A Novel Solution to Better Understand the Link Between T Cell Metabolism and Function

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Introduction

Cellular metabolism is a known driver of immune cell fate and function. and represents a key attribute to be assessed during optimization of process design and development of immune cell-based therapies.

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 a comprehensive assessment at different time points of the bioenergetic profile of Human Pan T cells expanded at different culture expansion conditions (media formulation, cytokines) previously reported to influence T cell persistence *in vivo*. In addition, we showed that T cell expansion under chronic CD3/CD28 stimulation impairs T cell metabolic fitness when compared with traditional acute CD3/CD28 activation followed by expansion in the presence of IL-2, highlighting how T cell metabolic profile assessment can contribute to improving T cell therapy production process development.

Experimental

XF T Cell Metabolic Profiling Kit Workflow

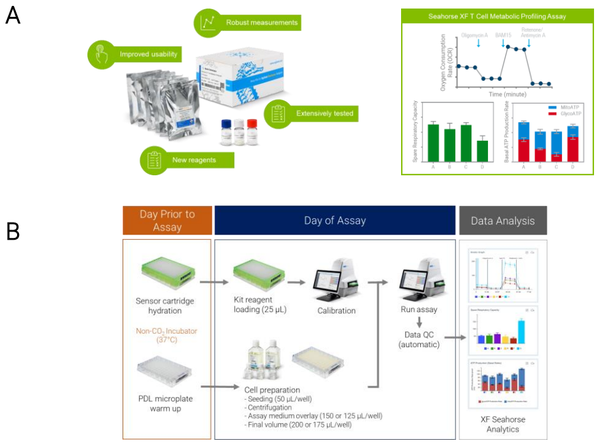
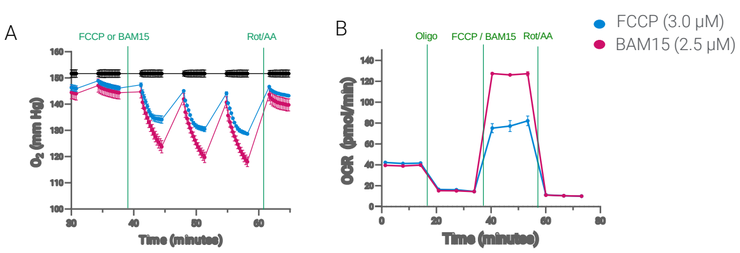


Fig 1: (A) The XF T Cell Metabolic Profiling kit contains new reagents optimized to allow for complete measurement of T cell metabolism along with a dedicated analysis tool, Seahorse Analytics. (B) Schematic workflow for running and XF T Cell Metabolic Profiling Kit assay.

BAM15 is an improved uncoupler for T cells and NK cells

Mouse Naïve CD8 T Cells



Human NK cells (HS Mini)

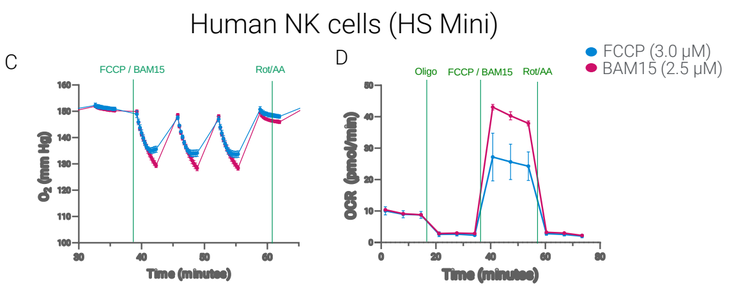
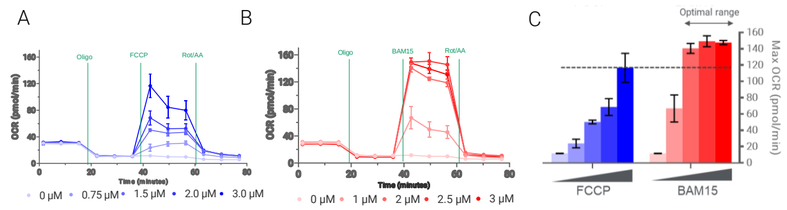


