

Amino Acid Analysis Indicates Metabolic Differences in Multi-Cytokine Backpack-Manufactured CAR T-Cells

Biaggio Uricoli¹, Heather Lin², Milla Neffling³, A. K. M. Nawshad Hossian², Ji Young Anderson-Czajkowski³, Graziella Piras³, Sarwish Rafiq^{2,4}, Erik Dreaden^{1,4,5,6}

¹Wallace H. Coulter Department of Biomedical Engineering, Emory University and Georgia Tech, Atlanta, GA

²Dept. Of Hematology and Medical Oncology, Emory University, Atlanta, GA

³908 Devices Inc, Boston, MA

⁴Winship Cancer Institute, Atlanta, GA

⁵Dept. Of Pediatrics, Emory University, School of Medicine, Atlanta, GA

⁶Aflac Cancer and Blood Disorder Center, Children's Healthcare of Atlanta, Emory University, Atlanta, GA

Repligen Corporation now owns the life sciences PAT product portfolio of 908 Devices Inc. Please contact Repligen for further inquiries.

Application Note

Highlights

With this work presented, we demonstrate a possible correlation between CAR T-cell activation, nutrient metabolism, and efficacy.¹ The potent, long-lived phenotypes of CAR T-cells showcased some differences in post-manufacturing spent media amino acid analysis as compared to less-active, exhausted phenotype CAR T-cells.



In addition, this work highlights the opportunity to optimize cell culture media for the *ex vivo* manufacturing of CAR T-cell therapies.

Overview

Novel reagents for consistent manufacturing of CAR T-cells with well-defined phenotypes are needed to improve treatment outcomes in patients. Traditional *ex vivo* expansion reagents provide only TCR activation and co-stimulation. Recently developed multi-cytokine backpacks (MCBs) shown in Figure 1 have been shown to provide CAR T-cell manufacturing with additional cytokine support, critical for cell expansion, differentiation, and the selection of potent, long-lived phenotypes.¹ A commonly used method for *ex vivo* activation is

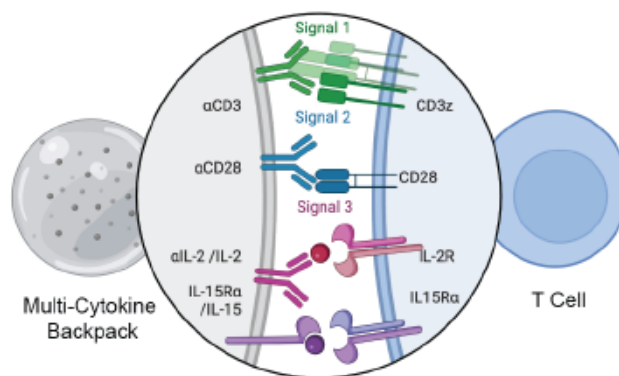


Figure 1. Multi-cytokine backpacks induce activation in CAR T-cell manufacturing with additional cytokine support, which is critical for cell expansion, differentiation, and the selection of potent, long-lived phenotypes.

using the cytokine IL-2 with Dynabeads (Thermo Fisher Scientific). In this project different combinations of immunomodulating proteins were used to create an MCB library.¹

The activated cells were tested for viability, CAR expression, phenotype and *in vivo* activity.¹ Here we describe the amino acid (AA) analysis done with Repligen's PATsmart™ REBEL® to assess differences in the metabolic activity in these activated cells. The differences in metabolism were measured for all groups with Dynabead + exogenous IL-2 as the control and a reference point.

Methods

The T-cells were isolated and transduced as described in Ref 1; Figure 2. After activation, the cells were expanded (RPMI-1640 with L-glutamine (VWR) with 10% FBS (Corning), 100 IU/ml penicillin and 100 µg/ml streptomycin (Gibco)) in a total volume of 200 µL.

