Allogeneic TCRα/CD38 Double Knockout T-cells bearing an anti-CD38 Chimeric Antigen Receptor (CAR): an improved immunotherapy for the treatment of T-cell acute lymphoblastic leukemia (T-ALL) and multiple myeloma (MM).

Poster 365

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#1 Introduction

Background: Adoptive immunotherapy with autologous T-cells expressing chimeric antigen receptors (CARs) targeting CD19 has achieved long-term remissions in patients with B-cell leukemia, pointing out that CAR technology may become a game changer in cancer treatment.

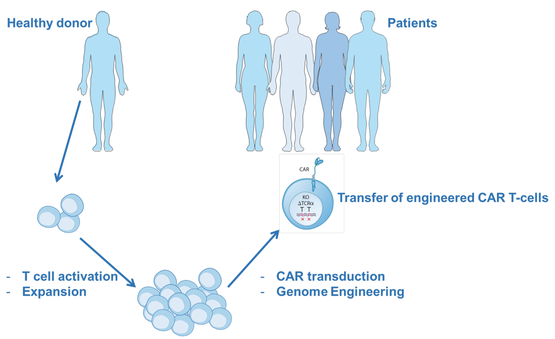
Aims: In the present study we have assessed the feasibility of CAR-mediated targeting of the CD38 antigen, which is highly expressed on tumor cells from most patients with acute lymphoblastic leukemia (T-ALL) and multiple myeloma (MM). However, expression of CD38 on normal activated T-cells is a significant hurdle for the development of CAR T-cells against this protein, since antigen-expressing T-cells will be targeted, potentially preventing the efficient production of anti-CD38 CAR T-cells.

Methods: To circumvent this issue we have used Transcription Activator-Like Effector Nuclease (TALEN®) gene editing technology to inactivate the CD38 gene (CD38 KO) in T-cells, prior to transduction with a lentiviral vector encoding an anti-CD38 CAR. To validate this approach, we have examined the capacity of CD38 KO cells expressing an anti-CD19 CAR to eliminate CD19+ cells in order to determine if the absence of CD38 has an impact on T-cell activity. Experiments in an orthotopic Burkitt's lymphoma mouse model showed that CD38 disrupted T-cells expressing anti-CD19 CAR were able to mediate an *in vivo* anti-tumor activity similar to unmodified T-cells expressing an anti-CD19 CAR. These results demonstrate that T-cells lacking CD38 are capable of mediating efficient *in vivo* anti-tumor activity.

Results: Gene editing technology can also be used to manufacture T-cells from healthy donors to generate allogeneic "off-the-shelf" engineered CAR+ T-cell-based frozen products. We have previously demonstrated that TALEN® mediated inactivation of the TCRα constant (TRAC) gene can be achieved at high frequencies and eliminates the potential for edited T-cells to mediate Graft versus Host Disease (GvHD). Furthermore, multiplex genome editing can lead to the production of double KO (TRAC and CD38) T-cells, allowing large scale manufacturing non allo-reactive CD38 specific T-cells. We will present data demonstrating that such gene-edited anti-CD38 CAR T-cells can be efficiently produced and display high cytotoxic activity.

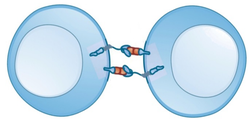
#2 Allogeneic approach to target CD38

One of the key barriers for adoptive transfer of 3rd party CAR T-cells can be overcome via TALEN® gene editing technology to disrupt expression of the TCR, allowing the use of any donors' T-cells and minimizing the risk of GvHD.



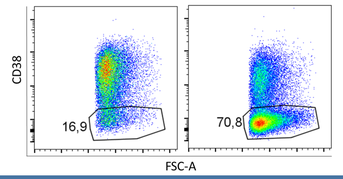
#3 CD38 gene inactivation in human T-cells

CD38 is expressed on activated T-cells, and these cells can be targeted during amplification of CAR T-cells



TALEN® targeting the coding sequence of the human CD38 gene were designed, and the corresponding mRNAs were transfected in primary T-cells using PulseAgile electroporation technology.

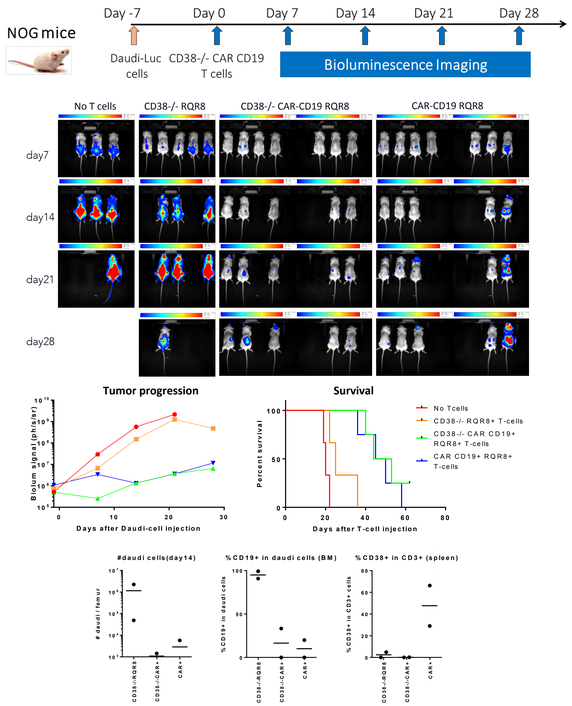
Flow cytometry analysis revealed high levels of CD38 gene inactivation T-cells.



