Acute Myeloid Leukemia (AML) immunotherapy: development of TIM3-CD33 targeted Dual CAR CIK cells for eradication of LSCs in the bone marrow niche

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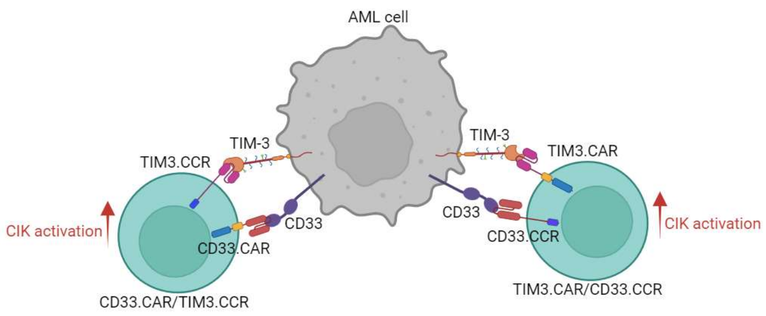
Introduction

Acute Myeloid Leukemia (AML) is a haematological malignancy characterized by high relapse rates, due to the persistence of chemoresistant Leukemic Stem Cells (LSCs). Alternative therapies are needed, and Chimeric Antigen Receptor (CAR)-T immunotherapy could be an innovative solution. However, AML heterogeneity and lack of tumor-specific antigens highlight the importance of the identification of more specific LSCs targets to match efficacy with safety, and the development of next-generation CAR molecules, such as armored or bi-specific CARs.

Therefore, we chose T-cell-Immunoglobulin-Mucin-3 (TIM-3), a checkpoint molecule overexpressed mainly by LSCs, as a novel target to be paired with the conventional CD33 for the design of two Dual CARs prototypes, composed by a second-generation CAR and a Chimeric-Costimulatory-Receptor (CCR).



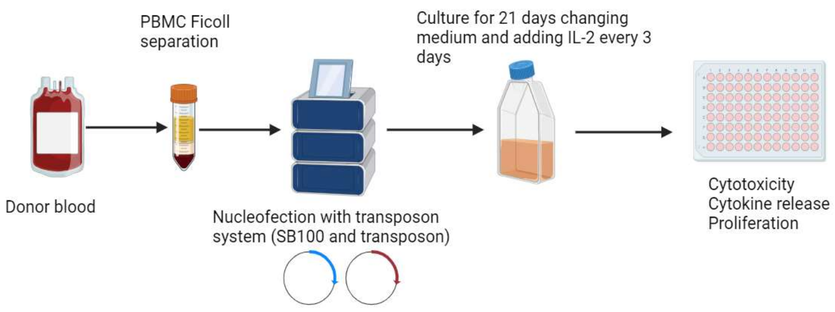
Aim

- We aim to develop two Dual CARs prototypes with double specificity for TIM-3 and CD33 (one of the main consolidated AML targets), in order to eradicate AML-LSCs whilst reducing the on-target off-tumor effect.
- Afterward, we want to assess the activation and killing activity of Dual CAR CIK cells against CD33$^+$ and TIM-3$^+$ AML cell lines and patients' blasts, with a major focus on LSCs compartment, *in vitro* and *in vivo*.
- Moreover, leveraging the checkpoint TIM3 targeting, this strategy aims at achieving the restoration of a proper antitumor response within an immunosuppressive TME.

Methods

CAR CIK production

50ml of donor PB were collected and PBMCs were isolated by Ficoll-Paque®. CIK differentiation with the addition of cytokines was coupled with electroporation of CAR construct with a non-viral Sleeping Beauty transposon system.



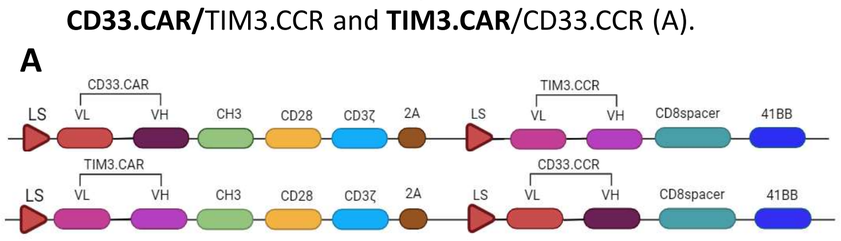
In vitro assays

At the end of CAR CIK cell culture cytotoxicity, cytokine release, proliferation and long-term assays were performed.

