ABSTRACT
LB210

Preclinical Development and Evaluation of Allogeneic CAR T Cells Targeting CD70 for the Treatment of Renal Cell Carcinoma

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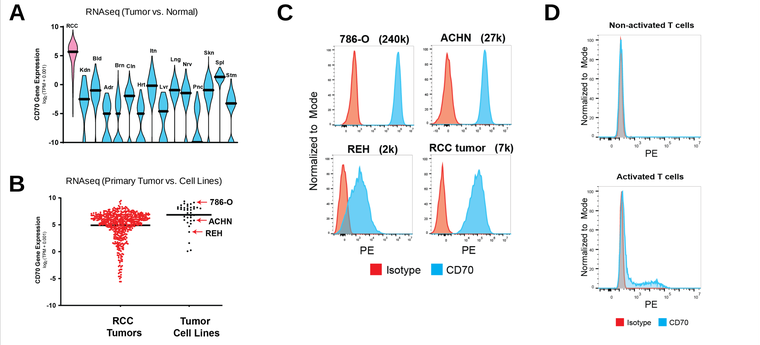
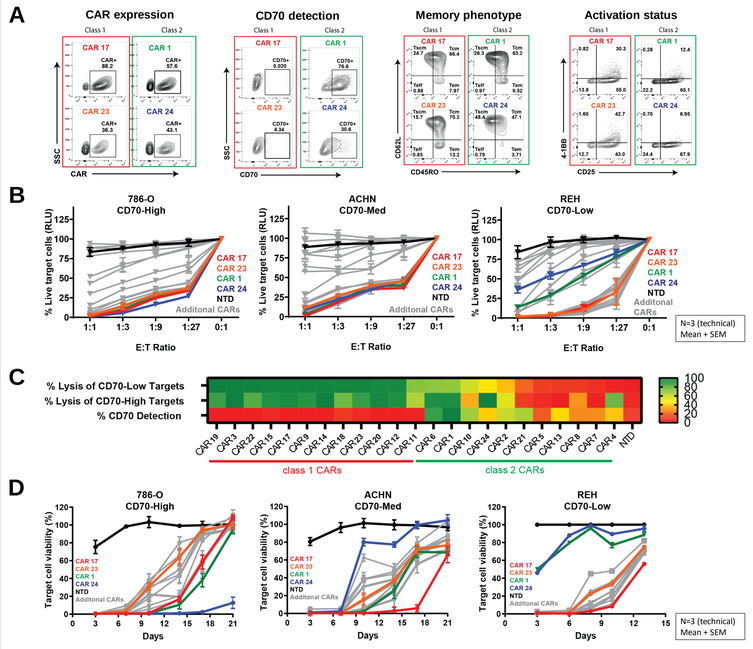
CD70 is highly expressed on renal cell carcinoma (RCC), with limited normal tissue expression, making it an attractive CAR T target for an immunogenic solid tumor indication. Here we generated and characterized a panel of anti-CD70 scFv-based CAR T cells. Despite the expression of CD70 on T cells, production of CAR T from a subset of scFvs with potent in vitro activity was achieved. Expression of CD70 CARs was found to mask CD70 detection in cis and provide protection from CD70 CAR T-mediated fratricide. Two unique classes of CAR T cells were identified with differing memory phenotype, activation status, and cytotoxic activity. Epitope mapping revealed CARs binding to the membrane distal region of the CD70 extracellular domain (ECD) fall into the more active and differentiated class, as compared to CARs binding the membrane proximal region of the CD70 ECD. CD70 CAR T cells were evaluated with rituximab-based off switches to provide control over CAR T function and displayed robust antitumor activity against RCC cell lines and patient-derived xenografts in mouse models. Tissue cross reactivity studies to evaluate off-target binding with two lead CARs showed membrane staining in rare tissue-resident lymphocytes, thus matching the known expression pattern of CD70. Expected findings related to T cell activation, and elimination of CD70-expressing cells were observed in a cynomolgus monkey CD3-CD70 bispecific toxicity study and included cytokine release and loss of cellularity in lymphoid tissues. Lastly, highly functional CD70 allogeneic CAR T cells were produced at large scale through elimination of the T cell receptor by TALEN® gene editing. Taken together, these efficacy and safety data support the evaluation of CD70 CAR T cells for the treatment of RCC and led to the advancement of an allogeneic CD70 CAR T candidate into Phase I clinical trials.

