Spent Media Analysis Aids in Determining Metabolic Mechanisms of Superior CAR T-cell Products

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Overview

As the use of CAR T-cell therapies advances, testing different CAR T-cells, and associating metabolic characteristics to well-performing, desired phenotypes of CAR T-cells enables optimization of CAR constructs and manufacturing processes.

Vasoactive intestinal peptide (VIP) is an emerging checkpoint pathway for T-cell function[1].

VIP is expressed by both T-cells[2,3] and tumor cells[1] and is upregulated during TCR activation[1,4,5]. Augmentation of the VIP/VIPR axis during expansion of primary human T-cells enhances less differentiated T cell phenotypes, anti-tumorcytotoxicity, and *in vivo* persistence.

By engineering CAR T-cells to secrete novel and potent VIPR antagonistic peptides, these cells can overcome the immunosuppression of VIP-rich tumor microenvironments (Figure 1).

Antagonization of VIP/VIPR axis in T-cells enhances T-cell-mediated anti-tumor function.

In this part of the project, we investigate enhancing CAR T-cell manufacturing processes by characterization of products through spent media amino acid analysis – correlating metabolism and metabolic needs with desired, less-differentiated and less-exhaustive phenotype CAR T-cell populations.

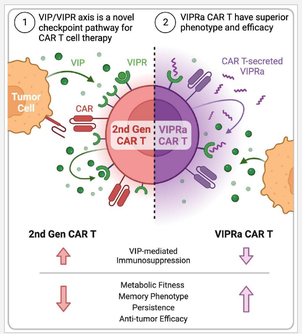


Figure 1. Antagonizing Vasoactive Intestinal Peptide (VIP) Receptors on CAR T Cells Improves Efficacy and Persistence

Methods

To interrogate if VIPR antagonism impacts metabolic pathways, spent media analysis was performed using Repligen's PATsmart™ REBEL® at-line amino acid analyzer to determine overall amino acid usage differences between CAR and CAR/VIPRa T-cells. Media analysis gives insights on consumption and accumulation of compounds in the extracellular environment of the cells.

Fresh and spent media analysis revealed differences in the consumption of amino acids such as alanine, glutamate, and serine. This prompted further investigation of the differences in metabolic pathway utilization and alterations in metabolites within these CAR T-cells. LC/MS metabolomics and cellular bioenergetics were used to elucidate differences between metabolic states of CAR and CAR/VIPRa T-cells.

The full work **(Lin *et al.* submitted)** included the following characterization steps

- *In vivo* –CAR T-cell functionality and potency, as well as longevity
  - Tumor imaging with fluorescence labels
- Microscopy – visual assessment of cells (numbers form, shape, expression of biomarkers)
  - evaluate secreted peptide interaction with T-cell
- RNA sequencing
- Seahorse Mitochondrial Stress Test – metabolic respiration states and stress
- Antigen-Stimulated Assays
  - Activation (such antigen-stimulated cytokine and activation assays)
  - Cytotoxity
  - Proliferation
- Flow Cytometry - number and proportions of cells with specific markers
- REBEL Analysis of amino acid from spent media
- Protein Binding Assays – potency and activity assays, with antigens, antibodies, substrates, etc.

REBEL at-line cell culture media analyzer: Actionable information for your bioprocess at the point of need

- Amino acids, vitamins, and biogenic amines analyzed at-line
- Minimal sample required: as low as 10 µL
- Simple sample prep: spin or filter and dilute
- Integrated analyzer includes autosampler, separation, detection, analysis and reporting
- Analysis run-time ~10 min per sample
- Consumable kit optimized for 200 analyses

Figure 2. REBEL at-line spent media analyzer, with the dedicated assay kit

Results

Figure 3.A

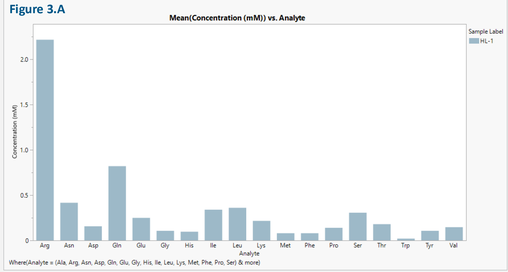
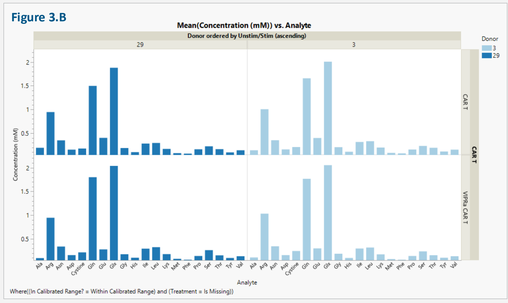


Figure 3.A. Fresh media (RPMI +10%FBS) was analysed for the starting concentrations of amino acids in the cell culture media. Noteworthy is the high level of arginine, and the absence of alanine.

