Application Note

Cell Analysis

Assessing NK Cell Bioenergetic Metabolism Using Extracellular Flux Analysis

Authors

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Abstract

Metabolism has emerged as a key driver of immune cell fate and function. NK cell function requires both glycolysis and OXPHOS for NK cell activation, proliferation, and effector functions. In addition, recent studies have highlighted the potential benefits of using engineered NK cells (CAR-NK) for cancer treatments and promising preliminary data have been demonstrated in early-phase clinical studies.

Modulating NK cell metabolism is a potential strategy to improve the efficacy of NK cell-based immunotherapy for cancer. The Agilent Seahorse XF NK cell metabolic profiling assay provides a complete assessment of NK cell metabolism in real time using the Agilent Seahorse XF analyzer and the Agilent Seahorse XF T Cell Metabolic Profiling kit. It allows for the simultaneous measurement of glycolytic and mitochondrial activity, and bioenergetic capacity in NK cell populations. The XF T Cell Metabolic Profiling kit uses an improved uncoupler (BAM15) for more consistent and accurate measurements of NK cell mitochondrial bioenergetic capacity, with minimal reagent optimization. This application note outlines the use of this assay to monitor and profile NK cell metabolism during cell expansion and after cytokine stimulation. It also demonstrates the potential use when designing and developing processes for the optimal production of NK cell therapy products.

Introduction

Natural killer (NK) cells are critical components of the innate immune response. They recognize specific antigenic peptides on the cell surface and bind ligands expressed on cells infected with viruses or bacteria, or transformed by oncogenes. They also play a key role in antiviral responses and supporting antitumoral activity. It was recently recognized that NK cells can contribute to shaping T cell responses, operating as part of the adaptive response. In addition, recent studies have highlighted the potential benefits of using engineered NK cells (CAR-NK) for cancer treatment, and promising preliminary data have been demonstrated in early-phase clinical studies.

Independently of the NK cell source (cord blood, iPSC, peripheral blood), the preparation of engineered NK populations requires extensive ex vivo expansion before patient infusion.[2] It is widely described that freshly isolated NK cells have low cytolytic capacity. Different expansion systems, including culture devices, media and cytokine composition, and coculture with feeder cell lines, can be used for NK cell expansion, resulting in cell products with differential attributes and in vivo efficacy. In addition, "priming" NK cells ex vivo by incubation with different cytokine combinations, such as IL-12, IL-15, and IL-18, induces a phenotypic change referred to as cytokine-induced memory-like NK cells. These "memory-like" NK cells showed increased responses in vivo against tumors.[3]

As has widely been demonstrated for other immune cells such as T cells, cellular metabolism plays a significant role in supporting and regulating NK cell function. Defects in metabolism can contribute to the generation of dysfunctional NK cells.[1] Expansion conditions can be optimized to increase the metabolic fitness of NK cells resulting in enhanced antitumor function in vivo.

This application note demonstrates the utility of the Agilent XF NK cell metabolic profile assay in enabling the simultaneous acquisition of robust measurements of glycolytic and mitochondrial activity, and bioenergetic capacity in NK cell populations. These measurements provide a complete characterization of the NK cell metabolic profile. This is done using a single assay that can monitor and optimize the metabolic fitness of expanded NK cell populations and select conditions that enhance the antitumor function of NK cell therapy products.

Experimental

Mice NK cell isolation

Mice NK cells were isolated from five fresh spleens of nine-week-old nonimmunized C57BL/6 mice (custom part number CF-1201; Hooke Laboratories, LLC; Lawrence, MA) using the "EasySep Mouse NK Cell Isolation Kit", according to the manufacturer's protocol (part number 19855; STEMCELL Technologies, Vancouver, Canada). The quality of the isolated mouse NK cells was confirmed by staining them with cell surface markers to define the percentage of the CD3-CD49+ and CD45+ population. Cells were stained using vendor-recommended concentrations of antibodies. TruStain FcX PLUS (antimouse CD16/32) antibody (part number 156603; BioLegend, San Diego, CA) was prepared in FACS buffer (DPBS, 5 % FBS, 0.1 % NaN_3) and incubated for 10 minutes on ice. A cocktail of the surface marker antibodies was added, incubated on ice for 20 minutes, and washed in FACS buffer three times. After the final wash, the cells were resuspended in 100 ng/mL DAPI and analyzed with the Agilent NovoCyte Advanteon flow cytometer, using the Agilent NovoExpress software. The following BioLegend antibodies were used: antimouse CD3-FITC (clone 17A2) (part number 100204), antimouse CD45-PE antibody (part number 103105), and antimouse CD49b-APC (Clone DX5) (part number 108909).

