271 DEVELOPMENT AND CHARACTERISATION OF HUMANISED SCFV CAR-T THERAPIES TARGETING BCMA AGAINST MULTIPLE MYELOMA

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Background Multiple myeloma (MM) remains an incurable disease despite advances in the development of new therapies. Currently, the development of new immune therapies based on chimeric antigen receptor-modified T lymphocytes (CAR-T therapies) targeting B-lymphocyte maturation antigen (BCMA) has revolutionized the treatment of MM. However, most patients continue to relapse, and this lack of long-term response calls for further improvement of these therapies.

The main objective of this work is to develop new CAR designs by combining humanized scFv that recognize different BCMA epitopes.

Methods Second-generation CAR constructs with 4–1BB as costimulatory domain targeting BCMA, have been designed using different humanized scFv. Both single and dual (with 2 scFv in tandem) CAR constructs have been generated. Phenotype analysis including subpopulations and markers of activation and exhaustion has been performed by flow cytometry. CAR-T cell functionality has been analyzed by cytotoxicity assays against MM lines using luciferase-based methodologies. *In vivo* antitumoral efficacy was evaluated in xenogeneic tumor models in mice.

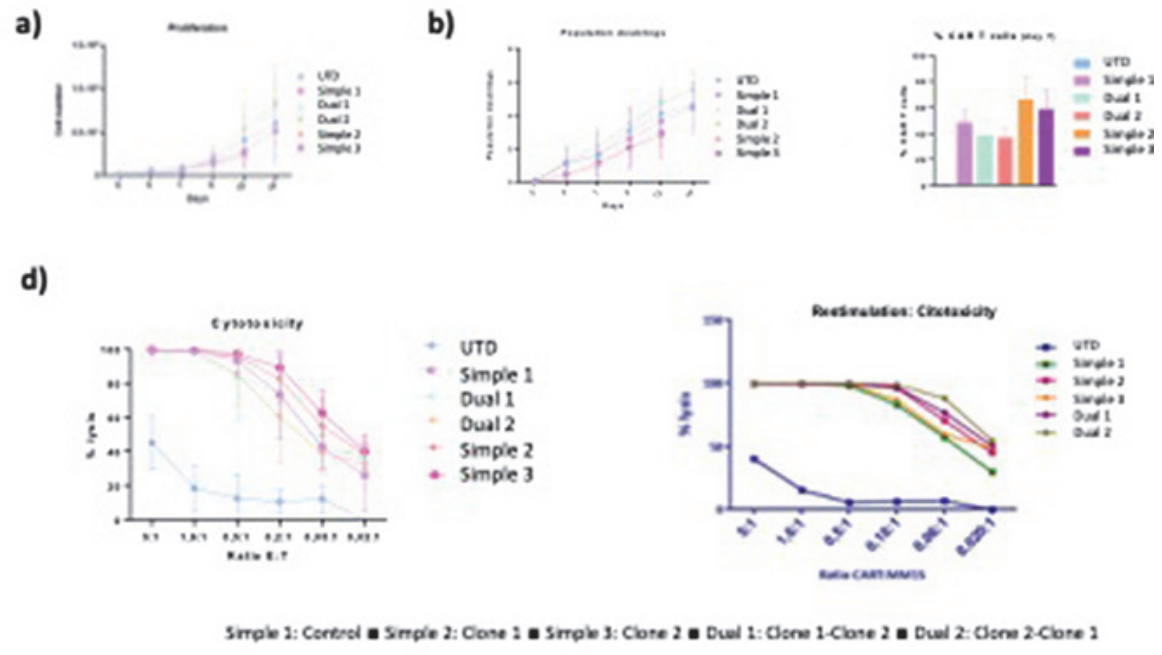
Results After the modeling and humanization process, first results have given us 108 humanized versions of the scFv for clone 1 and 54 versions for clone 2. To reduce the number of scFv to evaluate, we will focus on the analysis of 16 versions of each clone, using the 2 most conservative and the 2 most humanized versions of each of the HV and LV of each clone.

A total of 5 humanized CAR constructs were designed: three single and two duals.

In vitro experiments show a higher antitumor activity in single CAR-T cells with no significant differences. Although a lower level of transduction (% of CAR at day 7) is observed in the dual CAR-Ts, proliferation and efficacy are similar. Restimulation assays show increased efficacy of CAR-T simple 3 without statistical significance.

Finally, *in vivo* assays are performed with different survival rates depending on sex. In males, simple 1 CAR-T obtained longer survivals than the rest. In females, no deaths were observed at day 80 with the different constructs.

Conclusions The results of our study show that CAR-T with novel designs against BCMA are effective against MM cell lines. CAR-Ts with tandem dual humanized scFVs have not been shown to have greater efficacy than single CAR-Ts *in vitro*. More *in vivo* studies would be needed to gain a better understanding of the effects of sex. Also, the dose of CAR-T could be a reason of these small differences.



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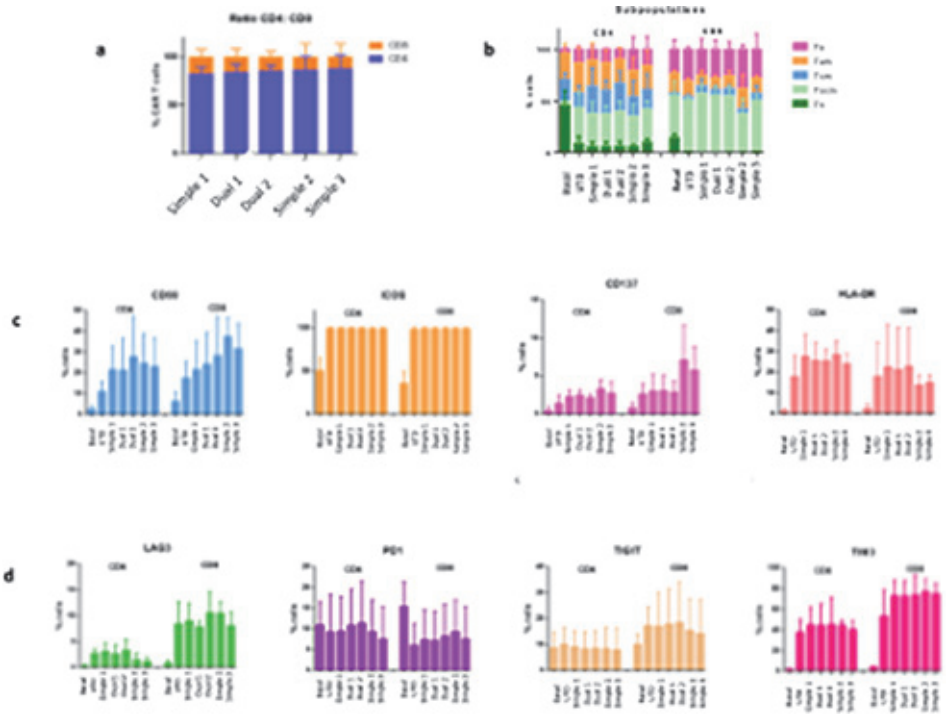

Abstract 271 Figure 2 Phenotypic characterization of CAR-T cells. (A) CD4:CD8 ratio of the different CAR-T. (B) Analysis of subpopulations of different CAR-T. (C) Analysis of the activation of the different CAR-T. (D) Analysis of the exhaustion of the different CAR-T.

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