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UCART20x22: First Allogeneic Dual CAR T-cell Therapy for the Treatment of B-cell malignancies

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#1 Abstract

Autologous CAR T-cell therapies have been transformative in the treatment of selected blood cancers. Despite this remarkable success, long term studies on patients treated with CD19 or CD22 CAR T-cells revealed the emergence of relapses that can be due to antigen loss. The therapeutic options after CAR T-cell relapses are limited, underscoring the urgent need to develop novel therapies to improve current survival rates. In addition, there is a need to develop allogeneic "off-the-shelf" therapies that are readily available at the time of treatment decision and that overcome development limitations of current autologous approaches. Here, we developed a dual allogeneic CAR T-cells with the potential to overcome current challenges in the treatment of B-cell malignancies.

Experimental Procedures

We generated UCART20x22, allogeneic CAR T-cells targeting CD20 and CD22, using a bicistronic lentiviral construct to express both CARs. TALEN® gene editing technology was used to mediate inactivation of the TRAC and CD52 genes: TRAC KO is used to prevent Graft-vs-Host Disease, and CD52 KO to allow deeper host lymphocyte depletion by conferring resistance to lymphodepletion regimens including an anti-CD52 monoclonal antibody such as alemtuzumab. To test UCART20x22 activity we use a combination of *in vitro* cytotoxicity assays against tumor cell lines, along with pre-clinical *in vivo* models assessing efficient dose/response and recapitulating antigen escape. We also performed *in vitro* activity assays against primary Non-Hodgkin Lymphoma (NHL) cells expressing different levels of the targeted antigens.

Results

We demonstrate that UCART20x22 displays strong activity against tumor cell lines expressing either a single antigen, CD20 or CD22, or both simultaneously. UCART20x22 also specifically releases IFNg in response to antigen specific stimulation. We also show that the specific activity of UCART20x22 persists over time against tumor cells expressing either one or both antigens. Our preclinical models illustrate that UCART20x22 dual CAR T-cells provide efficient *in vivo* clearance of tumor cells in a dose dependent manner. UCART20x22 efficiently eradicates tumors expressing both or a single antigen, and we observe persistence of UCART20x22 cells in the bone marrow after tumor clearance. Furthermore, *in vitro* assays against primary cells from Non-Hodgkin Lymphoma patients with diverse CD22 and CD20 antigen levels demonstrate that UCART20x22 has potent and specific cytotoxic activity as well as IFNg release against these panel of primary samples.

Summary of conclusions

We present a robust pre-clinical proof of concept of a potent allogeneic dual CAR T-cell product candidate, UCART20x22, with the potential to 1) overcome common mechanisms of resistance to CAR T-cell therapies in B-NHL, 2) enable the development of allogeneic CART option for B-NHL patients, 3) reduce the time from treatment decision to infusion.

#7 Conclusions

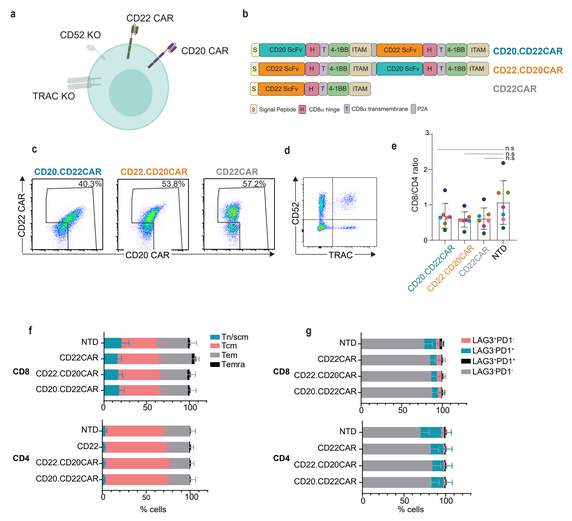
We provide pre-clinical proof of concept (POC) demonstrating the potent and persistent activity of UCART20x22 dual CAR T-cells for the treatment of B-cell malignancies. Our data shows that UCART20x22 displays:

- Robust *in vitro* cytolytic activity against tumors expressing different antigen combinations.
- Efficient and dose dependent tumor control *in vivo.*
- Efficient activity *in vivo* upon antigen loss.
- Potent activity against primary cells with heterogeneous levels of CD20 and CD22 expression.

