**Enhanced functionality of 3rd generation CAR-T cells mediated by activation of the IL23 cytokine pathway**

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### INTRODUCTION

**Background/significance to NETs:**

Neuroendocrine tumors (NETs) pose a colossal burden owing to the development of resistance to the existing therapy modalities. Currently the 5-year survival rates are poor for metastatic NETs.

Immunotherapy using CAR-T cells have proven very efficient in the treatment of many blood malignancies but still remain mostly ineffective for the solid tumors in general and the NETs in particular.

Our studies so far resulted in development of novel nanobody-directed CDH17 CART T cells to treat NETs in preclinical models.

Data from preclinical studies demonstrated that the 3rd generation CAR-T(3G) therapy surpassed the 2nd generation CARs(2G) in eradicating the NETs. Here, we further explore the mechanisms that power the 3rd generation CAR-Ts by using broad range sequencing approaches.

Massive sequencing approaches have been beneficial in identification of potential signaling pathways that can further improve the efficacy of CAR-T therapy for pancreatic NETs.

**CONCLUSIONS**

3rd generation CAR-Ts have proven to perform better in preclinical studies in comparison to the 2nd generation CAR-Ts.

Using sequencing approaches we were able to identify signaling pathways such as IL23 that can further improve the functionality of 3rd generation CAR-Ts.

Ex-vivo results indicate that the activation of IL23 could be beneficial for better CDH17-CART function.

### METHODS

**Schematic overview of the experimental background and methods**

**Figure legends:**

- Diagram representing 3G and 3G CARTs constructs.
- Increased cytokicity of 3G CARTs as compared to 2G.
- Enhanced cytokine response displayed by 3G as compared to 2G.
- In vivo NT-3 tumor response to 3G vs 2G CARTs.
- Immunofluorescent analysis of histological sections 3G vs 2G.
- Combination of Next Generation Sequencing (NGS) approaches to understand cellular regulators of enhanced 3rd generation CARTs.

**CONCLUSIONS**

Further confirm the results obtained so far using NET cell lines NT-3 & BON for cytotoxicity and cytokine release profiling.

Explore the role of CD226 in enhancement of cytokocity of 3G CARTs.

Perform in vivo studies to establish the efficacy of IL23 signaling pathway in improvement of function of CDH17-CARTs.

### RESULTS

**ScRNA Sequencing indicated upregulated Th17 response in 3G CARTs**

**Figure legends:**

- (G) Cluster analysis of ScRNA sequencing data showing the percentage of Th1 and Th17 cells in 3G vs 2G CARTs.
- (H) Graph showing increased ratio of Th17/T171 cells in 3G CARTs.
- (I) GSEA of RNA seq data to show the Th1 type immune response.
- (J) Heat map representation of the control vs 3G2G genes involved in a Th17 type immune response.

**CONCLUSIONS**

- Enhanced cytokocity of 3G CART VH1 with co-expression of p40 subunit in SKOV3-CDH17 cells.
- Cytotoxicity analysis of NBC4-CDH17 overexpression system using the CARTs that coexpressed both VH1 and p40.

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