**Abstracts**

**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 antitumor efficacy was evaluated in xenogenic 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.
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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