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

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RCC 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-oncologic agents and thus it may be amenable to 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 were utilized from in vitro, in vivo, and kinesin and levels of CD70 were utilized. CAR T cells were utilized in a low, moderate, and high anti-tumor activity model. These candidates performed better with CD70 knockdown and some work irrespective of knockdown. A comovesome monkey toxicity study was conducted with one clone formatted as a CD70-3bispecfic antibody and no unexpected findings were observed. Multiple off-switch CAR formats were evaluated. CD70 T CAR T cells were also successfully manufactured in a large-scale process. In summary, multiple CD70 T CAR T cells have been profiled and a subset selected for further investigation as potential clinical candidates.

**ABSTRACT #3716**

**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) Flowstream 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 displayed suitable transduction efficiencies, between 30-60%, 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) Short-term cytotoxicity assay was used to screen for potent CARs. (B) Long-term stress test was used to further rank potent CARs. (C) CD70 CARs show anti-tumor efficacy in multiple in vivo models and optimal Rituximab-based off-switch formats were identified.

**CONCLUSIONS**

- CD70 is expressed in RCC with normal tissue expression limited to activated lymphocytes
- CARs were commercially activated, 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 levels similar to that of primary patient samples
- Cytotoxicity study using a bispecific surrogate showed no unexpected findings
- CD70 CAR T cells were successfully manufactured in a large-scale process.