Bispecific Adaptor Molecule Controlled Folate Receptor CAR-T Cell Immunotherapy: *In-Vitro* Activity and T-cell Exhaustion Studies

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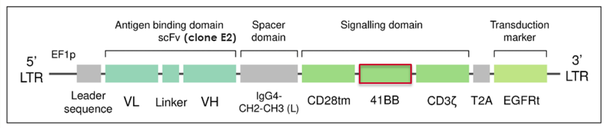
Abstract

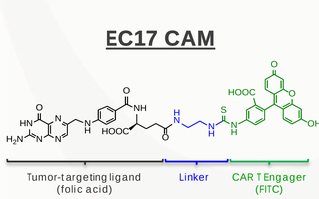
Chimeric antigen receptor (CAR) T-cell therapy has transformed personalized medicine by producing high remission rates and durable responses in CD19+ hematologic malignancies. Continuing efforts are being made to predict and manage toxicities associated with cytokine release syndrome and to improve efficacy against solid tumors where limited targets are available. In our CAR-T adaptor molecule (CAM)-based approach, a bispecific small-molecule ligand, EC17 (folate-FITC), is used to redirect fluorescein isothiocyanate (FITC)-specific CAR T cells against folate receptor (FR)-positive tumors. Our EC17/CAR T-cell therapy is able to elicit FR-dependent antitumor activity against human tumor xenografts of triple-negative breast, acute myeloid leukemia, osteosarcoma, and ovarian cancer. In tumor-bearing mice, EC17 CAM-triggered CAR T cell activation and cytokine release syndrome can be properly "tuned" in such a way that severe toxicity is avoided without sacrificing the efficacy. To further our understanding, we conducted a series of *in-vitro* activity and T cell exhaustion studies in human tumor cell lines that showed differential sensitivity to EC17/CAR T-cell therapy in xenograft transplants. We used T cells engineered to express a second-generation fully humanized FITC-specific (FITC-E2) CAR selected by the Jensen laboratory at Seattle Children's Research Institute. Our co-culture data suggested that although CAR T cell function/cytolytic activity is FR-dependent and EC17-driven, different tumor types elicit different CAR T cell responses in cytokine production, cytotoxicity, and T cell exhaustion.

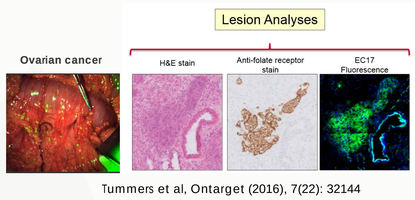
Principle Components of CAR-T Adaptor Molecule (CAM) Controlled Immunotherapy

Humanized anti-FITC scFv E2-CAR Construct



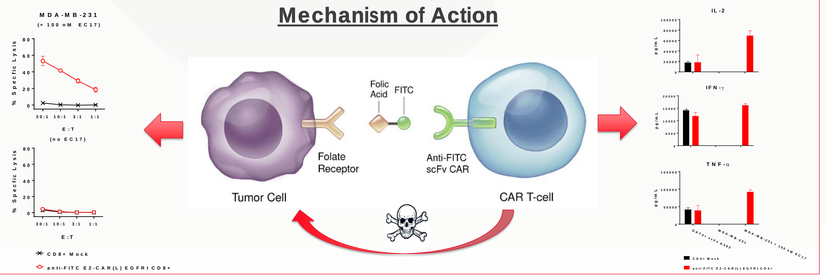
EC17 CAM



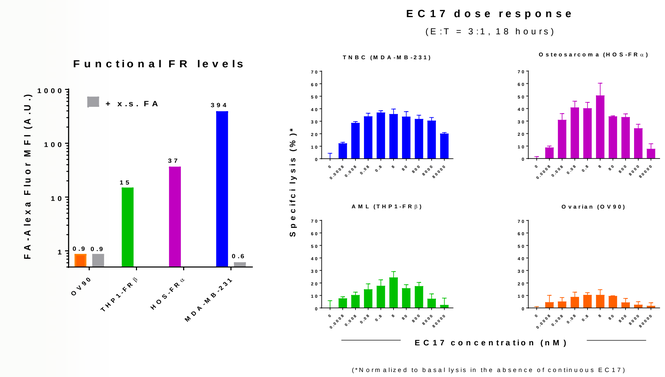


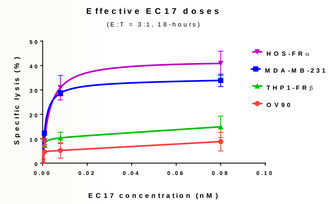
Tummers et al, Ontarget (2016), 7(22): 32144

