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

Siler Panowski1, Surabhi Srinivasan1, Silvia Tacheva-Grigorova1, Nguyen Tan1, Bryan Smith1, Dinali Wijewarnasuriya1, Jonathan Villanueva1, Shanshan Lang1, Yvonne Mak1, Zea Melton1, Thomas Van Blaricom1, Adit Ghosh1, Hongxiu Ning1, Tao Sai2, Jonathan Heyen2, Mathilde Dusseaux3, Roman Galetto3, Javier Chaparro-Riggers2, Barbara Saso1

1Allogene Therapeutics, Inc., South San Francisco, CA, USA; 2Pizer Inc., San Diego, CA, USA; 3Cellectis SA, Paris, France

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 T-cells 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-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 scale through elimination of the T cell receptor by TALEN® gene editing. Taken together, these efficacy and safety data support the evaluation of CD70 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 from TCGA and GTEx shows that CD70 is highly expressed in clear cell RCC, but low or absent in all normal tissues. (B) RNAseq data compiled from TCGA and COLE show that higher CD70 expression in primary tumors is slightly lower than that in ACCs. (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/CD8 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 proximal (CD70 class 1) or high CD70 (class 2). Class 2 CARs were more differentiated and activated. (B) Class 1 CARs display greater short-term cytolytic activity. (C) 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 preventing cells from fratricide. CD70 CAR+ T cells were co-cultured with CD70 cells expressing cis CARs or with CD70 cells expressing cis CARs but no CD70 detected in class 1 CARs using a cis 1 approach. (B) Representative flow plot. (C) Class 1 and class 2 CARs fail to detoxify CD70-mediated fratricide.

**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 766-O tumors were treated with CD70 CAR+ T cells modified using TALEN® to knockout either the TCR complex (TCR) alone or both TCR and CD70. When CD70 CAR T cells were treated with CD70 CAR T cells, genetically modified to knockout either TCR alone or both TCR and CD70. CD70 CAR T cells showed superior efficacy, irrespective of TCR KO. Statistically performed using RM one-way ANOVA, data shown as mean ± SEM.