Fig 2: BAM15 addition results in a more robust uncoupling response in T and NK cells compared with FCCP. BAM15 or FCCP were titrated in either mouse naïve CD8 T cells or human NK cells. (A), (C): Changes in extracellular oxygen levels after uncoupler addition, highlighting the more consistent rate during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

Results

BAM15 results in a broader effective range and simplified concentration optimization

Mouse CD8 T cells



Human NK cells (HS Mini)

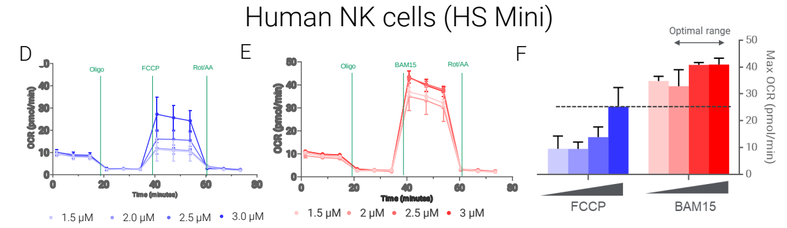


Fig 3. BAM15 at 2.5 µM shows the optimal response in human and mouse T cells and human NK cells, minimizing the requirement of concentration optimization for each cell sample. (A) Kinetic OCR trace of FCCP titration (0 – 3 µM) in mouse CD8 T cells. (B) Kinetic OCR trace of BAM15 titration (0 – 3 µM) in mouse CD8 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 human NK cells (data is overlayed from separate HS mini assays with cells from the same donor (F) Bar graph showing maximum OCR obtained with FCCP or BAM15 titration.

XF T Cell Persistence Assay can be used to evaluate different culture conditions for T cell expansion.

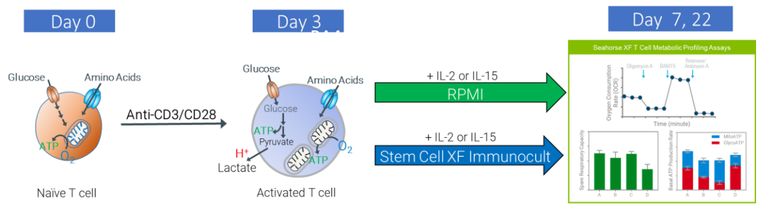


Fig 4 Schematic depicting experimental design to evaluate T cell expansion conditions. Naïve Pan human T cells were activated for 3 days using CD3/CD28 Dynabeads. Cells were then split into two different cell culture media conditions in the presence of IL-2 or IL-15. Seahorse XF T Cell Persistence assays were conducted at days 3, 7, 14, and 22 post-activation in XF RPMI pH 7.4 assay medium and analyzed using the dedicated views on Seahorse Analytics.

Cell culture media formulation and cytokines used during expansion influence T cell metabolic parameters associated with T cell persistence

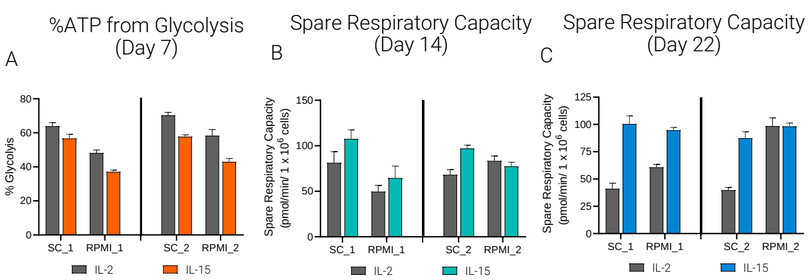
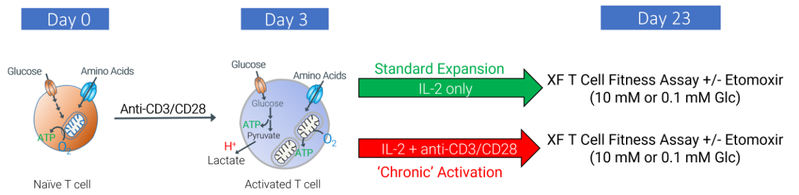


