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Design of innovative FAP-target modules for simultaneous delivery of CAR-T cell- and radiotherapy

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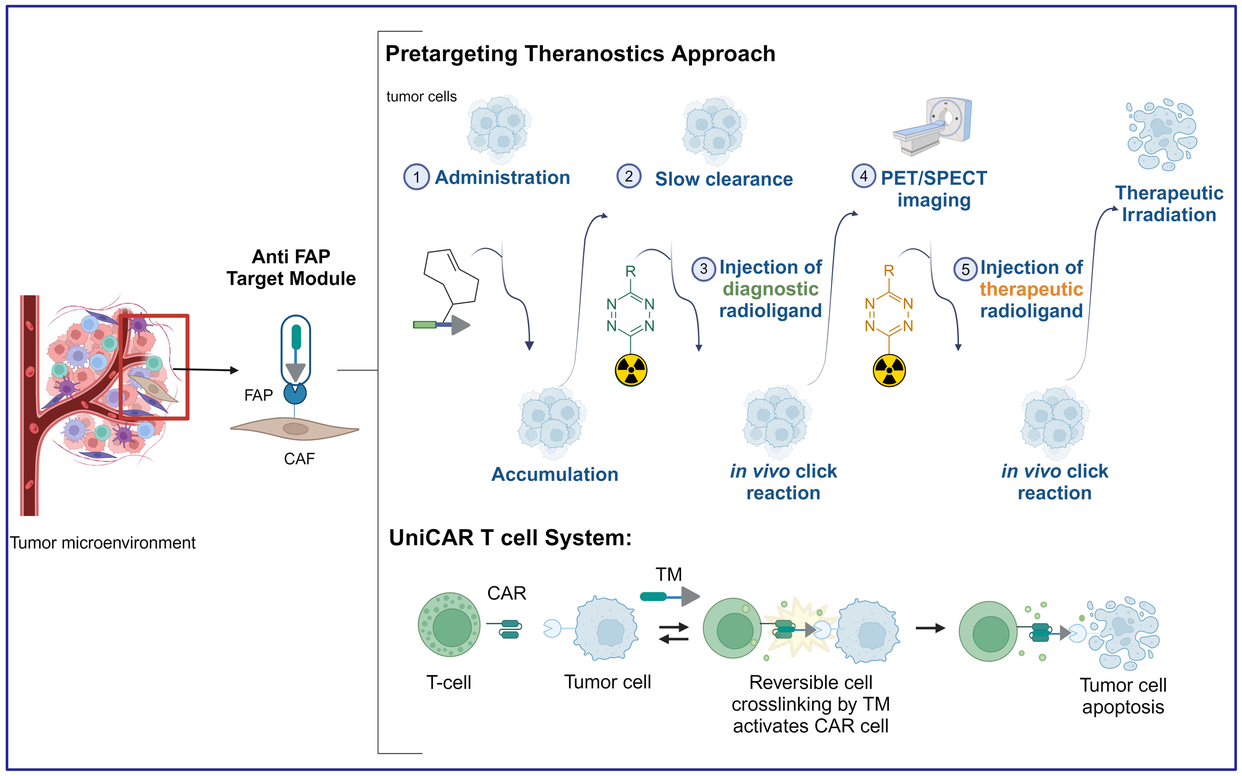
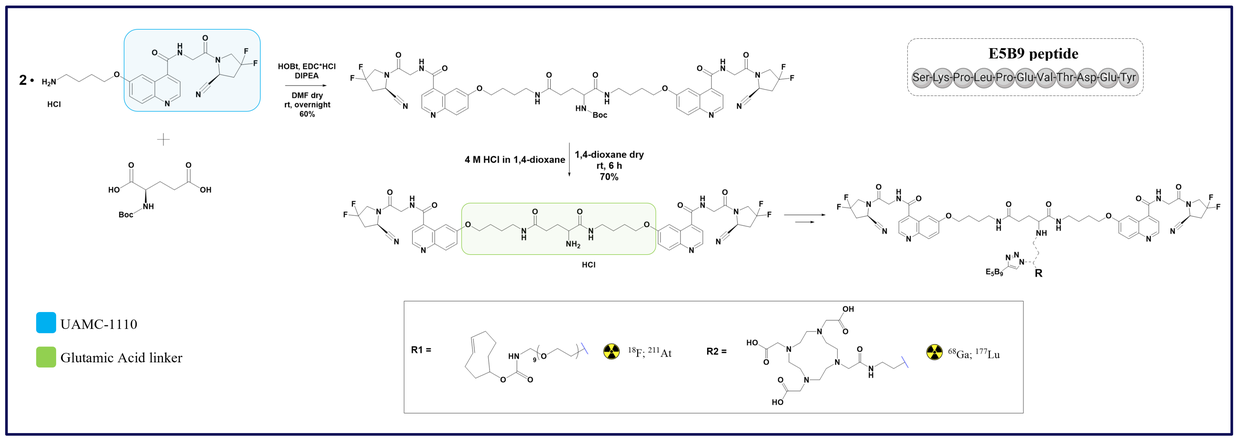
Objectives