AA concentrations were measured from the fresh and spent (end-point) media using the REBEL at-line spent-media analyzer. CAR T-cell products were isolated via centrifugation / 400xg for 5 minutes, and the cell-free supernatant was diluted 1:10 with manufacturer-provided diluent. Automated quantitation of AAs for each sample was achieved using embedded calibrations.

Two biological replicates of each sample were analyzed in triplicate using REBEL.

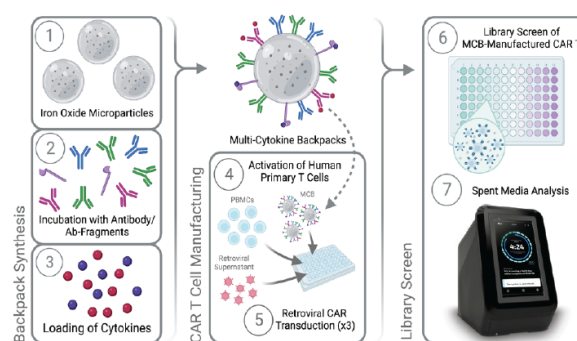


Figure 2. CAR T-cell manufacturing using the multi-cytokine backpacks for activation. The resulting T-cells were tested for viability, metabolic activity, CAR expression, phenotype and *in vivo* activity.¹

The nutrient compositions of the final CAR T-cell product spent media were analyzed to identify differences in the cell metabolism, and the measured levels were plotted using 908 Devices add-in tool for JMP statistical software. Please see further details, such as analyte panel and components in REBEL resources.

The nutrient compositions of the final CAR T-cell product spent media were analyzed to identify differences in their cellular metabolism. The ratio of the mean AA concentrations from the microparticle backpack library samples to the mean concentrations from the control samples (Dynabead + IL-2) were plotted.

