Quality Assessment of Metabolic Fitness during T Cell and NK Cell Expansion

Guoxiao Wang, Xiaoyu Zhang, Pamela Swain and Natalia Romero.

Cell Analysis Division, Agilent Technologies, Lexington, MA, USA

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Introduction

- Cell metabolism strongly influences immune responses and anti-tumor efficacy of cell therapies *in vivo*. Both glycolytic and mitochondrial activity play differential critical roles at various stages of cell differentiation and function.
- *Ex-vivo* expansion of cell therapy products is a common requirement for most adoptive cell therapies. Expansion conditions strongly influence the characteristics of the final cell product and represent an opportunity to metabolically reprogram adoptive cell therapies for increasing *in vivo* anti-tumor potency.
- Integrating Seahorse XF assays with NovoCyte Flow Cytometry immunophenotyping and xCELLigence RTCA functional killing assessment yields robust insights into three critical cell attributes: metabolic fitness, killing potency, and population characterization. Together, they provide unique insights for the development of successful immunotherapies.

Experimental

XF T Cell / NK cell Metabolic Profiling Kit Workflow

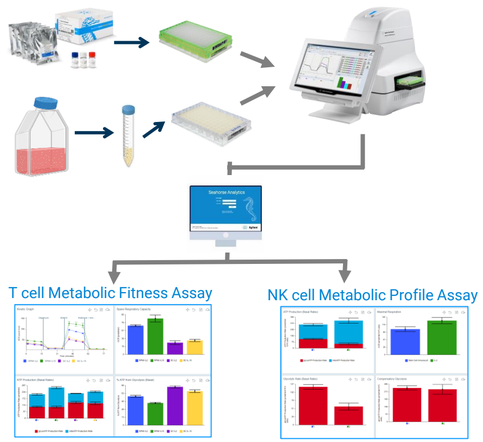

Fig. 1: The XF T Cell Metabolic Profiling kit contains new reagents optimized to provide comprehensive assessment of T cell and NK cell metabolism, including quantification of both glycolytic and mitochondrial pathways[1]. Along with a dedicated analysis tool, Seahorse Analytics, the kit supports dedicated assay workflows for T cells and NK cells that deliver quantitative characterization of basal metabolic poise, glycolytic and mitochondrial ATP production, compensatory glycolysis and robust quantification of the maximal mitochondrial capacity of the cells.

Results and Discussion

BAM15 delivers improved uncoupling performance and a broader effective range.

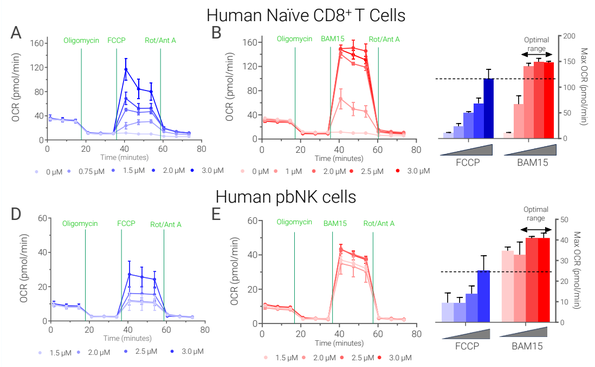

Fig 2. Comparison of BAM15 vs. FCCP uncoupling performance. OCR kinetic trace of FCCP (A, D) or BAM15 (B, E) titration of CD8+ T cells and pb NK cells. Maximum OCR after FCCP or BAM15 injection in T cells (C) and NK cells (F) at different uncoupler concentrations. In all cases, Oligomycin and Rotenone + Antimycin A concentrations used were 1.5 µM and 0.5 µM each, respectively. BAM15 at 2.5 µM shows the optimal response in naïve T cells and NK cells. Representative data of at least n=3 experiments for each cell type from different donors.

Composition of cell culture media during T cell expansion influences T cell metabolic profile

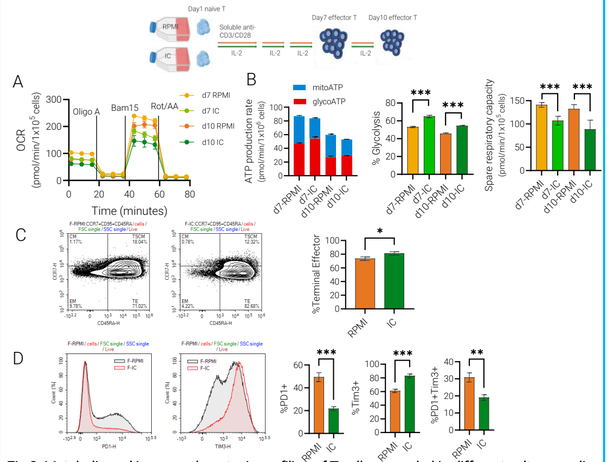

Fig 3. Metabolic and immunophenotypic profiling of T cells expanded in different culture media. Human panT cells were thawed in complete RPMI-1640 (RPMI) media or ImmunoCult™-XF T Cell Expansion Medium (Stem Cell Tech.) (IC) in T75 flasks. The next day, cells were activated with soluble anti-CD3/CD28 in respective media, and media were refreshed every two days. XF T cell persistence assay were performed on day7 and day10 using XF Pro analyzer. (A) OCR kinetic trace and (B) Seahorse analytics outputs for d7 and d10T. (C,D) Immunophenotyping of d10T cell using NovoCyte Advanteon. Data represents mean ± SEM of three independent expansions from the same donor. *p<0.05, **P<0.01, ***p<0.001. Data was normalized using NovoCyte Cell Count Protocol .[2]