Tumor-targeting therapies based on small molecules targeting **tumor-microenvironment (TME)**-specific proteases such as the **fibroblast activation protein (FAP)** have emerged as a suitable target for radioligand therapies (1). Switchable chimeric antigen receptor (CARs) T-cells such as the **Universal CAR-T cells**, that can be redirected to FAP-expressing cells of the TME with FAP-specific Target Modules (TMs), offer a promising approach for enhancing safety and effectiveness in cancer immunotherapy (2). This work aims to design and synthesize novel small-molecule-based FAP-specific TMs that allow for UniCAR T-cell therapy and the simultaneous delivery of radioligands for diagnostic and therapeutic purposes. To develop such a theranostic approach, we based our labeling strategy on the tetrazine ligation (3). The **tetrazine ligation** is a two-step reaction between a constrained trans-cyclooctene (TCO) and a 1,2,4,5-tetrazine (Tz). The reaction is initiated via an inverse-electron-demand Diels–Alder (IEDDA) reaction, followed by a spontaneous retro-Diels-Alder reaction, with the expulsion of nitrogen gas.

Schematic representation

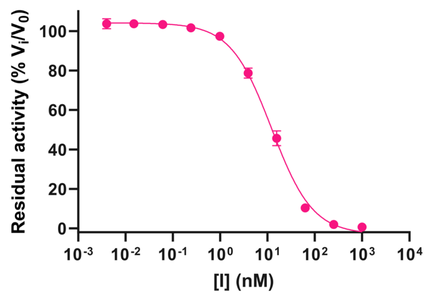


Design of the Target Modules



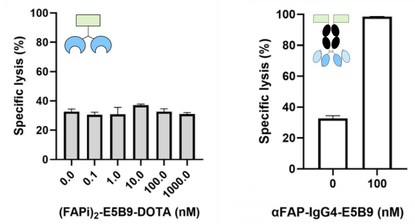
Results

Inhibition assay



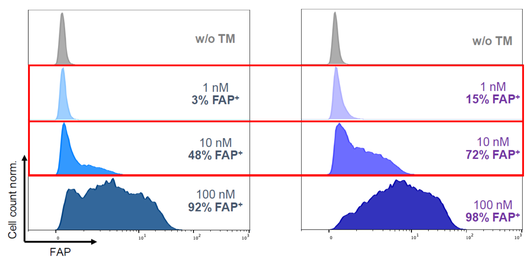
(FAPi)$_2$-E5B9-DOTA: **6 nM**

(FAPi)$_2$-E5B9-DOTA is not able to redirect UniCAR T-cells in vitro

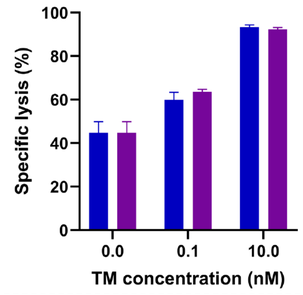


*Co-culture of UniCAR T-cells and HT1080-FAP in presence or absence of TM for **16h***

Successful binding of **new TMs** to FAP



Successful redirection of UniCAR T-cells by **new TMs**



Conclusions

Novel UniCAR TM based on homodimeric UAMC-1110 was synthesized. Biological studies evaluate the affinity of the Target Module for Fibroblast Activation Protein (FAP) in the nanomolar (nM) range. However, it was not able to redirect UniCAR T-cells to FAP expressing cells in vitro, giving a negative response to cytotoxicity analyses. Chemical modifications were applied to the final structure, leading to two new Target Modules compounds. First preliminary analyses were established, confirming the success of the strategy chosen. Positive binding to FAP and successful redirection of UniCAR T–cells make the new TMs promising. In the future, biodistribution studies using PET imaging will be conducted to provide insights into the in vivo pharmacokinetics of the TMs.

References

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