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 CAR 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

METHODS

Schematic overview of the experimental background and methods

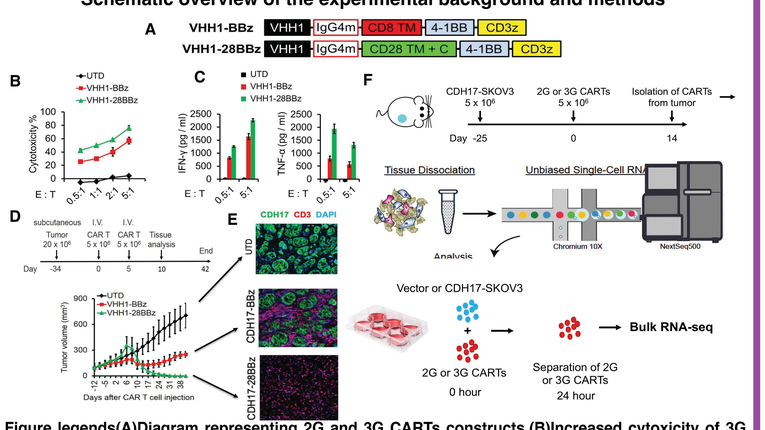


Figure legends(A)Diagram representing 2G and 3G CARTs constructs.(B)Increased cytoxicity of 3G CARTs as compared to 2Gs. (C)- Enhanced cytokine response displayed by 3Gs as compared to 2Gs.(D) In vivo NT-3 tumor response to 3G vs 2G CARTs.(E)Immunofluorsecent analysis of histological sections 3G vs 2Gs. (F)Combinatination of Next Generation Sequencing (NGS) approaches to understand cellular regulators of enhanced 3rd generation CARTs

RESULTS

ScRNA Sequencing indicated upregulated Th17 response in 3G CARTs

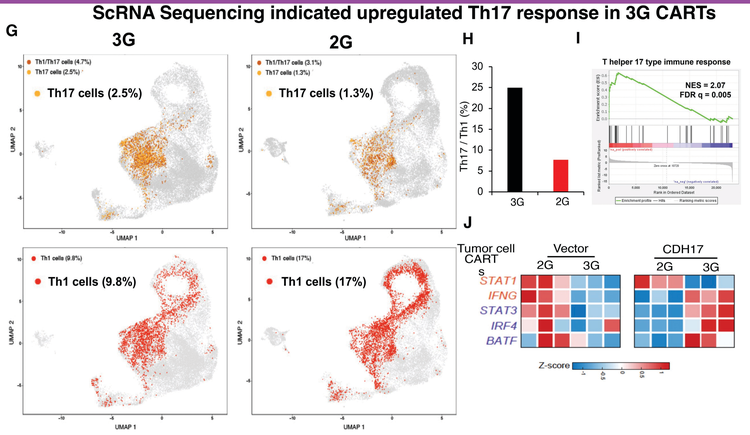


Figure legends: (G)Cluster analysis of ScRNA sequencing data showing the percentage of Th17 and Th1 cells in 3G vs 2G CARTs. (H) Graph showing increased ratio of Th17/Th1 cells in 3G CARTs. (I) GSEA of RNA seq data to show the Th17 type immune responses. (J) Heat map representation of the control vs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

scRNA-seq analysis showed that 3G has higher memory CD8 T cell population than the 2G CARTs