Human NK cell culture and expansion

Human peripheral blood NK cells (part number 70036; STEMCELL Technologies, Vancouver, Canada) were thawed following the manufacturer's instructions, and centrifuged at 300 × g for 10 minutes. They were then resuspended in a ImmunoCult NK Cell Base Medium (part number 100-0712; STEMCELL Technologies) at 1×10^6 cells/mL and recovered in a 25 mL culture flask overnight at 37 °C in 5% CO_2. The cells were used for assays on day 0 or expanded following the manufacturer's protocol for the ImmunoCult NK Expansion Kit (part number 100-0711; STEMCELL Technologies).

Briefly, nontissue culture-treated 12-well plates were coated with ImmunoCult NK Cell Expansion Coating Material for 2 hours (part number 100-0714; STEMCELL Technologies). On day 0, 1×10^6 NK cells resuspended in 1 mL of ImmunoCult NK Cell Expansion Medium (part number 100-0715; STEMCELL Technologies) were plated into the coated 12-well plates. On day 3, cells were fed by adding 1 mL of fresh Expansion Medium per well, and on days 7 and 10, NK cells were replated into freshly coated plates, 2 mL per well at 0.2×10^6 cells/mL. NK cells were expanded for a total of 14 days.

Human NK cell cytokine overnight stimulation

Human NK cells were incubated with a cocktail of cytokines, including rhIL-12 at 10 ng/mL (part number 78027; STEMCELL Technologies), rhIL-15 at 20 ng/mL (part number 78031; STEMCELL Technologies), and rhIL-18 at 100 ng/mL (part number 592104; BioLegend, San Diego, CA). These were prepared in ImmunoCult NK Cell Base media overnight at 37 °C in 5% CO_2.

Agilent Seahorse XF assays

Agilent Seahorse XF HS PDL cell culture miniplates (part number 103727-100) were warmed overnight in a 37 °C non-CO_2 incubator. Agilent Seahorse XFp sensor cartridges were hydrated and placed in a 37 °C non-CO_2 incubator overnight. On the day of the XF assay, NK cell total and viable cell numbers were determined using an Agilent NovoCyte Advanteon flow cytometer.[4] The Agilent Seahorse XF assay medium was prepared using Agilent XF RPMI pH 7.4 media (part number 103576-100) supplemented with 10 mM glucose (Agilent part number 103577-100), 2 mM glutamine (Agilent part number 103579-100), and 1 mM pyruvate (Agilent part number 103578-100), and prewarmed to 37 °C. Cell suspensions were centrifuged at 300 × g for 10 minutes in 15 mL conical tubes. The cell pellet was resuspended in the XF assay medium at 2.3×10^6 (day 0) or 1.2×10^6 (days 7 to 14) viable cells per mL. Cell concentration for data normalization was confirmed using the NovoCyte flow cytometer.[5] A volume of 30 µL of the appropriate cell suspensions were plated in the prewarmed XF HS PDL miniplates (Agilent part number 103727-100), resulting in 7.0×10^4 (day 0) or 3.5×10^4 cells (days 7 to 14) per well. After plating, the Agilent Seahorse HS miniplates were centrifuged to 300 × g for 2 minutes. The silicon mask was removed, and the well volume was adjusted to 200 µL for the final volume. Microplates were incubated at 37 °C in a non-CO_2 incubator for 45 to 60 minutes. The Agilent XF T Cell Metabolic Profiling kit (part number 103771-100) and the FCCP from the Agilent Seahorse XF Cell Mito Stress Test (part number 103015-100) reagents were resuspended in prewarmed XF assay media. To evaluate the uncoupler performance, BAM15 and FCCP were resuspended at 30 µM and diluted to 25 µM, 20 µM, and 15 µM to prepare 10x port stocks. For evaluation of the metabolic profile over several days, the concentration of BAM15 used was 2.5 µM (final volume) for all time points.[6]

Data analysis of the XF NK cell metabolic profiling assay

Agilent Seahorse Analytics (SHA), a web-based software platform, was used to analyze the XF results files. Within SHA, the dedicated analysis view for the XF NK Cell Metabolic Profile automatically calculates the key metabolic parameters when using the XF T Cell Metabolic Profiling kit reagents with NK cells (Figure 1).

