Integrating transcriptomics and proteomics for the discovery of novel antigen targets on the surface of malignant plasma cells amenable to CAR-T cell approach in the treatment of RRMM patients

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INTRODUCTION

- Multiple myeloma (MM) is still incurable, and outcomes are dismal when patients become refractory to treatments (1,2).
- Antibody-based therapeutics have now been FDA-approved but most patients relapse (3,4,5,6).
- New T cells therapy designs, such as HLA-independent T cell receptors (HIT receptors), showed a high efficacy in the context of low antigen density, making new targets now accessible for the treatment of tumors (7,8).

METHODS

Figure 1. Target discovery pipeline used to integrate proteomic and transcriptomic data and identify novel targets on the surface of malignant plasma cells. Patient datasets were downloaded from GEO, MMRF and GTEx databases (9).

AIM

- **To identify novel surface antigens amenable to Chimeric Antigenic Receptor-T (CAR-T) cell targeting for the treatment of patients with relapsed/refractory multiple myeloma (RRMM).**

RESULTS

- Using this analysis pipeline, we re-discovered well characterized targets, including B cell maturation antigen (BCMA, also called TNFRSF17) or SLAM Family Member 7 (SLAMF7), as well as prioritized **25 potential new targets**, such as C-C Motif Chemokine Receptor 10 (**CCR10**), Integrin Subunit Beta 7 (**ITGB7**) and Interleukin 6 Cytokine Family Signal Transducer (**IL6ST**) (Fig. 1-3).

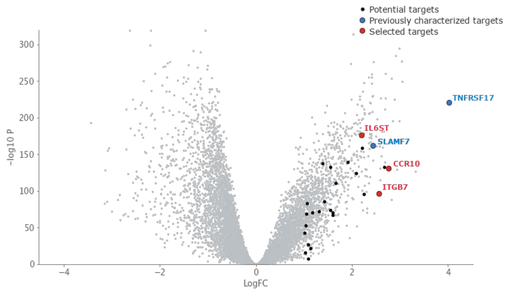

Figure 2. Volcano plot representation of the differential gene expression analysis highlighting potential targets passing all proteomic filters (yellow), previously characterized targets (blue) and targets selected for downstream validation (red).

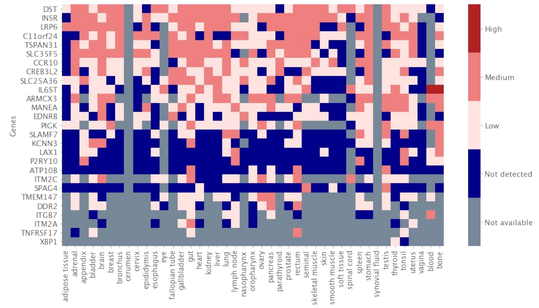

Figure 3. Protein expression level of potential targets, identified in both microarray and RNA-Seq, in healthy tissues. None of the selected targets exhibit a homogeneously high expression (red and dark pink) across tissues, thus mitigating the risk of elevated cytotoxicity.

- ITGB7 expression was higher in MM cell lines compared to other tumor cell lines, according to the Cancer Cell Line Encyclopedia (CCLE) database (Fig. 4), significantly associated with the progression free survival in MM patients, and maintained from diagnosis to relapse (data not shown).
- We experimentally validated the protein expression of these promising targets using flow cytometry in MM cell lines (MM.1S, OPM2) (Fig. 5).
- We established a **proof-of-concept CAR-T cell versus CCR10**, which exhibited *in vitro* killing activities against MM.1S cells (Fig. 6).

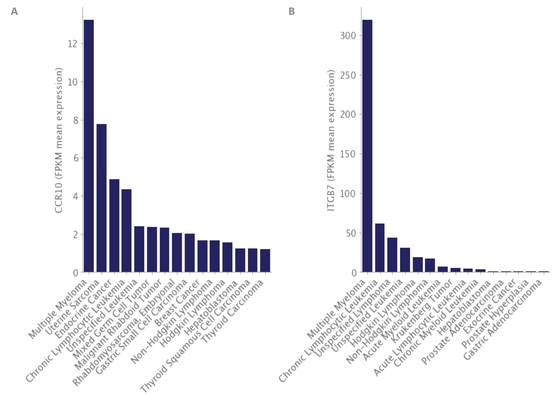

Figure 4. Top 15 indications ranked by the mean expression of A) CCR10 and B) ITGB7 in corresponding cell lines (CCLE data). Both proteins are highly expressed in MM cell lines (first indication).

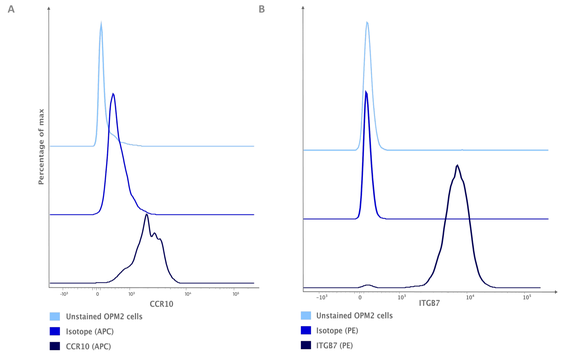

Figure 5. Expression of A) CCR10 and B) ITGB7 on the surface of MM cells (OPM2 cell line) measured by flow cytometry. Both proteins are highly expressed in OPM2 cells, making them attractive targets for therapy.

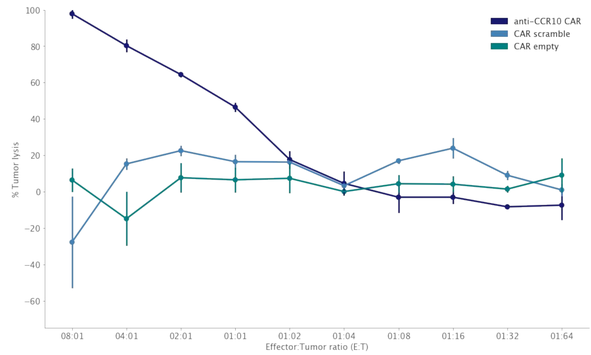

Figure 6. Percentage of specific lysis of anti-CCR10 CAR-T cells with CCR10 knockout (dark blue), CAR scramble cells (light blue) and empty CAR cells (green) against MM.1S-luciferase cells measured by luminescence after 24 hours of incubation (n = 3 technical replicates). Error bars represent +/− SD.

CONCLUSION

- **Our translational bioinformatics approach yielded novel surface antigens amenable to CAR-T cell targeting and HIT receptor approaches, potentially promising for the treatment of RRMM patients.**
- **With appropriate tissue specification, this strategy could generalize to other indications, including solid tumors.**

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