Moreover, UCART20x22 has been efficiently engineered through TALEN® mediated editing of TRAC and CD52 genes, to prevent Graft-vs-Host Disease and to provide resistance to alemtuzumab, which is used to achieve deeper host lymphocyte depletion prior to treatment with UCART20x22. This TRAC/CD52 KO scaffold has successfully been implemented during manufacturing of "off-the-shelf" CAR T-cells against other targets, which are currently being evaluated in ongoing clinical studies for safety and efficacy.

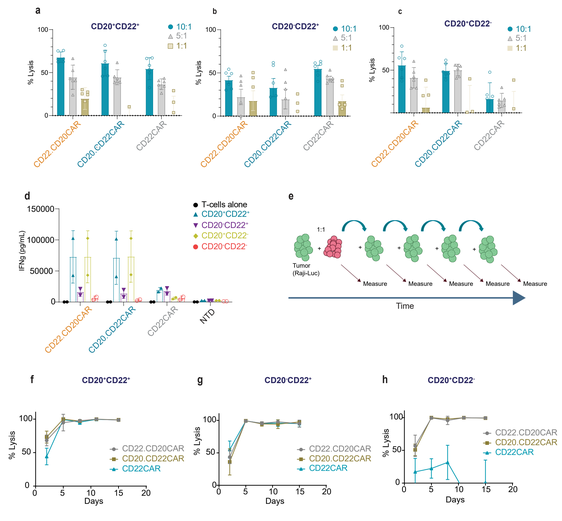
In sum, we show a robust pre-clinical POC for an efficient allogeneic UCART20x22 with the potential to overcome mechanisms of resistance to CAR T-cell therapy in B-NHL, such a single antigen escape, and to enable the development of an allogeneic CAR T-cell option for B-NHL patients.

#2 Allogeneic Dual CAR T-cells UCART20x22 can be efficiently generated



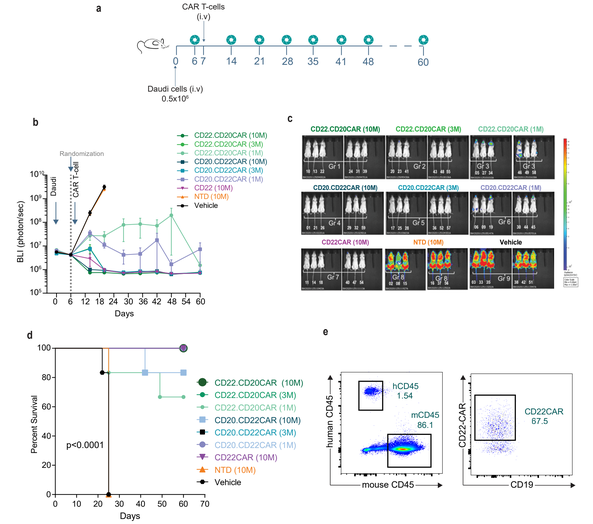
UCART20x22 can be efficiently generated. **a**. Diagram showing the attributes of the UCART20x22 product candidate. **b**. Constructs used in this study. **c**. Flow cytometry data showing CAR expression for dual CAR T-cells and single CD22 CAR T-cells. **d**. Flow cytometry data showing CD52 and TRAC knock-out efficiency before depletion of TRCab$^+$ cells. **e**. Ratio of CD8/CD4 cells in the productions indicated. **f**. Flow cytometry data showing differentiation status of CAR T-cells expressing the indicated CAR constructs. Tn/scm: Naive T-cells (CD45RA$^+$CD62L$^+$); Tcm: central memory T-cells (CD45RA$^-$CD62L$^+$); Tem: effector memory T-cells (CD45RA-CD62L-); Temra: effector memory T-cells RA (CD45RA$^+$CD62L$^-$). **g**. Expression of exhaustion markers PD-1 and LAG-3 in CD8$^+$ and CD4$^+$ cells.