CD70 is expressed in RCC with limited normal tissue expression



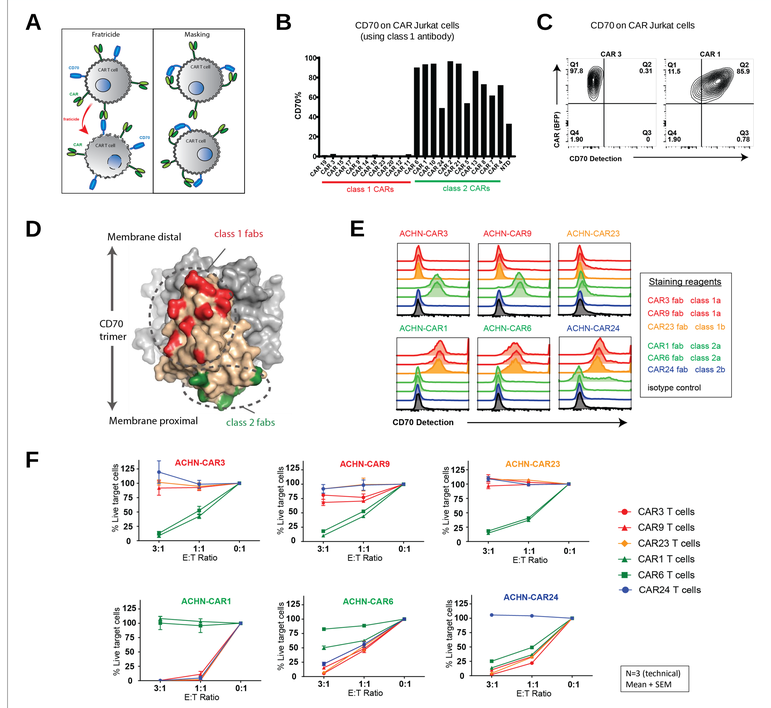
(A) RNAseq data compiled from TCGA and GTEX shows that CD70 is highly expressed in clear cell RCC, but low in almost all normal tissues. **(B)** RNAseq data compiled from TCGA and CCLE shows that median CD70 expression in primary tumors is slightly lower than that in ACHN. **(C)** RCC cell lines and primary RCC samples were analyzed by flow cytometry. **(D)** T cells were analyzed by flow cytometry for surface expression CD70 pre- and post-activation using CD3/CD28 beads.

Unique class of CD70 CARs with distinct attributes and enhanced cytolytic activity was identified



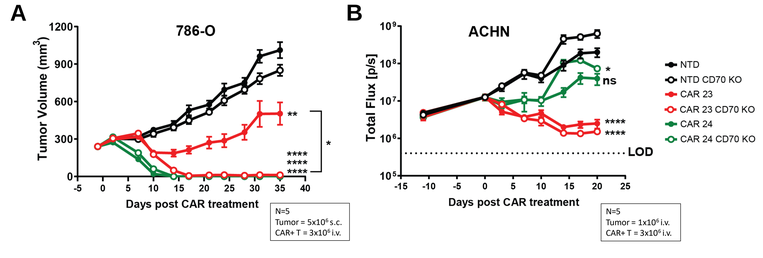
(A) CD70 CARs display suitable transduction efficiencies. CD70 expression analysis revealed unique classes of CARs with either minimal CD70 (class 1) or high CD70 (class 2). Class 1 CARs are more differentiated and activated **(B)** Class 1 CARs display superior cytolytic activity across a range of target expression over 72h **(C)** CARs with lower level of CD70 detection (class 1) had the greatest short-term cytolytic activity. **(D)** Class 2 CARs are highly active against CD70-high targets but perform poorly against CD70-low targets.