Results

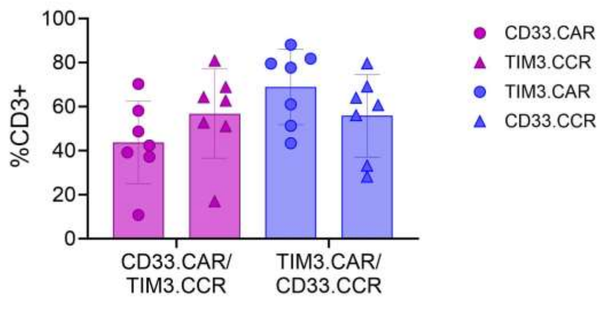
1) Relying on previous validation of single TIM3.CAR and CD33.CAR, we have developed two Dual CARs molecules: **CD33.CAR/**TIM3.CCR and **TIM3.CAR**/CD33.CCR (A).

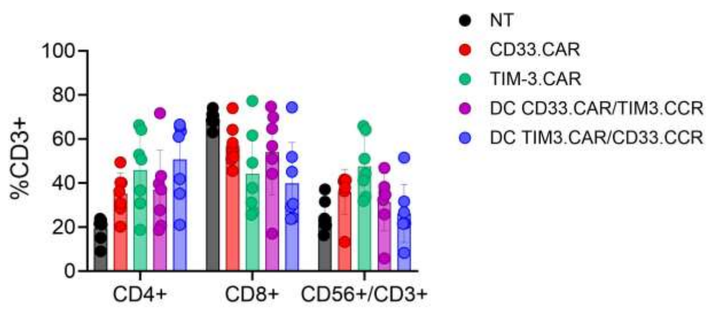
A



For each construct, the expression of CAR and CCR reached up to 60% at the end of CIK cells culture (B).

