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Regulation of CAR-T cell therapy in real time using bispecific small molecule adaptors and monospecific competitors

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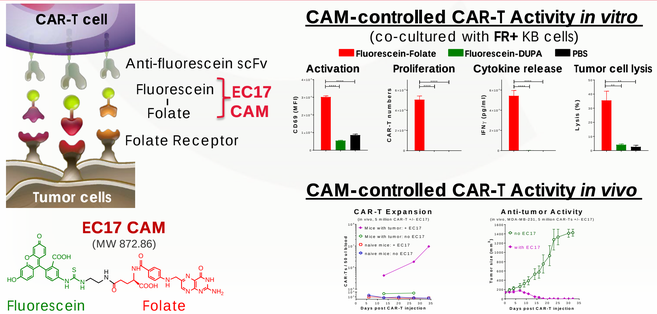
Abstract

Chimeric antigen receptor modified T cell (CAR-T) therapy has revolutionized personalized cancer treatment. The recent FDA approval of two CAR-T therapies for hematological cancers has marked a pivotal milestone in this new era of cellular therapies. CAR-T cells are genetically modified to become activated and proliferate in the presence of tumor antigens. In patients with high tumor burden and rapid T cell expansion, however, severe side effects including severe cytokine release syndrome (sCRS) and life-threatening cerebral edema may occur.

To “fine-tune” CAR-T cell activity in real time, we devised a three-pronged therapeutic strategy by using (1) *4-1BB*-CD3ζ CARs expressing anti-fluorescein (anti-FITC) scFv instead of an anti-tumor ligand/self-antigen scFv, (2) low-molecular-weight bi-specific CAR-T adaptor molecule (CAM) comprised of a FITC hapten linked to a tumor-specific ligand, and (3) competitors that can interfere with the adaptor/CAR-T interaction with tumor cells. Using folate-FITC (EC17) as the bi-specific CAR-T adaptor molecule, we evaluated the performance of FITC-CAR-T cells in various folate receptor (FR)-positive tumor models. Our results show that CAR-T activation and proliferation *in-vivo* was EC17 CAM- and tumor FR-dependent. Treatment-related toxicity (sCRS) was observed but could be easily controlled, or even prevented, by adjusting the concentration, dosing frequency or timing of EC17 CAM administration. Importantly, animals experiencing sCRS could be rescued by administering competitors of the bi-specific CAM. In the case of EC17 CAM/CAR-T therapy, improved animal behavior was observed as early as a few hours after dosing with a rescue agent and a rapid reduction of interferon-γ was also detected. Circulating CAR-T cells remained functional, and could be re-activated following subsequent EC17 CAM doses.

Taken together, our CAM/CAR-T approach offers a unique combination of a man-made hapten (FITC), flexible dosing control, and sCRS prevention/rescue without compromising antitumor activity or permanently removing CAR-T cells. Further studies are warranted to provide a rational basis for translating such therapeutic practice to the clinic.

CAR-T Adaptor Molecule (CAM) controlled CAR-T platform

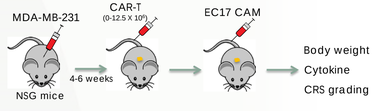


CAM-controlled CAR-T Activity *in vitro*

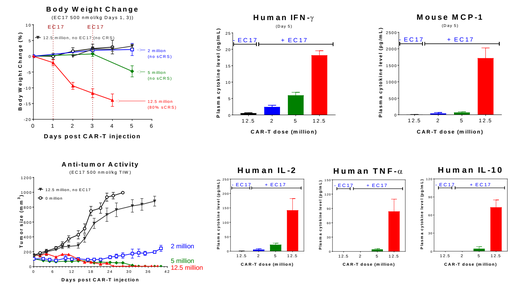
(co-cultured with **FR+** KB cells)

CAM-controlled CAR-T Activity *in vivo*

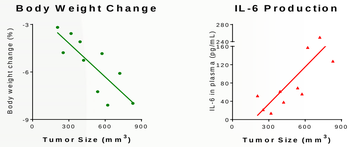
Severe cytokine release syndrome associated with CAR-T therapy