Fig 5: Cell culture medium and cytokine composition can influence T cell bioenergetic profile during expansion. (A) IL-15 expanded T cells show a more aerobic metabolic profile (expressed as reduced % ATP from glycolysis) at day 7 post-expansion across two separate donors and two different media culture compositions. (B) Expansion in media supplemented with IL-15 results in higher spare respiratory capacity at day 14 post activation in ImmunoCult™-XF T Cell Expansion Medium (Stem Cell technologies, SC) across two donors. © At day 22 post activation, a more pronounced increase in spare respiratory capacity in IL-15 expanded cells is observed, particularly in ImmunoCult™-XF T Cell Expansion Medium

Results

XF T Cell Fitness Assay can be used to assess metabolic fitness of T cells under different environmental conditions



'Chronic' stimulation of T cells results in decreased metabolic fitness

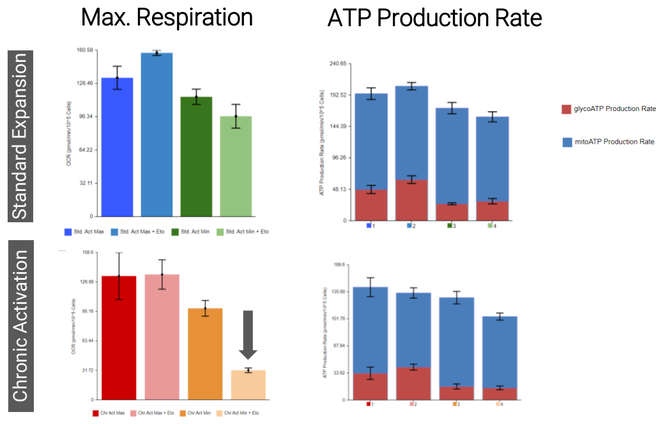


Fig 6: Chronic stimulation of T cells results in decreased metabolic fitness. Pan T cells were activated acutely with CD3/CD28 Dynabeads (standard activation) or under persistent stimulation. After 22 days of expansion cells were assessed for metabolic fitness by interrogating the ability to maintain bioenergetic capacity and maximal mitochondrial capacity under standard assay media conditions (max) or under fuel restricted conditions (min) (XF RPMI + 0.1 mM glucose, without Glutamine , -/+ Etomoxir). Acute injection of etomoxir under minimal supplemented assay media on persistent stimulated T cells results in reduced maximal respiration and basal ATP production.

Methods

Cell Culture

Human Pan T cells and NK cells were obtained from Stem Cell Technologies. For NK cell experiments, cells were thawed and rested overnight in RPMI + 10% FBS + 2 mM Gln. For T cell expansion experiments, Pan T cells were rested overnight in Stem Cell Technologies Immunocult XF medium. On day 1, T cells were activated using CD3/CD28 Dynabeads (Thermo Fisher). After 3 days, Dynabeads were 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. For chronic T cell antigen stimulation experiments, beads were not removed on day 3 and were replenished when cells were split. Mouse CD8 T cells were magnetically purified from splenocytes obtained at Hooke Laboratories and XF assays were run the same day.

Seahorse XF Assays

Expanded human T cells and naïve mouse CD8 T cells were resuspended in Seahorse XF RPMI, pH 7.4 medium, and seeded at 100,000 cells and 200,000 cells per well, respectively in XF96 PDL microplates. For detailed information see XF T Cell Metabolic Profiling Kit user guide at Agilent.com. Human NK cells were resuspended in Seahorse XF RPMI, pH 7.4, and seeded in HS PDL mini plates at 70,000 cells per well.

Conclusions

- BAM15 demonstrates significant benefit over the traditional uncoupler, FCCP, when assessing T cell and NK cell mitochondrial function.
- Medium composition and cytokine formulation during T cell expansion can result in profound differences in basal metabolic poise (expressed as % of ATP production from glycolysis) and mitochondrial bioenergetic capacity (expressed as Spare Respiratory Capacity). The presence of IL-15 promotes an aerobic program previously associated with increased T cell persistence (doi: 10.1158/2326-6066.CIR-18-0466).
- Chronic stimulation of T cells results in impaired metabolic fitness, with a higher dependency on fatty acid oxidation for maintaining aerobic capacity in minimal supplemented assay medium which could be representative of metabolically restricted environments, like the tumor microenvironment.
- The data presented show the impact of cell culture conditions during expansion on the resulting T cell metabolic profile and fate, highlighting the importance of including metabolic profiling to monitor and tune culture conditions during T cell therapy development.