Mechanism of Action

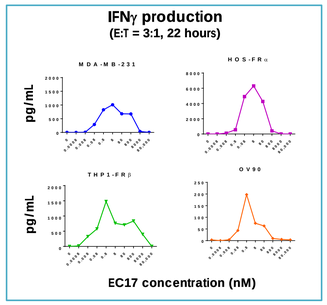


EC17 Induces a Potent FR-dependent Tumor Cell Killing by CAR T Cells



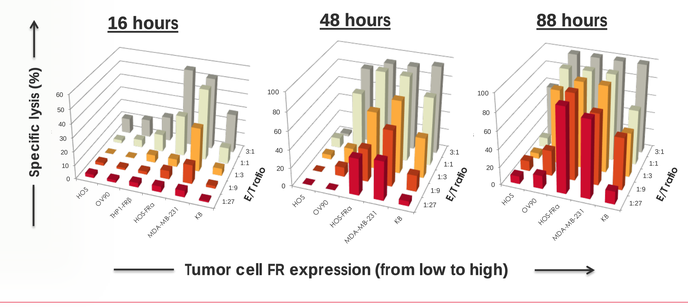


	HOS-FRα	MDA-MB-231	THP1-FRβ	OV90
Bmax (%)	43	34	10	4.7
EC50 (pM)	3.1	1.4	0.27	N/A

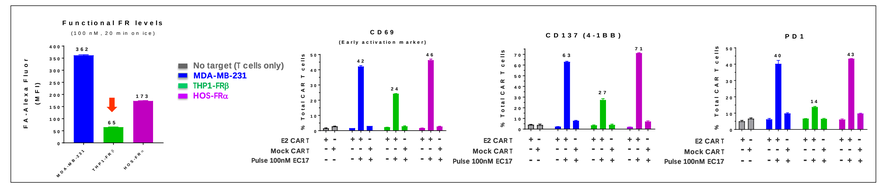


Positive Correlation of Cytolytic Activity of CAR T Cells with Functional FR Levels on Tumor Cells

Kinetics of target cell lysis in coculture

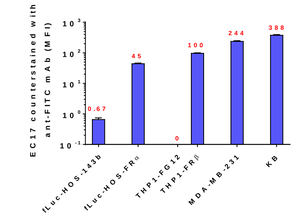


EC17/FR-dependent CAR T-cell Activation and Exhaustion Vary in Tumor Types with Different Sensitivity

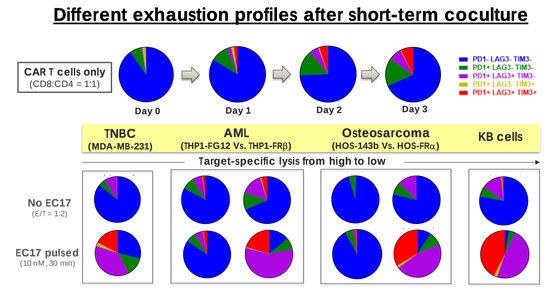


Real-time FR levels

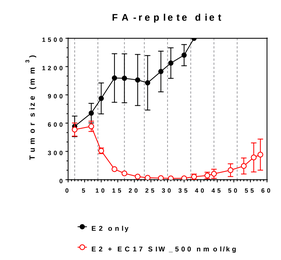
(10 nM, 30 min pulse, 37°C)

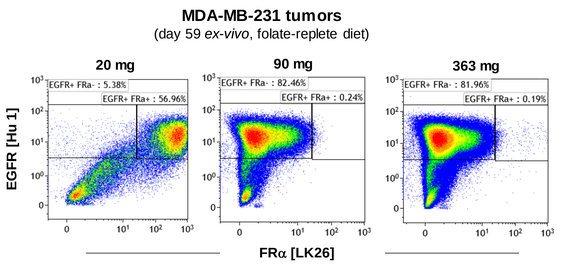


Different exhaustion profiles after short-term coculture



Loss of FR Expression is a Potential Mechanism of Tumor Relapse *In-vivo*





Conclusions

- As a pseudo-tumor antigen, monovalent EC17 CAM in its free form does not automatically activate FITC-specific CAR T cells (data not shown).
- EC17 triggers potent FR+ tumor cell killing by CAR T cells at low pico-molar concentrations.
- Different FR+ tumor types display different sensitivities to EC17-directed CAR T cell killing.
- In general, there is a strong positive correlation between FR expression and EC17-elicited CAR T-cell cytokine production, activation/exhaustion, and/or tumoricidal function.
- Multiple CAMs should be explored to overcome loss of FR expression causing tumor escape.

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