ABSTRACT #3716

AlloCAR T™ TARGETING CD70 FOR THE TREATMENT OF RENAL CELL CARCINOMA

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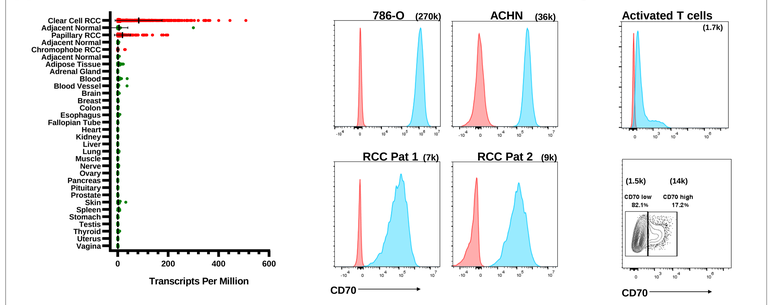
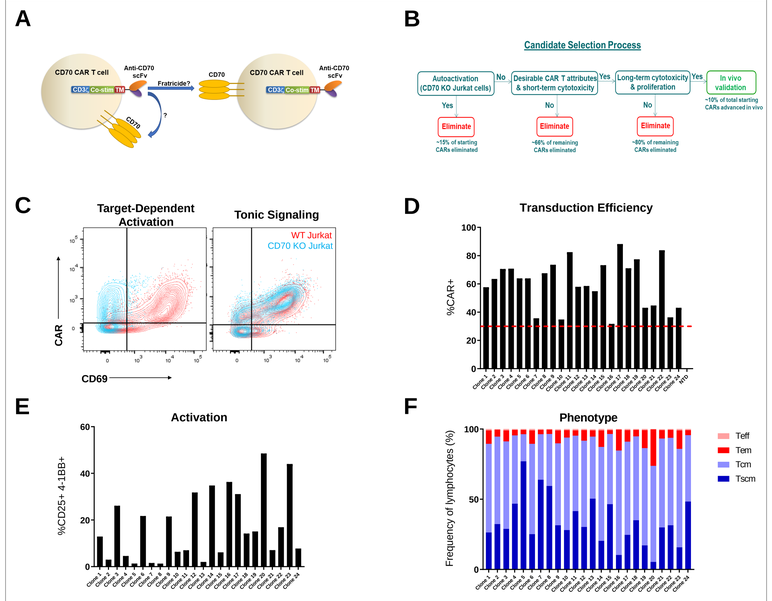
Renal Cell Carcinoma (RCC) represents a substantial patient population, with 65,340 new cases estimated in the US in 2018. Current treatment for advanced disease improves overall survival, but disease relapse is common and additional treatments are needed. RCC is a highly T-cell infiltrated tumor type with responsiveness to immuno-oncology agents and thus it may be amenable to a T-cell based therapy. T cells can be genetically modified to express chimeric antigen receptors (CARs), and adoptive transfer of CAR T cells is showing great promise in hematologic malignancies. To translate this approach for RCC treatment, expression data were mined and CD70 was identified as an antigen expressed in a high proportion of patients with RCC, with limited normal tissue expression on a fraction of activated lymphocytes and dendritic cells. Since CD70 expression is present on activated T cells, targeting it with a CAR could lead to fratricide and T cell exhaustion. Screens were specifically designed to identify CARs that were less impacted by these issues. A large panel of scFvs that bind to CD70 were generated and formatted into CARs. CD70 CAR T cells were ranked based on tonic signaling, transduction efficiency, phenotype, activation status and expansion. A subset of CD70 CAR T cells were moved into in vitro short and long-term cytotoxicity assays. Target cells expressing high, medium, and low levels of CD70 were utilized. CAR T cells were evaluated in vivo and robust anti-tumor activity was observed. Some candidates performed better with CD70 knockout and some worked irrespective of knockout. A cynomolgus monkey toxicity study was conducted with one clone formatted as a CD70-CD3 bispecific antibody and no unexpected findings were observed. Multiple off-switch CAR formats were evaluated. CD70 CAR T cells were also successfully manufactured in a large-scale process. In summary, multiple CD70 CAR T cells have been profiled and a subset selected for further investigation as potential clinical candidates.