Figure 3.B



Figures 3.B and 3.C. Spent media analysis from different donors (3.B; here, donors 29 and 3) with the two different constructs (3.C – donor 51), showed very similar aa concentration levels at the end-point of manufacturing. Noteworthy is the production/secretion of alanine and significant consumption of arginine from the fresh media, as well as differences between the stimulated and unstimulated cell samples.

Figure 3.C

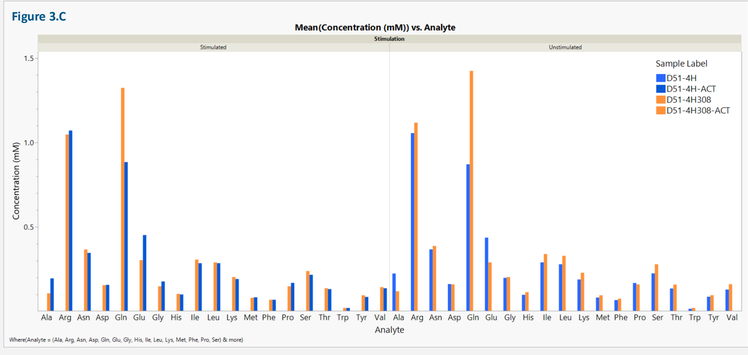


Figure 3.D

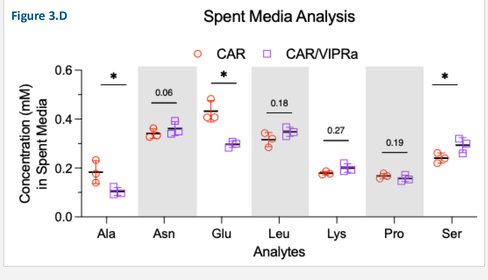


Figure 3.D. Further investigation of REBEL spent media analysis of amino acids of manufactured CAR T-cells with and without VIP receptor antagonistic peptide construct revealed differences in the levels of amino acids such as alanine, glutamate, and serine at the manufacturing end-point.

Results, cont'd.

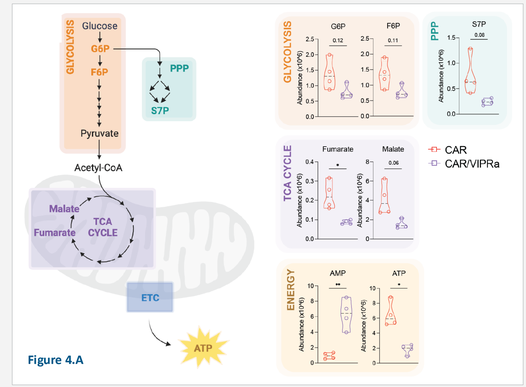


Figure 4.A. The intracellular cell metabolism was investigated with LCMS analysis to determine utilization of different metabolic pathways (glycolysis, TCA, PPP, AMP/ATP) by measuring the abundance of intracellular metabolites

Figure 4.A

Figure 4.B

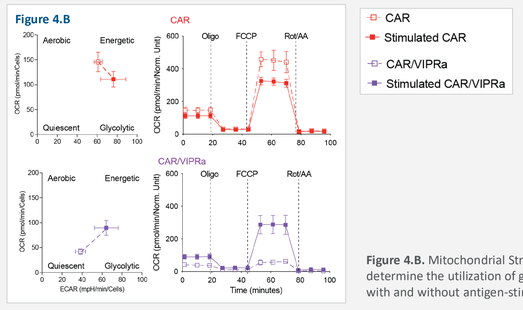


Figure 4.B. Mitochondrial Stress Test was performed to functionally determine the utilization of glycolysis and oxidative phosphorylation with and without antigen-stimulation.

Summary

We found that when the manufactured cells are antigen-stimulated, CAR/VIPRa T-cells become significantly more energetic. In contrast, CAR T-cells become less energetic and more glycolytic, which are known features of T-cell exhaustion.

Further, metabolic assessment with multiple methods produced findings that correlated with this assessment, such oxygen consumption and certain amino acid levels in spent media.

Finally, CAR T-cell activity, potency, and cytotoxicity assessments showed a correlation with anti-tumor activity.

Together, this work demonstrates that distinct metabolic features allow CAR/VIPRa Tcells, when antigen-stimulated, to have enhanced functions against tumors.

Conclusions

We've investigated the mechanisms by which CAR T-cells engineered to secrete potent VIPR antagonistic peptides (CAR/VIPRa) can overcome the immunosuppression of VIPrich tumor microenvironments.

At-line spent media analysis of amino acids, in combination with other assays and measurements, was used to understand, characterize and optimize CAR T-cell manufacturing process, correlate with cell performance, and develop bioprocess parameters.

References

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