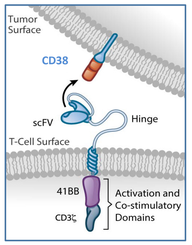
#4 CD38-KO T cells anti-tumor activity *in vivo*

NOG mice were sublethally irradiated (1,44 Gy) 8 days before injection of T-cells. At Day (-7) 5x10^5- Daudi Luciferase cells/mice were iv injected. Mice were then infused with wt or CD38-/- CAR T-cells. Bioluminescent signal was assessed at D(-1), D7, D14, D21 and D28 post injection of T-cells. At D14, 2 mice from the groups CD38-/- RQR8, CD38-/- CAR-CD19 RQR8 and CAR-CD19 RQR8 were sacrified for flow cytometry analysis.

CD38-KO doesn't affect the anti-tumor activity of CAR T-cells *in vivo*.

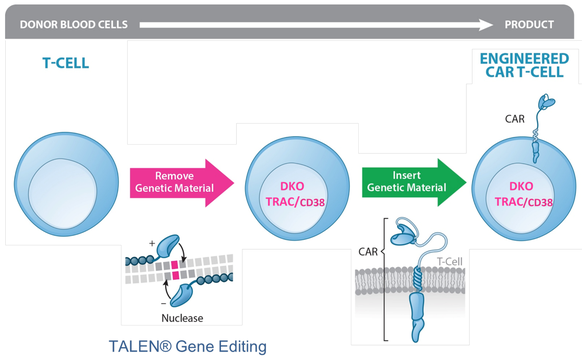


#5 Generation of UCART38 cells



An scFv targeting the extracellular domain of the human CD38 antigen was used to construct a chimeric antigen receptor containing a 41BB co-stimulatory domain and the CD3z activation domain.
The anti-CD38 CAR was coexpressed with the suicide gene RQR8

UCART38 cells can be produced using cells from a healthy donor in a process designed for GMP compatibility.
The TCRα and CD38 KO is performed prior to CAR transduction, and the TCRαβ KO cells are purified at the end of the production process.

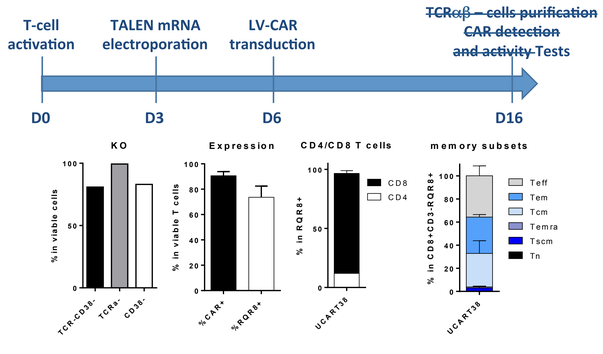


TALEN® Gene Editing

#6 UCART38 expression and phenotype

T-cells were transfected with mRNAs encoding the TALEN® targeting the CD38 and TCRα genes, and transduced 3 days later with the lentiviral vector encoding the anti-CD38 CAR. T-cells were amplified during 10 days in culture in presence of IL2. At the end of the culture process, expression of the CAR and the suicide gene RQR8 was assessed by flow cytometry.

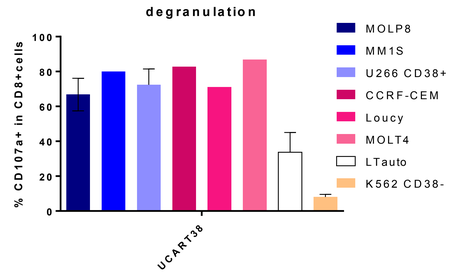
At the end of the R&D process, 99,4% of T-cells were TCRαβ negative and 80% of T-cells were CAR+ and RQR8+. UCART38 cells were mainly memory (Tem and Tcm) CD8+ T-cells.



UCART38 *in vitro* anti-tumor activity

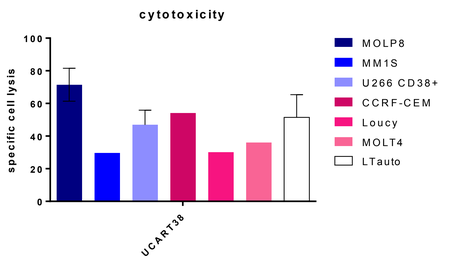
Degranulation activity upon co-culture of CAR T-cells with CD38 expressing MM cells (MOLP8, MM1S, U266-CD38+) or T-ALL cells (CCRF-CEM, Loucy, MOLT4) and autologous activated **T-cells** was measured by detecting CD107a expression in CD8+ cells after 5h of co-culture. CD38- cells were used as a control.

UCART38 cells are able to degranulate in the presence of the CD38 antigen presented by MM cells lines and T-ALL cell lines and T-cells.



Cytotoxic activity against MM and T-ALL cell lines was also measured by flow cytometry, upon co-culturing the effector and target cells for 4h.

UCART38 was able to induce lysis of MM and T-ALL cells.



#10 Conclusions and Perspectives

1) **We show that TALEN®–mediated disruption of the CD38 gene in T-cells is efficient and doesn't affect CAR activity.**
2) **We have previously demonstrated that TALEN® mediated inactivation of the TCRα constant (TRAC) gene can be achieved at high frequencies and eliminate the potential for edited T-cells to mediate Graft versus Host Disease (GvHD).**
3) **Multiplex genome editing can lead to the production of double KO (TRAC and CD38) T-cells, allowing large scale manufacturing of allogeneic, non alloreactive CD38 specific T-cells that display anti-tumor activity.**
4) **) Gene editing technology offers the possibility of developing an off-the-shelf CAR T-cell based frozen product that would be immediately available for administration to a large number of MM and T-ALL patients.**