CD70 CAR masks CD70 in cis and protects CAR T cells from fratricide



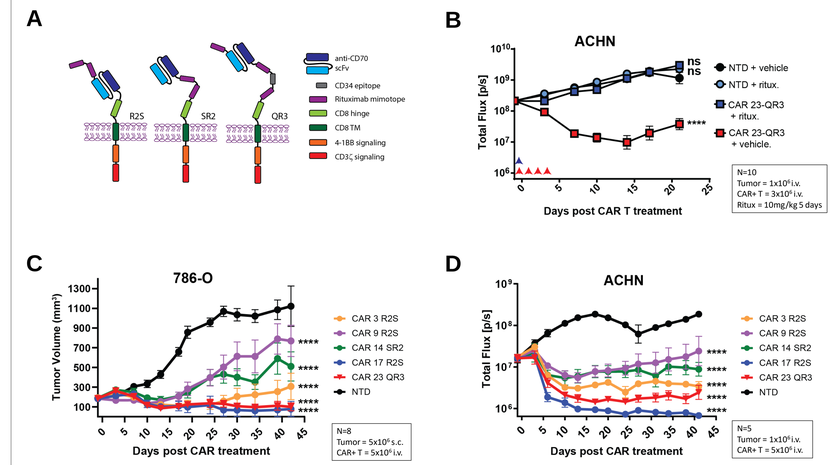
(A) CD70 CAR could either bind to CD70 on neighboring CAR T cells thus leading to fratricide or it could bind to CD70 in cis thereby protecting cells from fratricide. **(B)** Jurkat CAR cells, incapable of killing or fratricide, show similar CD70 expression pattern as T cells with no CD70 detected on class 1 CARs using a class 1 antibody. **(C)** Representative flow plot. **(D)** Class 1 and class 2 CARs fall into distinct CD70 epitope bins that are membrane distal or membrane proximal, respectively. **(E)** Class 1 Fabs are unable to stain ACHN cells expressing class 1 CARs but able to stain cells expressing class 2 CARs and vice versa. **(F)** Cells expressing class 1 CARs are protected from killing by class 1 CAR T cells but not protected from class 2 CAR T cells and vice versa.

CD70 KO enhances function of certain CARs in CD70-high model but no difference observed in model with lower CD70 expression



(A) NSG mice implanted with subcutaneous 786-O tumors were treated with CD70 CAR T cells modified using TALEN® to knockout either the T cell receptor (TCR) alone or both TCR and CD70. While CAR 23 benefits from CD70 KO, CAR 24 is efficacious regardless of CD70 KO, suggesting that not all CARs require and benefit from CD70 KO. **(B)** Mice injected with intravenous ACHN cells were treated with CD70 CAR T cells genetically modified to knockout either TCR alone or both TCR and CD70. CAR 23 shows superior efficacy, irrespective of CD70 KO. Statistics were performed using RM one-way ANOVA, data shown as mean ± SEM.

CD70 CARs with optimal off-switch showed anti-tumor efficacy in multiple in vivo models



(A) Each CD70 CAR was engineered with various Rituximab-based off-switch formats and tested since optimal format needs to be determined on a per CAR basis. **(B)** Activity of the off-switch was confirmed in ACHN model where dosing of Rituximab completely abrogates anti-tumor activity. In vivo efficacy of CAR T cells with optimal off-switch formats was evaluated using **(C)** 786-O subcutaneous model and **(D)** ACHN intravenous model. Statistics were performed using RM one-way ANOVA, data shown as mean ± SEM.