CAR-T Dose Dependence of CRS

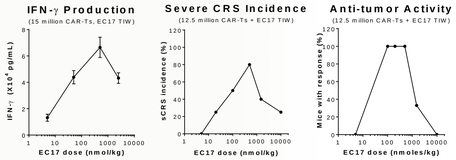


Tumor Burden Dependence of CRS



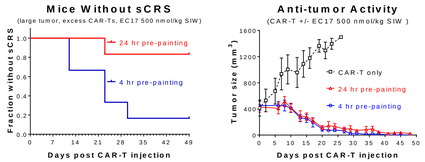
Antigen control through CAM administration can limit adverse events

EC17 CAM Dose Level



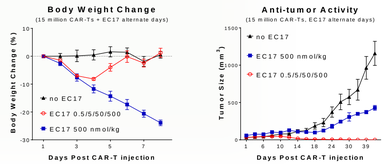
(EC17 CAM 500 nmol/kg dose peaks CAR-T activity and related toxicity)

Pre-painting of Solid Tumor With EC17 CAM



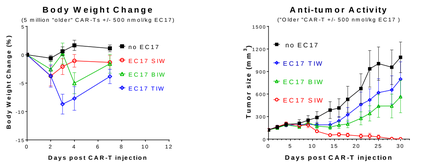
(Pre-painting with EC17 CAM helps CAR-T trafficking to tumors only)

EC17 CAM Dose Escalation



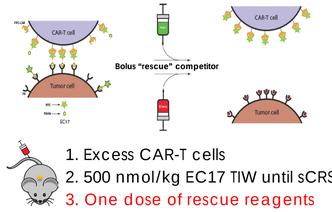
(CAM dose escalation prevents CAR-T AICD and/or cell exhaustion)

EC17 CAM Dose Frequency

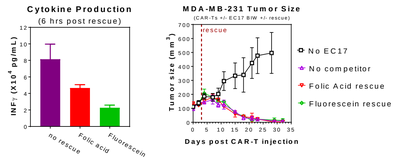


(Less frequent activation of CAR-T prevents CAR-T cell exhaustion)

Regulation of CAR-T therapy using competitors to bi-specific CAM

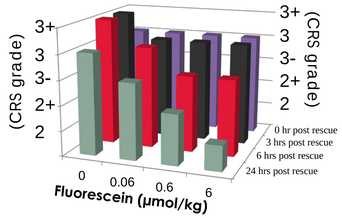


One Dose of Competitors Can Rescue sCRS Mice Without Compromising CAR-T Anti-tumor Activity



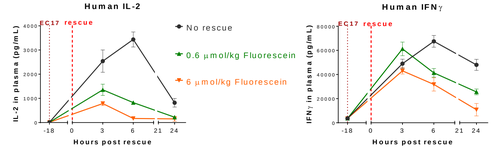
~250 µmol/kg i.v. used for angiography

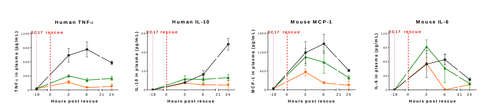
sCRS Mice Rescued With Fluorescein Had Improved Clinical Behavior in 6 Hours



CRS Grading Scale					
0	1	2	3	4	5
Normal	~pilo ~greasy still active	pilo greasy less active	pilo, greasy bunched, thin not active	moribund bunched, pale lethargic	Death

sCRS Mice Rescued With Fluorescein Had Reduced Cytokine Production in 3 Hours





Activity in various models

Conclusions

1. A three-pronged strategy is devised to fine-tune CAR-T therapy *in vivo*: CAR-T, bi-specific CAM, and the monospecific competitors to the CAM.
2. FITC-CAR-T activation and proliferation *in vivo* are CAM- and tumor antigen- dependent.
3. Anti-tumor activity of CAM/CAR-T therapy can be optimized by controlling CAM administration.
4. Treatment-related toxicity (e.g. CRS) was observed but could be easily prevented or controlled by adjusting CAM administration.
5. Administering competitors of CAM can rapidly rescue animals experiencing sCRS without compromising subsequent CAR-T anti-tumor activity.

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