B

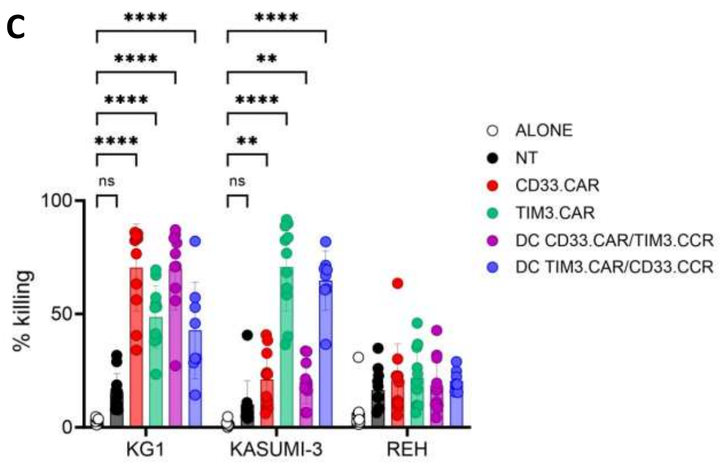




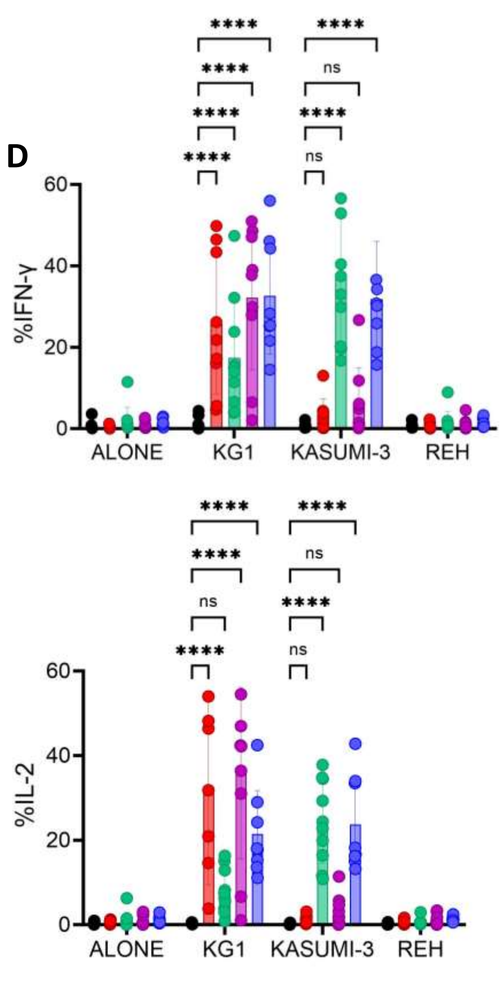
2) CD33.CAR/TIM3.CCR CIKs showed anti-leukemic activity, in terms of cytotoxicity (C), cytokine releasing (D) and proliferation (E) against AML cells lines KG1 (CD33$^+$ TIM-3^{dim}), while their effector functions were strongly reduced against KASUMI-3 cells (CD33$^+$ TIM-3$^+$). Since KASUMI-3 cell line overexpresses TIM-3 and Gal9 as compared to other AML cell lines, the data so far obtained suggest that the TIM-3/Gal9 axis could play an inhibitory role on CAR CIKs that is more evident by using KASUMI-3 cell line.

TIM3.CAR/CD33.CCR CIKs exhibited higher killing activity against KASUMI-3 than KG1 (C), but elevated later effector functions against both cell lines (D, E).

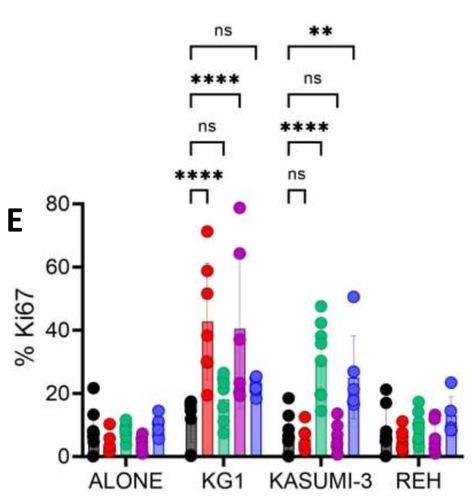
C



D

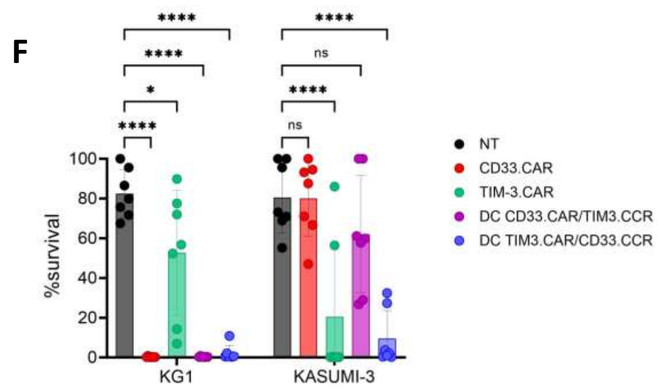


E



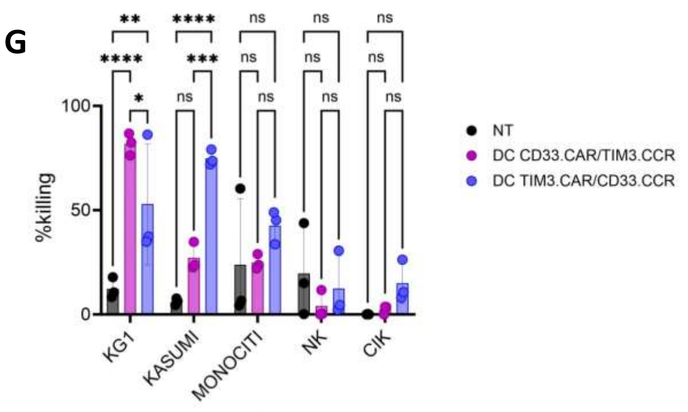
3) TIM3.CAR/CD33.CCR CIKs showed significative long-term effector functions against KASUMI-3 and KG1 cells, unlike single TIM3.CAR CIKs and despite the low TIM-3 expression level of KG1 (F).

