^{1792\Delta/\Delta}$ (grey, left), wt (black), and $T^{1792tg/tg}$ (grey, right) $CD4^+$ T cells activated for 48h with plate-bound αCD3/αCD28 mAbs. Error bars show means ±SD, Dunn's multiple comparison test, p values: ns=not significant, *<0.05 **<0.002 ***<0.0002 ****<0.0001. Data represent 2-3 independent experiments with 3-4 biological replicates per group.

Figure 2. miR-17~92 expression modifies metabolism in activated CD4 T cells. $CD4^+$ T cells from $T^{1791\Delta/\Delta}$ (light grey), wt (black), $T^{1792/tg/tg}$ (grey) were assessed for their metabolic activity by

mitochondria stress test with seahorse machine. A) Mitochondria stress test measured with a 96-well seahorse in naïve $CD4^+$ T cells. Two experiments with 3-4 biological replicates per group and experiment are shown. left: Extracellular acidification rate, right: Oxygen consumption rate. Both parameters are overlapping in the three genotypes. B) Basal respiration and ATP coupled respiration in naïve $CD4^+$ T cells. C) Mitochondria stress test measured in $CD4^+$ T cells activated for 48h and D) Basal respiration and ATP coupled

CLAIMS

1. Use of a calcineurin inhibitor (CNI)-resistant immune cell in which regulatory activity of the miR-17-92 cluster or paralogs thereof is increased in the manufacture of a medicament for use in combinatorial therapy of adoptive cell transfer therapy in a subject in need thereof, wherein said medicament is to be administered in combination with a CNI in said subject.

2. The use of claim 1 wherein said immune cell is engineered to overexpress at least one miRNA selected from the group consisting of miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92a-1, miR106a, miR-18b, miR-19b-2, miR-20b, miR-92a-2, miR-363, miR-106b, miR-93, miR-25, preferably miR-17 or miR19.

3. The use of claim 2 wherein said immune cell is engineered by introducing into said immune cell a nucleic acid construct comprising at least one miRNA sequence selected from the group consisting of: SEQ ID NO: 17 to 46.

4. The use of claim 3 wherein said nucleic acid construct comprises at least one pre-miRNA sequence selected from the group consisting of: SEQ ID NO: 1 to 16.

5. The use of claim 3 or 4 wherein said nucleic acid construct is introduced into said immune cells by electroporation.

6. The use of claim 1 wherein said immune cell is engineered to inactivate the expression of at least one miR-1792 cluster target gene, preferably Rcan3 gene.

7. The use of claim 6 wherein said immune cell is engineered to introduce Cas9/CRISPR complex able to target Rcan3 gene into said immune cell.

8. The use according to any one of claims 1 to 7 wherein said calcineurin inhibitor is selected from the group consisting of: cyclosporine A, FK506 and CTLA-4 Ig.

9. The use according to any one of claims 1 to 8 wherein said immune cell is selected from the group consisting of: T cell, B cell, Tumor infiltrating lymphocytes, NK cell, macrophage and regulatory T cell.

10. The use of claim 9 wherein said immune cells originate from said subject or a donor.

11. The use according to any one of claims 1 to 10 wherein said immune cell further express an recombinant antigen receptor, preferably a chimeric antigen receptor.

12. The use according to any one of claims 1 to 11 wherein said adoptive cell transfer therapy is used for the treatment of a cancer, an autoimmune disease, an inflammatory disease, an infectious disease, a disease requiring hematopoietic stem cells transplantation (HSCT) or the prevention of organ rejection, preferably a disease selected from the group consisting of: graft versus host disease, hematologic malignancy or posttransplant lymphoproliferative disease, an autoimmune disease.

13. A pharmaceutical composition comprising the CNI resistant immune cell as defined in any one of claims 1 to 12, a calcineurin inhibitor and a pharmaceutically acceptable carrier.

14. Use of the pharmaceutical composition of claim 13 in the manufacture of a medicament for adoptive cell transfer therapy in a subject in need thereof.