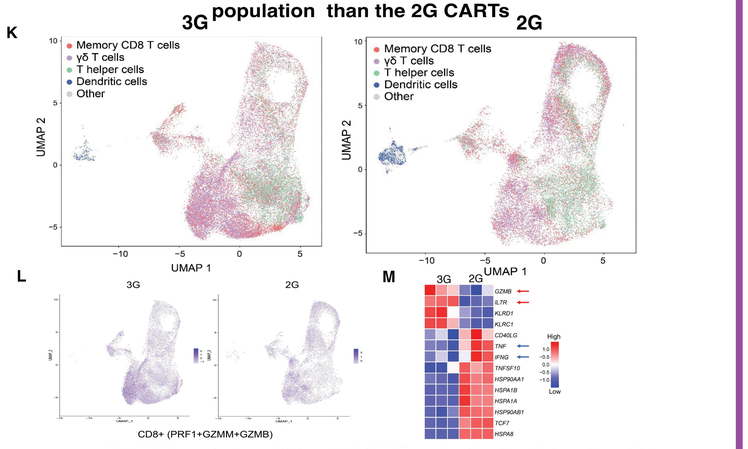


Figure legends: (K) UMAP clustering of 3G and 2G CARTs indicating the distribution of the classes of immune cells. (L) UMAP showing enhanced cytolytic enzymes in 3G vs 2G CARTs. (M) Heatmap analysis of 2G vs 3G RNA seq data showing differential expression of cytokines and granzymes

Bulk RNA sequencing data analysis of 3G and 2G CARTs

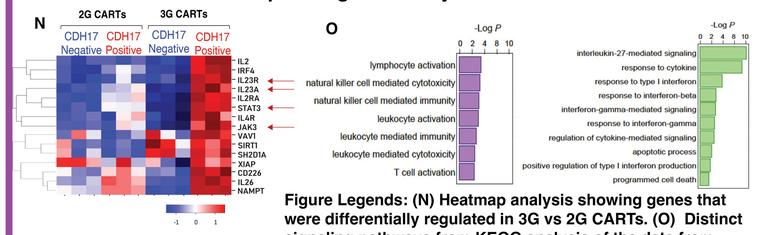
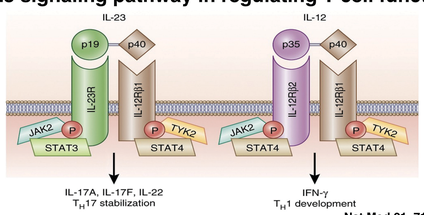


Figure Legends: (N) Heatmap analysis showing genes that were differentially regulated in 3G vs 2G CARTs. (O) Distinct signaling pathways from KEGG analysis of the data from RNA seq

IL23 signaling pathway in regulating T cell functions



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Role of IL23 signaling pathway in the enhanced 3G CART cell activity

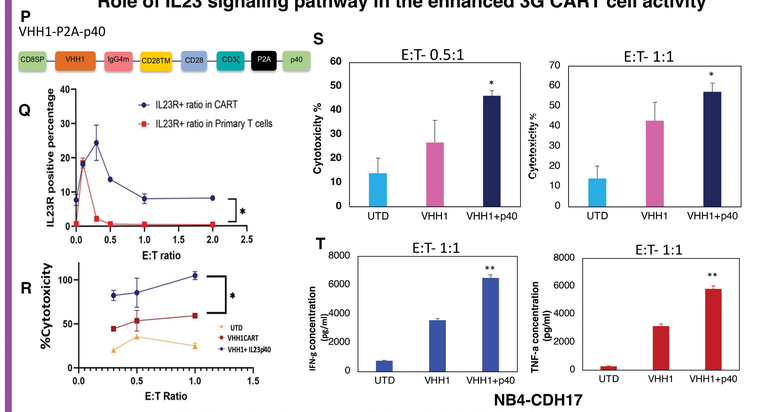

Figure legends: (P) Construct design of the VHH1-P2A-p40 used for expression of VHH1 and p40.(Q). IL23R percentage expression in CART cells.(R). Enhanced cytotoxicity of 3G CART VHH1 with co-expression of p40 subunit in SKOV3-CDH17 cells (S). Cytotoxicity analysis of NB4-CDH17 overexpression system using the CARTs that coexpressed both VHH1 and p40

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.

FUTURE DIRECTIONS

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 cytotoxicity of 3G CARTs

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

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