#3 UCART20x22 exhibits potent *in vitro* cytolytic activity



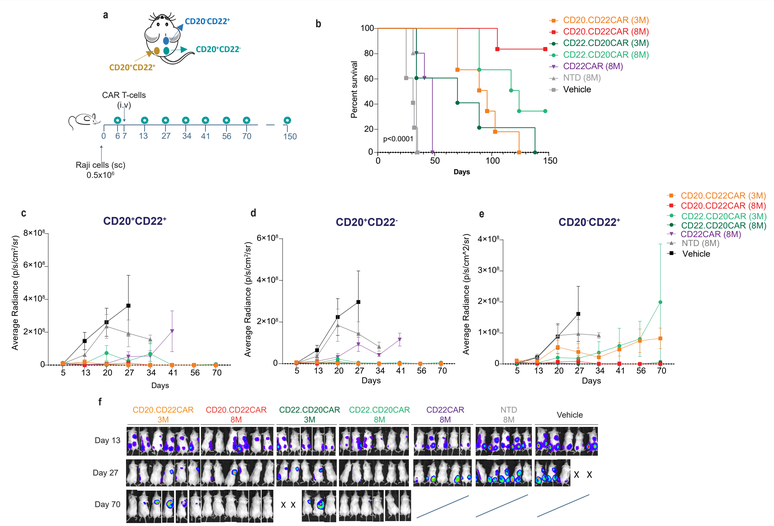
UCART20x22 exhibits robust cytolytic activity *in vitro*. a. Cytotoxic activity against Raji (Burkitt lymphoma) cells expressing CD20 and CD22. **b, c** Cytotoxic activity against Raji cells expressing CD22 or CD20. **d**. IFNgamma release after overnight incubation at a effector:target ratio 1:1. **e**. Schematics representing serial killing assay. **f**. Serial killing assay against CD20$^+$CD22$^+$ Raji cells. **g,h** Serial killing assay against CD22$^+$ or CD20$^+$ Raji cells.

#4 UCART20x22 exhibits robust activity *in vivo* in a dose dependent manner



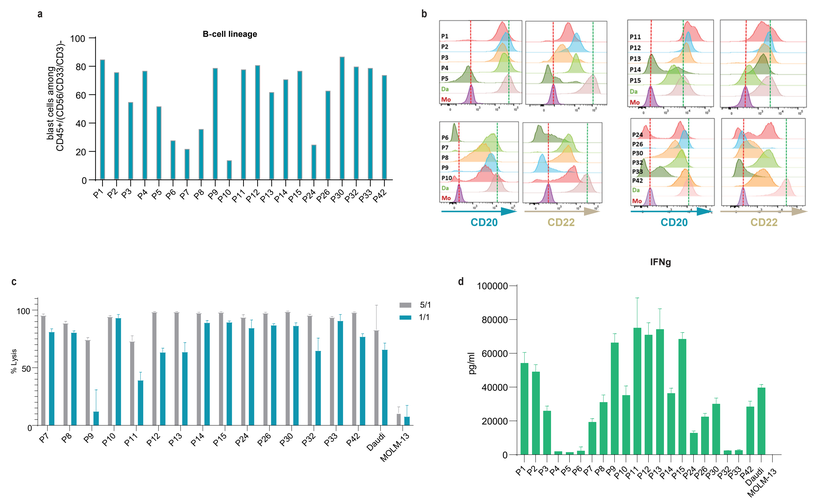
UCART20x22 efficiently targets tumors *in vivo*. **a**. Schematic showing experimental design with NSG mice engrafted with Daudi cells (Burkitt Lymphoma). **b**. Tumor control over time. **c**. Bioluminescence imaging of the different groups at day 20 of the study. **d**. Kaplan-Meier curves showing animal survival. **e**. Flow cytometry data gating strategy for human CD45+ cells and CAR T-cells as detected with CD22 protein in bone marrow of animals treated with UCART20x22.

#5 UCART20x22 displays strong activity against different target combinations



In vivo **tumor antigen model shows strong activity of UCART20x22 against tumors expressing different antigens.** a. Schematic showing experimental design with NSG mice injected subcutaneously with Raji cells (Burkitt Lymphoma). **b**. Kaplan-Meier curves showing animal survival. **c,d,e**. Graph showing tumor control for CD20$^+$CD22$^+$ cells, CD20$^+$CD20$^-$ and CD20$^-$CD22$^+$. **f**. Bioluminescence imaging measured at different time points.

#6 UCART20x22 effectively kills primary NHL cells with diverse CD20 and CD22 expression levels



UCART20x22 efficiently targets primary B-NHL cells. **a**. Graph showing percentage of blast cells present in B-NHL primary cells. **b**. Flow cytometry data representing the level of CD20 and CD22 expression in samples from panel a. Intensity of expression in a positive (Daudi, Da) and negative (MOLM-13, Mo) cell line is represented by the green and red dotted lines respectively. **c**. UCART20x22 cytotoxic activity against B-NHL cells. **d**. UCART20x22 IFNgamma release upon exposure to primary B-NHL samples.