No unexpected findings observed in a cyno CD3 bispecific toxicity study

	Findings	
Tissue	**Vehicle**	**CD70-CD3 bispecific**
blood	no findings	severe at 100µg/kg dose (n=2) / cytokine release
bone marrow	no findings	Grade 1 (n=2) / increased myeloid/erythroid ratio
gut associated lymphoid tissue	no findings	Grade 2 (n=2) / decreased cellularity (lymphocytes)
pancreas	no findings	Grade 2 (n=2) / decreased zymogen content
salivary gland	no findings	Grade 2 (n=2) / decreased zymogen granules
spleen	no findings	Grade 2 (n=2) / decreased cellularity (lymphocytes)
thymus	no findings	Grade 2 (n=1) Grade 4 (n=1) / decreased cellularity (lymphocytes)
tonsil	no findings	Grade 1 (n=1) / decreased cellularity (lymphocytes)

No suspected article-related macroscopic or microscopic findings were observed in adrenal, bone, bone marrow, brain, colon, cervix, duodenum, epididymis, eye, heart, ileum, jejunum, kidney, liver, lung, nerve, ovaries, pancreas, pericardial sac, pituitary gland, salivary gland, stomach, testis, thyroid, trachea, urinary bladder, uterus, vagina

CD3-CD70 bispecific activity was confirmed based on CD69 expression and secretion of cytokines such as IL-6 (N=2)

Tissue Cross-Reactivity assay was performed to assess safety profile

Cell line/Tissue	CAR3	CAR17	CAR23
ACHN (CD70+)	1-3+, freq, M	2-3+, freq, M	1-3+, freq, M
Raji (CD70+)	3-4+, freq, M	3-4+, freq, M	3-4+, freq, M
786-O (CD70+)	2-4+, freq, M	2-4+, freq, M	2-4+, freq, M
293T (CD70+)	negative	negative	negative
786-O CD70 KO (CD70-)	negative	negative	negative
Kidney, epithelial tubules	negative	negative	2-4+, occas, C
Lymph Node (leukocytes)	1-3+, rare, M	1-3+, occas, M	1-3+, rare to occas, M
Spleen (leukocytes)	negative	negative	1-3+, freq, M
Thymus (epithelial-reticular cells)	2-3+, occas, C	2-3+, freq, C	2-3+, occas, C
Thymus (leukocytes)	negative	1-3+, occas, M	negative

1+ = weak, 2+ = moderate, 3+ = strong, 4+ = intense, negative (0%), rare (>1-5%), rare to occas (>5-25%), occas = occasional (>25-50%), freq = frequent (75-100%), M = membrane staining, C = cytoplasmic staining

CAR 17 displayed 1-3, freq cytoplasmic staining in all tissues evaluated

CAR 3 and CAR 23 displayed no staining in adrenal, bladder, blood cells, blood vessels, bone marrow, brain - cerebellum, brain - cerebral, breast, colon, eye, fallopian tube, GI tract - esophagus, GI tract - small intestine, GI tract – stomach, heart, liver, lung, ovary, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, spinal cord, striated muscle, testis, thyroid, tonsil, ureter, uterus – cervix, uterus – endometrium

TCR assay was performed using soluble binding domains from CARs 3, 17, and 23 to assess binding against a panel of 36 normal tissues from 3 healthy donors.

CONCLUSIONS

- CD70 is expressed in RCC with normal tissue expression limited to activated lymphocytes
- A unique class of CARs (class 1), that display distinct attributes and superior cytolytic activity was identified
- Both class 1 and class 2 CARs mask CD70 in cis thus protecting CAR T cells from fratricide
- Class 1 CARs bind membrane distal region while class 2 bind membrane proximal region
- CD70 KO benefits class 1 CARs against CD70-high tumors but no benefit observed in models with lower CD70 levels
- Optimal off-switch addition renders class 1 CARs highly effective in multiple RCC models across range of target levels
- Cyno toxicity study using a bispecific surrogate showed no unexpected findings
- Tissue Cross-Reactivity assay showed acceptable safety profile