F



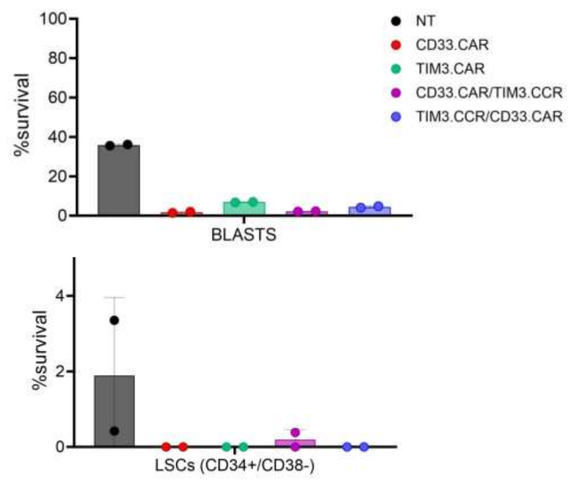
4) In the safety profile evaluation assay, Dual CAR CIKs exhibited their ability to spare healthy TIM-3$^+$ cells, such as monocytes and NKs. Moreover, Dual CAR CIKs showed no killing activity against CIK cells, demonstrating the absence of fratricide (G).

G

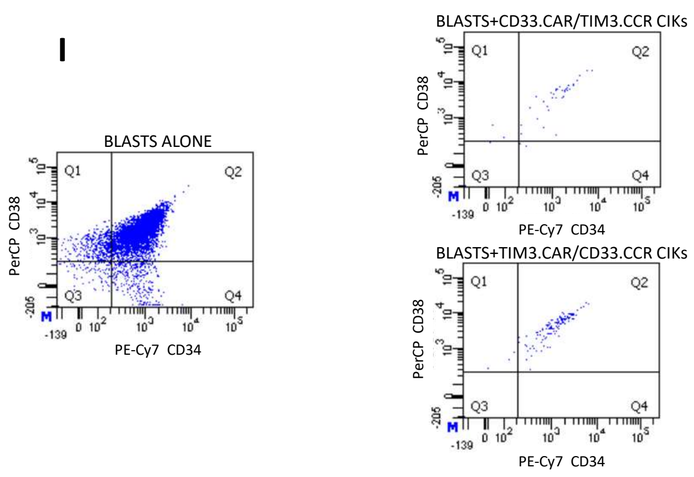


5) The preliminary results of Dual CAR CIKs cytotoxicity against primary AML blasts highlight CIKs anti-leukemic activity (H) and their eradication of LSCs compartment (I).

H



I



Conclusions and future perspectives

- We successfully developed Dual CD33-TIM3 CAR CIKs with specific and potent anti-leukemic activity against AML cell lines and primary blasts expressing CD33 and TIM-3.
Moreover, we are evaluating the role of TIM-3/Gal9 axis in the inhibition of CD33.CAR and **CD33.CAR**/TIM3.CCR CIKs activation against KASUMI-3
- We highlighted the benefit of Dual targeting in the AML context, suggesting a better performance of **TIM3.CAR**/CD33.CCR CIKs over **CD33.CAR**/TIM3.CCR CIKs.
- We assessed the safety profile of Dual CAR CIKs, and we are investigating the mechanism behind the differential recognition of healthy and leukemic TIM-3.
- We are going to test the efficacy and safety of both Dual CAR CIKs *in vivo*, using NSG-PDX and humanized mice. Furthermore, humanized mice will be used to analyse the impact of TIM-3 targeting in the modulation of the immunosuppressive TME

Acknowledgements

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