Flow cytometry analysis

Human NK cells were incubated with BD Golgi Plug reagent (containing Brefeldin A) for 4 hours at 37 °C in 5% CO_2, following the BD Cytofix/Cytoperm Plus protocol (part number 555028; BD Biosciences, San Jose, CA). NK cells were washed in DPBS (Gibco, Billings, MT), stained for dead cells using the Zombie Aqua Fixable Viability kit (part number 423101; BioLegend, San Diego, CA) at 1/1000 dilution for 15 minutes at room temperature, and washed in FACS buffer. TrueStain FcX (part number 422302; BioLegend) was added to block Fc-receptors and incubated for 10 minutes on ice. A cocktail of anti-CD3, anti-CD56, and anti-NKG2D was added, along with corresponding FMOs, and incubated on ice for 20 minutes. Following three wash steps with FACS buffer, the cells were fixed and permeabilized with BD Cytofix/Cytoperm reagent for 20 minutes on ice and washed with BD Perm/Wash buffer. Fixed and permeabilized cells were stained with antihuman IFN-γ for 30 minutes on ice and washed with BD Perm/Wash buffer. All samples were analyzed with the Agilent NovoCyte Advanteon flow cytometer using the Agilent NovoExpress software. The following antibodies were used: antihuman CD3- FITC Antibody (part number 344804; BioLegend), antihuman CD56- PE (NCAM) (part number 318306; BioLegend), CD314 (NKG2D) Monoclonal Antibody PerCP-eFluor 710 (part number 46-5878-42; Thermo Fisher Scientific, Carlsbad, CA), and antihuman IFN-γ Antibody (part number 502512; BioLegend).

