A Novel Solution to Assess T Cell Metabolic Phenotype for the Optimization of Antitumor T cell Therapy Products

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Introduction

Cellular metabolism is a known driver of T cell fate and function. For this reason, it represents a key attribute to be assessed during T cell therapy optimization and production.

Agilent Seahorse XF T Cell Metabolic Profiling kit is a robust new solution that allows for the simultaneous measurement of basal metabolic requirements, metabolic poise, and mitochondrial bioenergetic capacity in T cells. These parameters have previously been shown to correlate with increased T cell persistence and improved metabolic fitness.

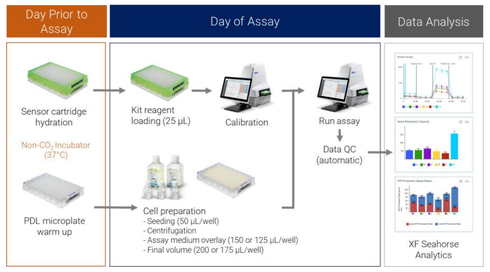
In this study, we used the assay for comprehensive assessment of the bioenergetic profile at different time points of human T cells when expanded in different cell culture media and cytokine formulations that were reported to increase T cell persistence in adoptive T cell therapy transfer experiments.

Experimental

A

XF T Cell Metabolic Profiling Kit Workflow

B



C

Fig 1: (A) The XF T Cell Metabolic Profiling kit contains new reagents optimized to allow for complete measurement of T cell metabolism along with a dedicated analysis tool, Seahorse Analytics. (B) Schematic workflow for running and XF T Cell Metabolic Profiling Kit assay. (C) The kit supports two dedicated assay workflows that deliver quantitative characterization of basal metabolic poise, that is, the balance between glycolytic and aerobic ATP production and robust quantification of the maximal mitochondrial capacity of the cells, parameters associated with either T Cell Persistence or T cell Fitness.

Results and Discussion

BAM15 is an Improved Uncoupler for T cells

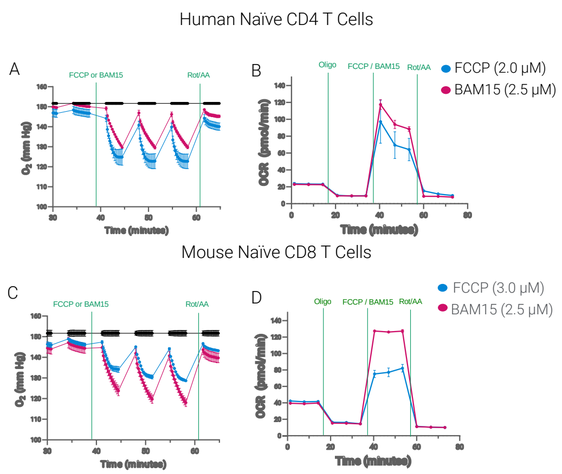


Fig 2: BAM15 addition results in a more robust uncoupling response in T cells compared with FCCP. BAM15 or FCCP were titrated in either human naïve CD4 T cells or mouse naïve CD8 T cells. (A), (C): Changes in extracellular oxygen levels after uncoupler addition, highlighting the more consistent rate during the 3 minutes of instrument measurement obtained with FCCP. (B), (D): OCR kinetic profile in naïve human CD4 T cells. and mouse naïve CD8 T cells, respectively illustrating underestimation of Max respiration obtained when FCCP is used us uncoupler in the assay.

BAM15 Results in a Broader Effective Range and Simplified Concentration Optimization

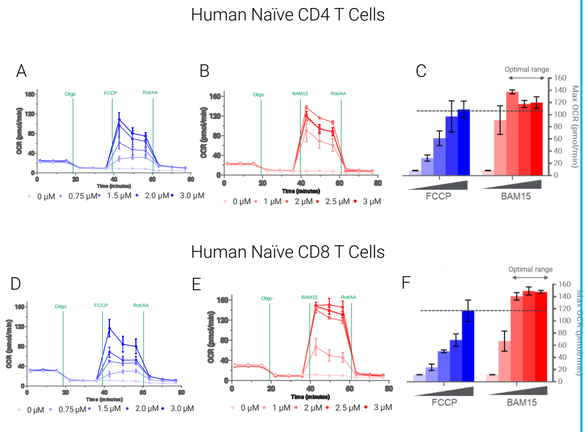


Fig 3: BAM15 at 2.5 µM shows the optimal response in naïve T cells, minimizing requirement of concentration optimization for each cell smaple: (A) Kinetic OCR trace of FCCP titration (0 – 3 µM) in naïve CD4 T cells. (B) Kinetic OCR trace of BAM15 titration (0 – 3 µM) in naïve CD4 T cells. (C) Bar graph comparing maximum OCR obtained with FCCP or BAM15 titration. (D) Kinetic OCR trace of FCCP titration (0 – 3 µM) (E) Kinetic OCR trace of BAM15 titration (0 – 3 µM) in naïve CD8 T cells. (F) Bar graph showing maximum OCR obtained with FCCP or BAM15 titration.