CD70 is expressed in RCC with limited normal tissue expression



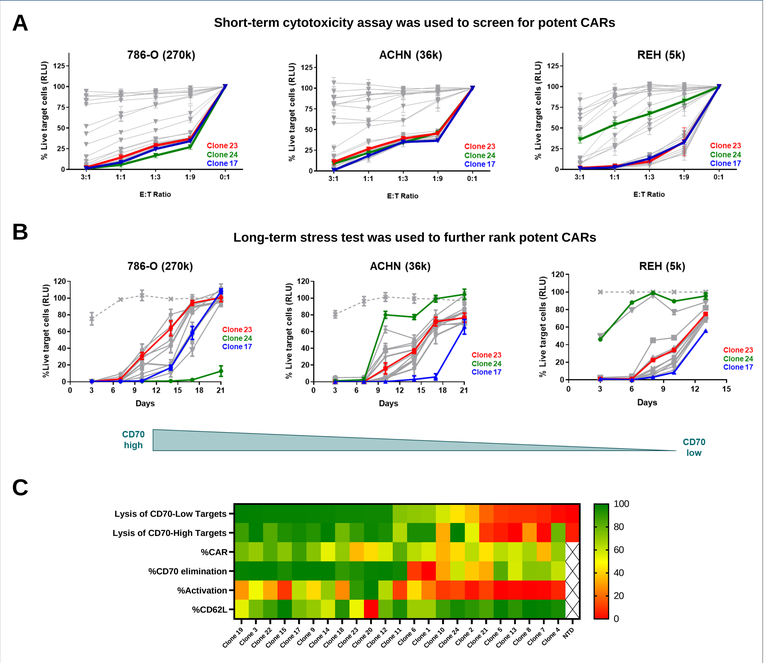
(A) RNAseq data compiled from TCGA and GTEX shows that CD70 is highly expressed in clear cell RCC, but low in almost all normal tissues. **(B)** RCC cell lines and primary RCC samples were analyzed by flow cytometry. **(C)** T cells were activated using CD3/CD28 and analyzed after 5 days by flow cytometry for surface expression and quantification of CD70 (numbers in parenthesis indicate Antibody Binding Capacity).

Screens were developed to select CARs based on tonic signaling, transduction efficiency, activation, and phenotype



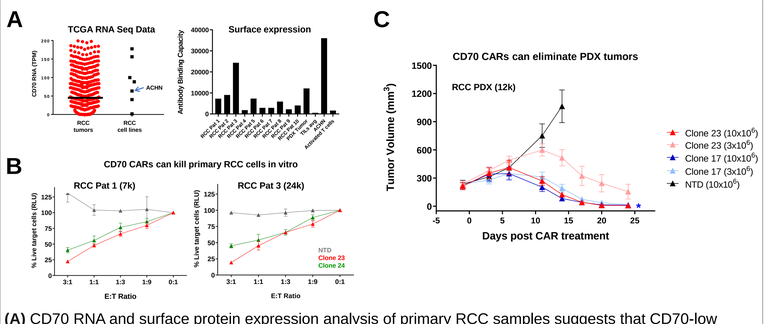
(A) CD70 CAR T cells can interact with CD70 in trans and possibly cis, potentially leading to fratricide and/or exhaustion. **(B)** Flowchart of CD70 CAR candidate selection process. **(C)** Tonic signaling and target-dependent activation were distinguished by CD69 expression in WT and CD70 KO Jurkat cells, and auto-activating clones were eliminated. **(D)** CD70 CARs display suitable transduction efficiencies, between 30-90%, in primary T cells. Transduction was determined by co-expression of BFP. **(E)** Activation status (CD25 and 4-1BB expression) and **(F)** phenotype (memory subsets as determined by CD62L and CD45RO) in primary T cells are informative as to which CARs may be more sensitive to CD70 expression in culture and may help predict success in cytotoxicity assays and in vivo studies.

In vitro cytotoxicity assays using cell lines with different target densities separate CARs based on their activity