Results and Discussion

NK cells expanded *in vitro* and after stimulation with IL12/15/18 showed increased metabolic fitness

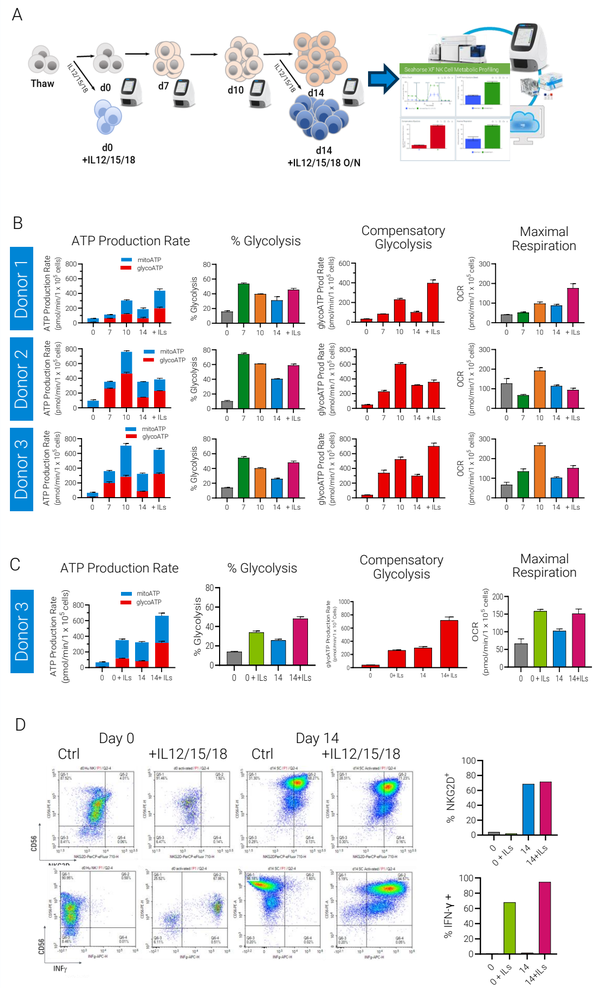

Fig 4: Evaluation of metabolic changes of NK cells during in vitro expansion using the XF NK cell Metabolic Profile assay. (A) Experimental design to evaluate Hu pbNK cells during *in vitro* expansion using ImmunoCult NK expansion kit (StemCell Technologies). On days -1 pre-expansion and day 13 post-expansion, a subset of cells were stimulated overnight with IL12/15/18 (ILs) (BioLegend) at a concentration of 10/20/100 ng/mL, respectively. (B, C) XF NK Metabolic Profile Assay was performed using the XF T cell Metabolic Profiling kit on the XF HS mini analyzer at the indicated time points. Data were analyzed using Seahorse Analytics (SHA). (D) Flow Analysis of NK cells was performed using the NovoCyte Advanteon before and after stimulation with IL12/15/18 before and after expansion, showing the increase in the expression of NKG2D and intracellular IFN-γ.

Results and Discussion

Development of an integrated workflow for in-process analytics of CART cells during manufacturing

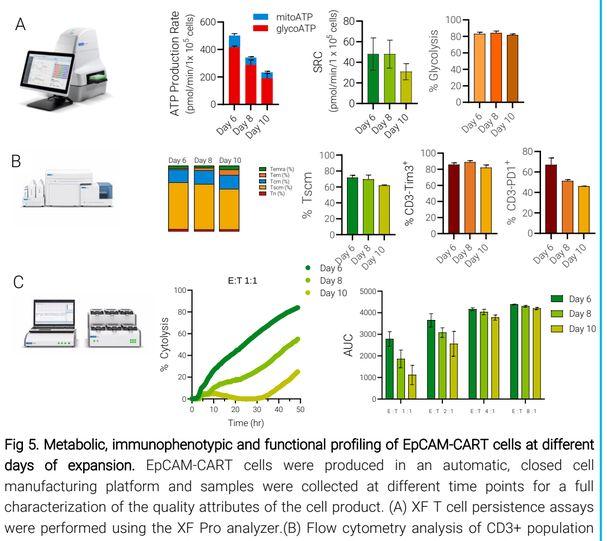

Fig 5. Metabolic, immunophenotypic and functional profiling of EpCAM-CART cells at different days of expansion. EpCAM-CART cells were produced in an automatic, closed cell manufacturing platform and samples were collected at different time points for a full characterization of the quality attributes of the cell product. (A) XF T cell persistence assays were performed using the XF Pro analyzer.(B) Flow cytometry analysis of CD3+ population was performed using the Agilent NovoCyte Quanteon Flow Cytometer. (C) The killing potency of the samples was performed using an impedance-based RTCA xCELLigence system[3]. Data represents mean±SEM of three independent expansions from the same donor.

Conclusions

- BAM15 demonstrates significant benefit over the traditional uncoupler FCCP, when assessing T cell and NK cell metabolism.
- The XF T Cell Metabolic Profiling kit combined with the new outputs delivered in Seahorse Analytics (ATP Productions Rate, % Glycolysis, Compensatory glycolysis) may offer unique early insights into metabolic fitness to monitor and tune cell expansion conditions during T cell and NK cell therapy development.
- The data presented highlights the importance of including metabolic profiling, combined with functional real time killing activity and immunophenotyping for a comprehensive assessment of critical quality attributes of cellular therapies.

References

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