Metabolic evaluation of T cell expansion conditions

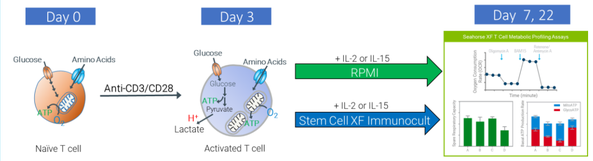


Fig 4: Schematic depicting experimental design to evaluate T cell expansion conditions. Naïve Pan human T cells were activated for 3 days using CD3/CD28 Dynabeads. Cells were then split into two different cell culture media conditions in the presence or absence of IL-2 or IL-15. Seahorse assays were conducted at day 3, 7, 14 and 22 post activation.

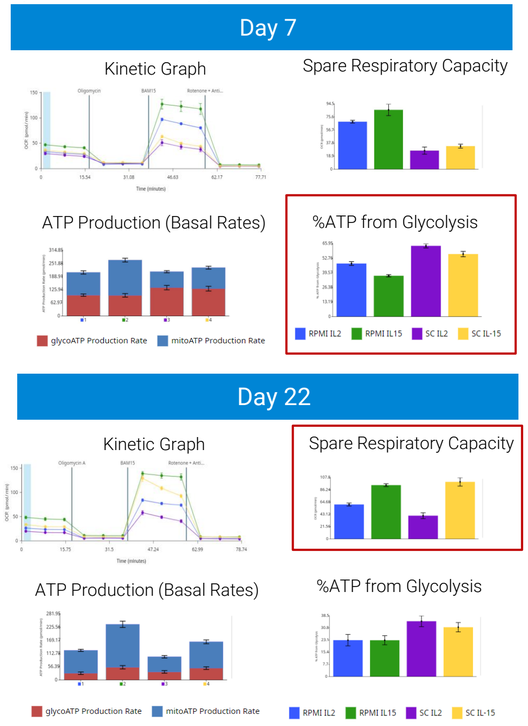


Fig 5: Cell culture medium composition can have profound effects on the metabolic profile of human Pan T cells during a 22-day expansion period. Data displayed show OCR kinetic graphs, spare respiratory capacity, ATP Production rates and % ATP Production from glycolysis at day 7 and day 22. Graphs were generated using Seahorse Analytics.

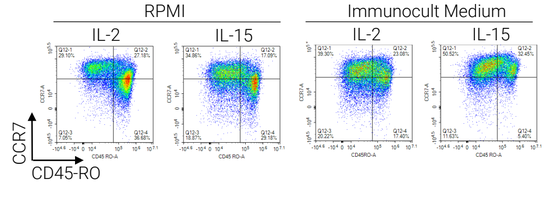


Fig 6: Flow cytometry scattered plot showing the highest % central memory (CD45RO+/CCR8+) in T cells cultured in Immunocult medium with IL-15. Day 22 T cells (CD3+) were stained for CCR7 and CD45 RO at 4 degrees for 20 mins. Cells were washed and analyzed using a Novocyte Advanteon Flow cytometer. Data shown is a representative experiment.

Conclusions

- BAM15 demonstrates significant benefit over the traditional uncoupler, FCCP, when assessing T cell oxidative metabolism. It supports more robust oxygen consumption rates over measurement time and allows for accurate measurement of ATP production rates.
- Higher spare respiratory capacity has often been associated with better T cell persistence *in vivo.* At day 7 post-activation, IL-15 cultured cells have a lower % of ATP production from glycolysis, indicating a more aerobic poise. By day 22, this results in IL-15 cultured cells having significantly higher spare respiratory capacity than IL-2 cultured cells. This parameter may offer unique early insight into T cell metabolism during expansion.
- The Agilent XF T Cell Metabolic Profiling kit allows for simultaneous evaluation of glycolytic and mitochondrial activity and capacity, providing a robust and simple assay for comprehensive profiling of T cell populations.
- The data presented show the impact of cell culture conditions during expansion on the resulting T cell profile and fate, highlighting the importance of including metabolic profiling to monitor and tune culture conditions during T cell therapy development.

Methods

Cell Culture

Human T cells (CD4 and Pan T cells) were obtained from Stem Cell Technologies. For naïve T cell experiments, T cells were thawed and rested overnight in Stem Cell XF Immunocult Medium. For T cell expansion experiments, Pan T cells were rested overnight in Stem Cell Technologies Immunocult XF medium. On day 1 cells were activated using CD3/CD28 dynabeads (Thermo Fisher). After 3 days, dynabeads were magnetically removed and cells were put into either RPMI + 10% FBS or Immunocult XF culture medium with either IL-2 or IL-15 and maintained over 22 days. Mouse CD8 T cells were magnetically purified from splenocytes obtained at Hooke Laboratories and XF assays were run the same day.

Seahorse XF Assays

Naïve human T cells, expanded T cells and mouse CD8 T cells were resuspended in Seahorse XF RPMI, pH 7.4 medium and seeded at 200,000 cells, 100,000 cells and 150,000 cells per well respectively in PDL-coated microplates. Assays were carried out using a Seahorse XF Pro. For detailed information see XF T Cell Metabolic Profiling Kit user guide at Agilent.com

References

Select publications relating to the utility of T cell metabolic measurements for T cell therapy

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