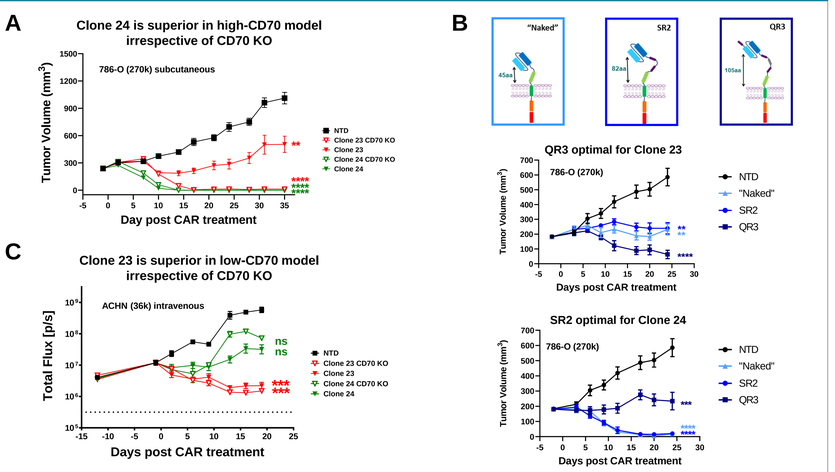
(A) CARs were further ranked in an in vitro short-term cytotoxicity assay by mixing freshly thawed CAR T cells with luciferase-labeled targets expressing high, low, or very low levels of CD70 at different E:T ratios for 72 hours. A subset of CARs that were highly active against all three targets were advanced into **(B)** a long-term stress test where freshly thawed CAR T cells were mixed with targets and re-challenged every 2-3 days. CARs that were highly active against all three targets are currently being investigated in vivo. **(C)** Profiling various CARs shows differences in their cytotoxic activity in relation to their phenotype during CAR T cell production.

CD70 CAR T cells are efficacious against primary RCC samples



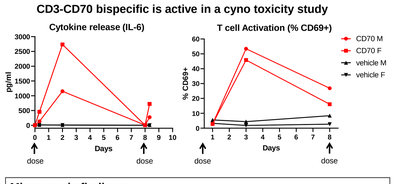
(A) CD70 RNA and surface protein expression analysis of primary RCC samples suggests that CD70-low ACHN model is relevant for evaluating CD70 CAR T candidates. **(B)** CD70 CAR T cells were able to kill primary RCC cells in an in vitro short-term cytotoxicity assay and **(C)** were efficacious against Patient Derived Xenograft tumors in vivo (statistics were performed using RM one-way ANOVA, data shown as mean ± SEM).

CD70 CAR T cells show anti-tumor efficacy in multiple in vivo models and optimal Rituximab-based off-switch formats were identified



(A) NSG mice implanted with subcutaneous 786-O tumors were treated with CD70 CAR T cells that were modified using TALEN® to knockout either the T cell receptor (TCR) alone or both TCR and CD70. While Clone 23 CAR benefits from CD70 KO, Clone 24 is efficacious regardless of CD70 KO, suggesting that not all CARs require and benefit from CD70 KO. **(B)** CD70 CAR T were engineered with off-switch formats and tested in the subcutaneous 786-O model. The QR3 format was optimal for Clone 23 and the SR2 format was optimal for Clone 24, suggesting that optimal format needs to be determined on a per CAR basis. **(C)** Mice injected with intravenous ACHN cells were treated with CD70 CAR T cells that were genetically modified to knockout either TCR alone or both TCR and CD70. Clone 23 CAR shows superior efficacy, irrespective of CD70 KO. Statistics were performed using RM one-way ANOVA, data shown as mean ± SEM.

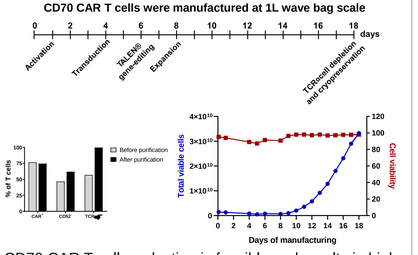
No unexpected findings observed in a cyno CD3 bispecific toxicity study



Microscopic findings:

- Minimal to moderate decreased cellularity of lymphocytes in GALT, spleen, thymus and tonsil
- Mild decreased zymogen granules in salivary gland, pancreas likely secondary to clinical condition
- No signs of non-lymphocyte targeting of tissues/organs

CD70 CAR T cells can be manufactured at large-scale



CD70 CAR T cell production is feasible and results in high cell yield, viability, knockout, and transduction efficiencies.

CONCLUSIONS

- **CD70 is expressed in RCC with normal tissue expression limited to activated lymphocytes**
- **CARs were screened, characterized, and ranked against targets using in vitro cytotoxicity assays**
- **CD70 CAR T cells are efficacious in multiple in vivo models, including a PDX model**
- **Long-term efficacy results suggest that it is possible to select CARs that are highly active despite potential fratricide**
- **CARs were selected based on their activity against targets with CD70 expression level similar to that on primary patient samples**
- **Cyno toxicity study using a bispecific surrogate showed no unexpected findings**
- **CD70 CAR T cells were successfully manufactured in a large-scale process**