ImmunoTools *special* Award 2024

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Innovative Target Modules for FAP-targeting UniCAR T therapy

Over the past decades, various strategies have been implemented to reprogramme immune effectors against tumour cells. Among them, chimeric antigen receptor (CAR) T-cells have shown highly promising clinical results in haematological malignancies, leading to the approval of several CAR T-cell therapies since 2017. Nevertheless, CAR T-cells can be associated with significant side effects, e.g. cytokine release syndrome or neurotoxicity (Sterner et al., 2021). To circumvent these safety issues, our group has developed modular CAR platforms like the Universal CAR (UniCAR) system. In this system, T-cells are genetically modified to express a UniCAR, which is not directed to a tumour-associated antigen but to the E5B9 epitope derived from the nuclear La/SS-B protein. The activation of UniCAR T-cells relies on bifunctional adaptor molecules, called Target Modules (TMs). Each UniCAR TM contains the E5B9 epitope, specifically recognized by the UniCAR, and a binding moiety that binds specifically to the target antigen. Since only the appropriate cross-linkage of the TM to the UniCAR T-cells and target cells activates the effector functions of the UniCAR T-cells, adverse events can be rapidly switched off by TM withdrawal. Different structures based on e.g. small-molecules or antibody-derived fragments allow for control of the affinity of the TM to the target, its half-life and valency. In contrast to conventional CAR T-cell therapies, UniCAR T-cell potency can thus be modulated for better safety, controllability and flexibility (Bachmann et al, 2019).

To date, CAR T-cells have shown very limited activity in solid tumours due to several hurdles, e.g. the immunosuppressive tumour microenvironment (TME). Since the TME is a major barrier to effective CAR T-cell response, new strategies focusing on TME-associated targets are being explored. The Fibroblast Activation Protein

(FAP), a biomarker of the TME overexpressed in the majority of solid cancers, has emerged as a promising candidate for diagnostic and therapeutic applications (Liu et al., 2015).

Given the promising characteristics of the UniCAR system and the emergence of FAP as a pan-tumour target, we have recently developed antibody-based theranostic UniCAR TMs directed to FAP, which allow for both diagnostic imaging and UniCAR T-cell therapy (Loureiro et al, 2023; Boutier et al. 2024). Our current aim is to construct novel FAP-specific TMs based on FAP inhibitors already under pre-clinical and clinical investigation.

The reagents provided by ImmunoTools will allow for the:

- In-depth characterization of the binding of FAP-specific TMs to UniCAR T-cells and target cells by flow cytometry (anti-streptavidin antibody).
- Study of the phenotype of redirected UniCAR T-cells using antibodies recognizing activation/exhaustion markers such as HLA-DR & PD-1 and memory markers such as CD62L & CD45RO.
- Absolute quantification of cytotoxic proteases (granzyme B) expressed by UniCAR T-cells upon target cell killing.
- Assessment of the effect of IFN exposure on the expression of PD-L1 on tumor cells and the promotion of UniCAR T-cell exhaustion.

Key references:

- Bachmann M. The UniCAR system: A modular CAR T cell approach to improve the safety of CAR T cells. *Immunol Lett*. 2019, 211:13-22.
- Boutier H & Loureiro LR, et al. UniCAR T-Cell Potency-A Matter of Affinity between Adaptor Molecules and Adaptor CAR T-Cells? *Int J Mol Sci*. 2024, 30;25(13):7242.
- Liu F & Qi L, et al. Fibroblast activation protein overexpression and clinical implications in solid tumors: a meta-analysis. *PLoS One*. 2015 16;10(3):e0116683.
- Loureiro LR & Hoffmann L et al. Immunotheranostic target modules for imaging and navigation of UniCAR T-cells to strike FAP-expressing cells and the tumor microenvironment. *J Exp Clin Cancer Res*. 2023, 15;42(1):341.
- Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J*. 2021, 6;11(4):69.

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ImmunoTools *special* AWARD for **Hugo Boutier** includes 8 reagents

PE - conjugated anti-human HLA-DR, CD45RO, Goat anti-mouse (IgG), Strepavidin

APC - conjugated anti-human CD62L, Streptavidin

Purified anti-human PD1 (3D1)

Recombinant human cytokines: rh IFNγ

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