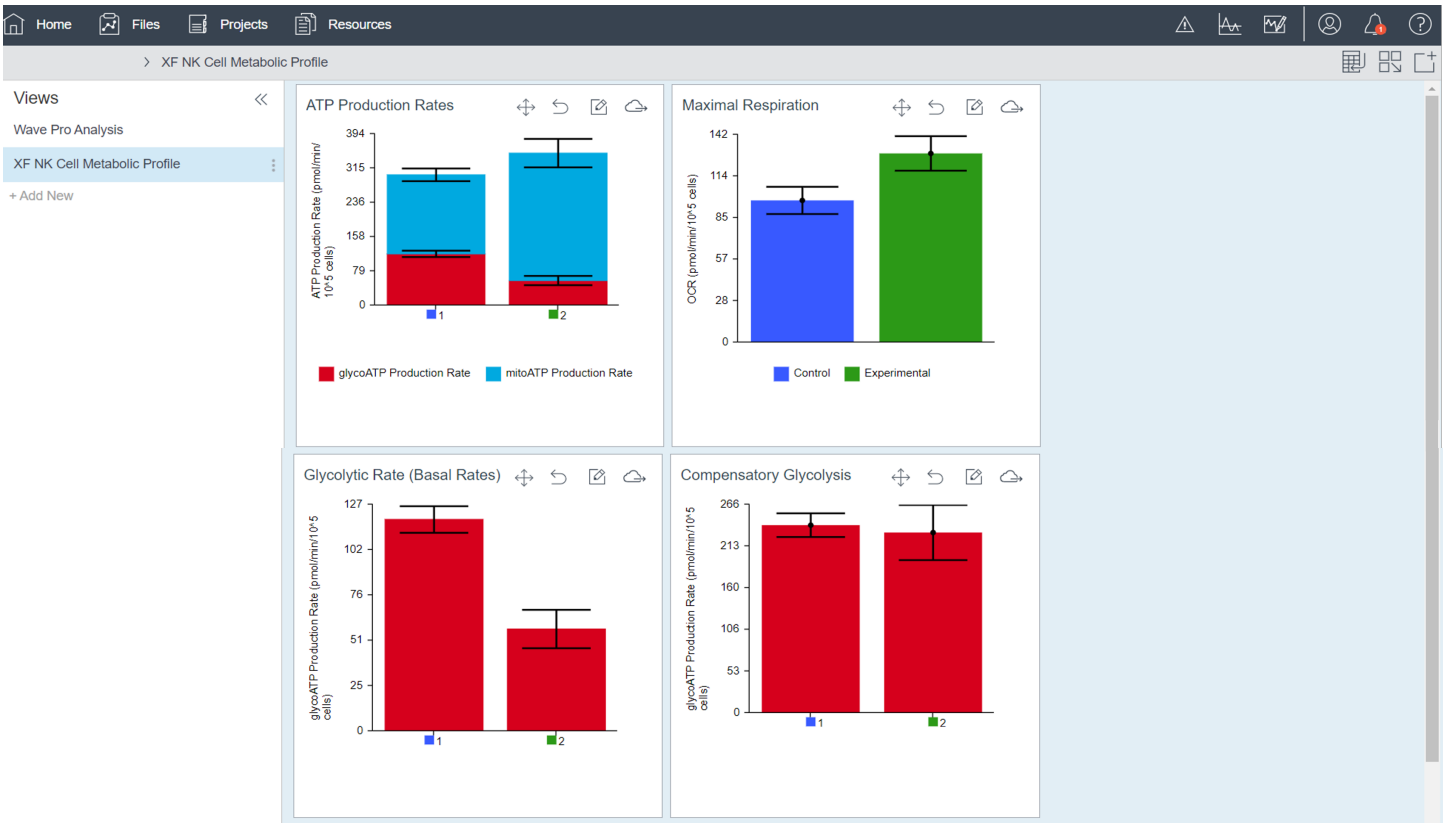

Figure 1. Screenshot of Agilent Seahorse Analytics software dedicated analysis view for NK Cell Metabolic Profile assay.

Results and discussion

Evaluation of BAM15 uncoupler activity in NK cells

The use of mitochondrial uncouplers is a frequent practice when evaluating the mitochondrial function of live cells. Using the Agilent Seahorse Cell Mito Stress Test, a kit containing FCCP (carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone) as uncoupler reagent, combined with Agilent Seahorse XF analyzers, has provided the foundational knowledge about immune cell energy metabolism and its role in directing cell fate and function.[7,8]

Recently, we demonstrated that FCCP does not provide optimal mitochondrial uncoupling performance when used in human or mouse T cells. This results in the underestimation of maximal respiratory capacity.

However, the XF T cell Metabolic Profile kit, containing the uncoupler BAM15 ((2-fluorophenyl){6-[(2-fluorophenyl)amino] (1,2,5-oxadiazolo[3,4-e]pyrazin-5-yl)}amine) delivers a more accurate and precise determination of maximal respiration. It offers a more robust solution for assessing mitochondrial bioenergetic capacity in T cells.[9]

Hence, we evaluated the performance of the XF T Cell Metabolic Profiling kit reagents containing the uncoupler BAM15, in human and mouse NK cells, and compared them with the metabolic parameters obtained when using the uncoupler FCCP, present in the Seahorse XF Cell Mito Stress Test kit.

