Dual CAR-engineered NK92 Cells: An Off-The-Shelf Cell Therapy for Cancer

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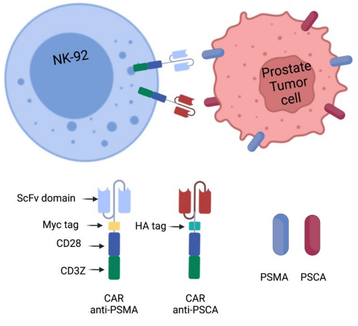
Abstract

The chimeric antigen receptor NK92 (CAR NK92) cell targeting the prostate-specific membrane antigen (PSMA) has shown antitumor effects, *in vitro* as *in vivo* (1-3). However, the expression changes of PSMA could affect CAR NK92 therapeutic activity with a relapsed/refractory prostate tumors. We observed that a dual CAR NK92 cells targeting simultaneously two antigens overexpressed on prostate tumors: PSMA and PSCA (prostate stem cell antigen) antigens improve the antitumor efficacy against prostate tumors, compared to CAR-NK92 cells targeting only one antigen, which is an extraordinary translational value in the clinical treatment of CRPC.

Background

Autologous chimeric antigen receptor T (CAR-T) cell therapy is an effective treatment for relapsed/refractory acute lymphoblastic leukemia (r/r ALL). However, certain characteristics of autologous CAR-T cells can delay treatment availability. Relapse caused by antigen escape after single-targeted CAR-T therapy is another issue. Moreover, their therapeutic efficacy against solid tumors is limited. To overcome current limits, other cellular effectors than T lymphocytes are under study as possible candidates for CAR-engineered cancer immunotherapy. A novel approach involves the NK92 cell line, which mediates strong cytotoxic responses against a variety of tumor cells but has no effect on non-malignant healthy counterparts. CAR-NK cells have a more favorable toxicity profile compared to CAR T cells, especially in avoiding adverse effects such as cytokine release syndrome and since NK92 cell line can be easily expanded to clinical grade numbers under current Good Manufacturing Practice (cGMP) conditions, treatment with CAR modified NK92 should be considered a treatment option for patients with lymphoid and solid malignancies.

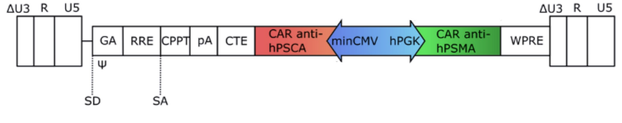
Objective



We have recently reported a novel therapeutic approach against Prostate cancer based on engineering of NK92 cells with a CAR recognizing the prostate-specific membrane antigen (PSMA), which is overexpressed in prostatic neoplastic cells (2). However, in attempt to improve efficacy of our NK-92/CAR cells and to prevent some weaknesses in targeting one antigen, such as “on-target off-tumor” cytotoxicity and the tumor escape, we have developed a dual CAR-NK92 cells targeting simultaneously PSMA and PSCA.

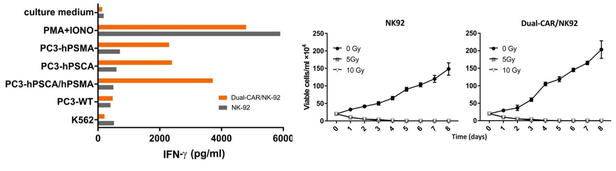
Materials & Methods

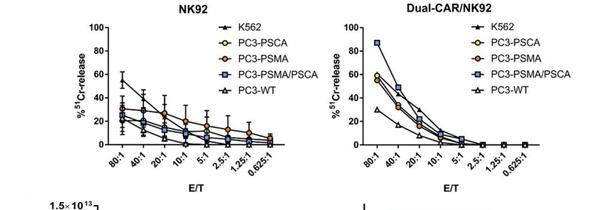
To express the Dual CAR, we used a Lentiviral Vector (LV) carrying a bidirectional promoter that drives the simultaneous expression of the anti-PSMA CAR and the anti-PSCA CAR.
The expression of CAR molecules on single and dual CAR-T cells was detected by flow cytometry. The killing capacity and activation of NK-92/CAR cells were measured by cytotoxic assays and cytokines release assays *in vitro* towards different prostate tumor targets expressing only one or both the antigens.

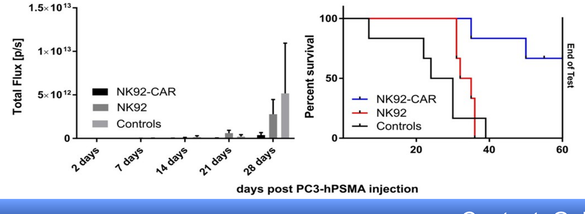


Results

Dual CAR/NK-92 cells acquired high and specific lytic activity that is higher in presence of both the antigens. Moreover, degranulate and produce high levels of IFN-γ in response to antigen recognition. Lethal irradiation of the effectors, a safety measure requested for the clinical application of retargeted NK92 cells, fully abrogated replication but did not impact on phenotype and short-term functionality. Despite high remission rates following chimeric antigen receptor T cell (CAR-T) cell therapy relapse due to loss of the targeted antigen is increasingly recognized as a mechanism of immune escape. Moreover, dual CAR/NK92 cells in prostate cancer-bearing mice not only restrained tumor growth but more importantly statistically improved survival.







Summary

The emergence of target antigen loss and expression downregulation highlights the critical need to anticipate antigen escape. The PSMA/PSCA bivalent CAR provides an opportunity to test whether simultaneous targeting may reduce the risk of antigen loss and relapse of the tumor. The results obtained from these preliminary experiments demonstrate that dual CAR/NK92 cell therapy mediates potent lytic activity against the prostate cancer cells and is a breakthrough in the development of an “Off-the-Shelf” adoptive cell therapy. So, the main advantages of this therapy are not only the possibility to obtain a “ready-to-use” drug immediately available for every prostate cancer patient but also the synergistic activity of the two CARs to avoid the tumor escape mechanism. In conclusion, the potentiality of this approach bears great promises for a future clinical application.

Discussion

The robust *ex vivo* expansion of NK92 cells and their exquisite safety profile, as well as the ease of genetic modification, make this cell line an ideal platform for the development of an Off-The-Shelf therapeutic CAR-engineered variants to target other tumors. Overall, CAR NK92 cells can be produced at much lower cost compared to CAR T cells and will be a ready to use drug, immediately available for cancer patients, with a significant implication in their survival.

References

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3. Zuccolotto G, et al. PSMA-Specific CAR-Engineered T Cells for Prostate Cancer: CD28 Outperforms Combined CD28-4-1BB "Super-Stimulation". Front Oncol. 2021



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