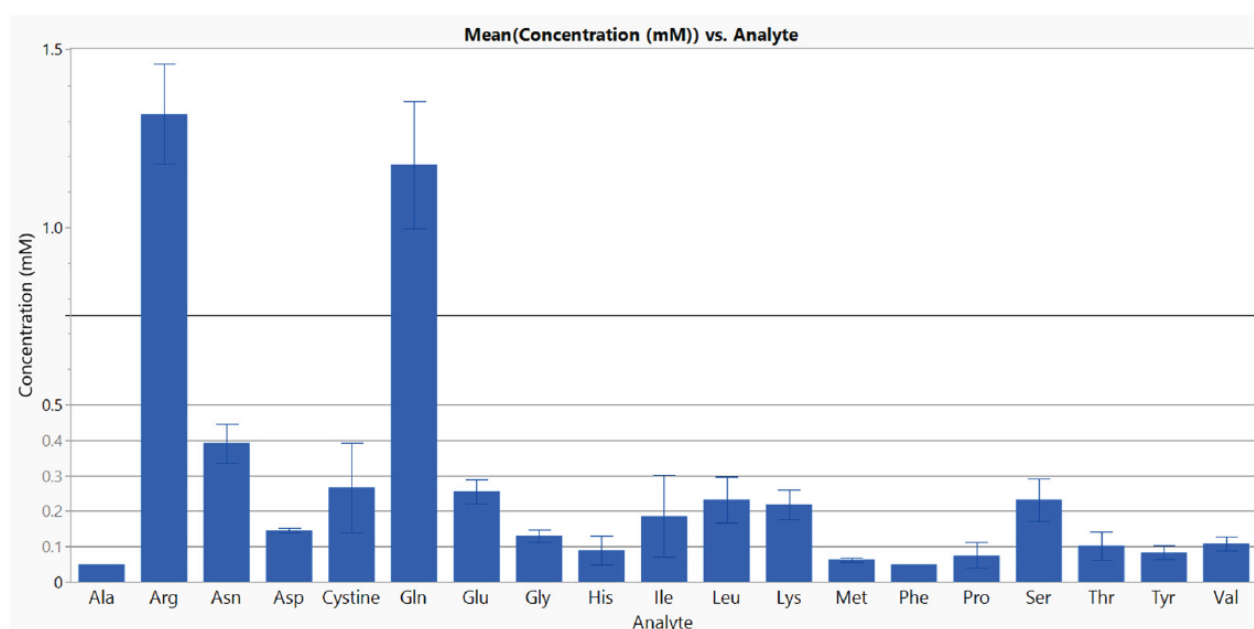


Figure 3. The amino acid levels in the fresh cell culture medium used in the multi-cytokine backpack T-cell activation. Cystine (dipeptide) is measured instead of Cysteine, and Tryptophan was below the detection range. The cell culture medium contains low levels of amino acids, including low levels of essential amino acids.

Results

Based on several biological assays, compounds from the multi-cytokine backpack library were categorized using cluster analysis. Compounds resulting in different phenotypes, with respect to CD8+ and CD4+ levels for less-differentiated CAR T-cell phenotypes as were clustered as “high,” “intermediate,” and “low” performance, indicating less-differentiated CAR T-cell phenotypes (T central memory cells (TCM) and T stem cell memory cells (TSCM)).¹

The fresh (unused) media for the experiment was checked for its amino acid content. The levels are shown in Figure 3.

Control samples for activated CAR T-cells – fresh and spent media analysis

The nutrient compositions of the final CAR T-cell product spent media were analyzed to identify differences in their cellular metabolism. The AA levels of all spent media samples were compared with Dynabead and exogenous IL-2-activated T-cell spent media as the control and a reference point. The

different controls and comparison conditions in spent media analysis AA levels are shown in Figure 4. It was observed that some AAs (ie. Ala, Gly, Pro) were accumulating in the media during culture during the manufacturing process in all samples, whereas some (Arg) were consumed (Figure 4).

Multi-cytokine backpack-activated CAR T-cell spent media analysis

A focus on the top candidates in the “high” group (#09, #16, #27, and #43) spent media samples interestingly showed differences as compared to the control in concentration for the AAs Ala, Asp, Gly, Pro, Thr (see selection shown in Figure 5).

This analysis may indicate that certain metabolic pathways specific to amino acids (such as Proline) are active in T-cells.²

This may indicate different metabolic pathways being active in less differentiated CAR T-cell products.

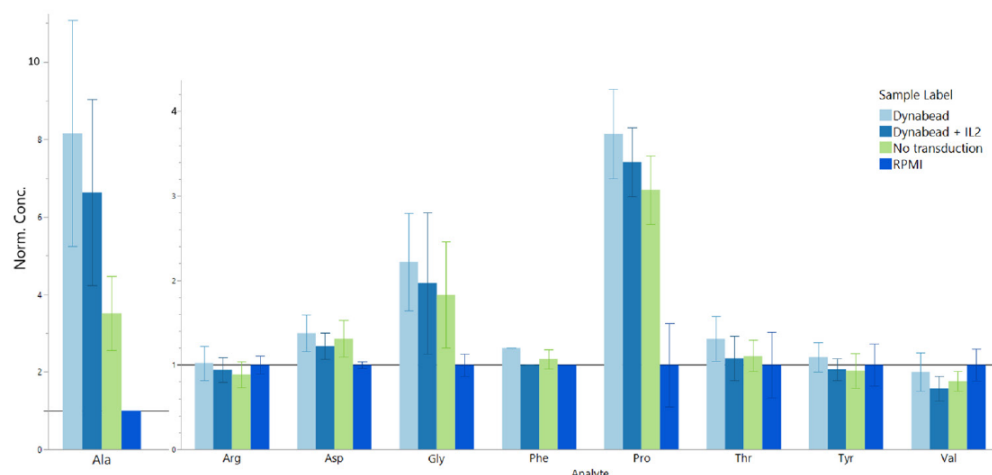


Figure 4. AA levels of the spent media from the control samples were compared to the fresh medium used for CAR T-cell manufacturing. Please note that the AA levels have been normalized to the fresh media concentration (“RPMI” – bright blue), as well as the difference in scale for the AA Ala. It was observed that some AAs (ie. Ala, Gly, Pro) were accumulating in the media during culture in samples, whereas some (Arg, Val) were consumed.

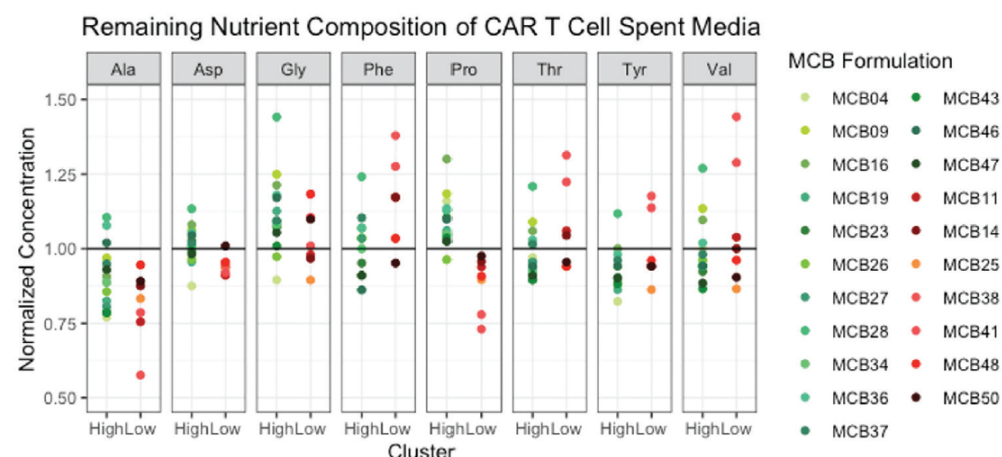


Figure 5. Eight amino acids selected here showed some differences in spent media levels as compared to the control Dynabead + IL-2 activated T-cell spent media (concentrations used for normalization, black line) as well as between the spent media samples from the “High” and “Low” MCB library compounds. For example, Ala was accumulating less in Dynabead+IL-2 than in the MCB activated T-cells. Pro was accumulating more in the “High” group (less differentiated T-cells) than in the “Low” group.



Conclusions

AA analysis is not regularly performed as part of CAR T-cell experiments due to the complexity of many traditional analysis methods and sample volume limitations. With automated quantitation and low sample volume requirements, the REBEL device enables rapid analysis at the bench side with no requirement for prior mass spectrometry expertise.

Studies to define relationships between AA dynamics, T-cell metabolism, and CAR T-cell biology are currently in progress; however previous findings indicate that AAs are critical for efficient T-cell activation and proliferative responses.³

With this work presented here, we demonstrate a possible correlation between CAR T-cell activation, nutrient metabolism, and efficacy. The “high”, and “low” activity MCB compounds with respect to CD8+ and CD4+ less-differentiated CAR T-cell phenotypes (TCM and TSCM) showcased some differences in post-manufacturing spent media amino acid analysis.¹ This also highlights the opportunity to optimize cell culture media for the *ex-vivo* manufacturing of CAR T-cell therapies.

References

1. Lin HK and Uricoli B et al. *Adv Healthc Mater.* 2024 Jan 21:e2302425. 2024 Jun;13(14):e2302425. doi: 10.1002/adhm.202302425.
2. Ye L et al. *Cell Metab.* 2022 Apr 5;34(4):595-614.e14. doi: 10.1016/j.cmet.2022.02.009.
3. Cobbold SP et al. *Proc. Natl. Acad. Sci. U.S.A.* 2009 Jul 21;106 (29) 12055-12060, <https://doi.org/10.1073/pnas.0903919106>.

Contact

Repligen Corporation
685 Route 202/206
Bridgewater, NJ, USA 08807
analytics-support@repligen.com
(908) 707-1009