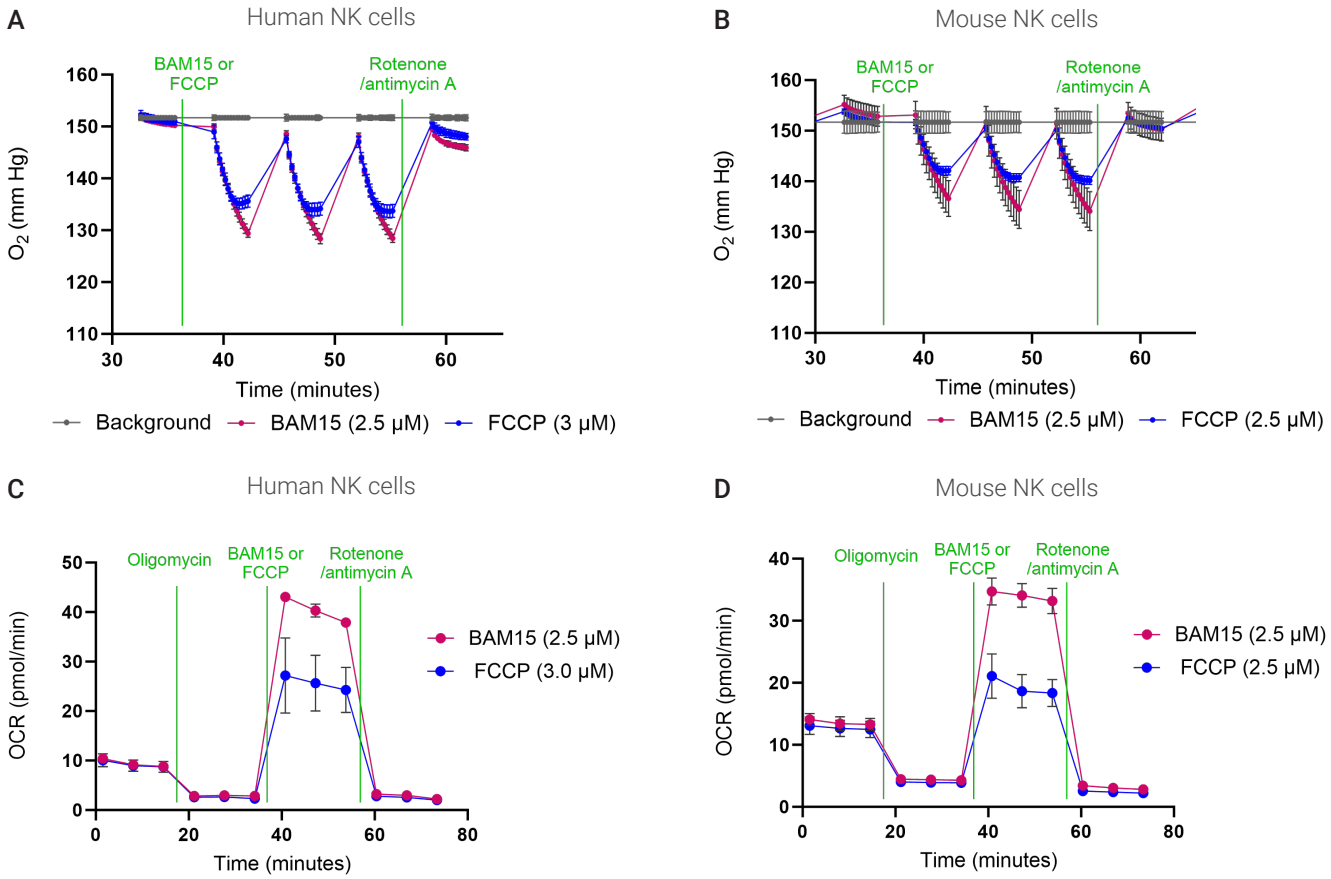

Figure 2. Comparison of oxygen consumption measurements using uncouplers FCCP and BAM15. Changes in extracellular oxygen level are shown in human NK from (A) PBMC and (B) mouse NK cells from the spleen of nonimmunized C57BL/6 after the addition of optimal FCCP (blue) and BAM15 (red) uncoupling concentrations. Oxygen consumption rate (OCR) obtained from the Mito Stress Test (blue line) or NK Cell Metabolic Profiling assay using T cell Metabolic Profiling kit (red line) is illustrated in (C) human NK and (D) mouse NK cells.

As shown in Figure 2A to B (blue lines), when human or mouse NK cells are tested with the XF Cell Mito Stress Test kit, the rates of change in extracellular O_2 levels during the 3 minutes of instrument measurement after FCCP injection are not consistent or linear. This results in variable OCR calculations and underestimation of maximal respiration (Figures 2C and 2D, blue lines). However, when the uncoupler BAM15 is used instead of FCCP, a stable decrease in the extracellular O_2 level is obtained after BAM15 injection during the 3 minutes of instrument measurement (Figures 2A and 2B, red lines), resulting in a more accurate and precise determination of OCR and maximal respiration (Figures 2C and 2D, red lines).

To confirm the improved performance of BAM15 as a mitochondrial uncoupler compared to FCCP in NK cell's metabolic profiling, we performed side-by-side uncoupler titrations using human and mouse NK cells from at least three different donors or mice isolations. As shown in Figure 3, we consistently obtained higher maximal respiration and smaller standard deviations when BAM15 was injected as the uncoupler, compared to those obtained at the optimal FCCP concentrations. Titration experiments also demonstrated that the range of optimal BAM15 concentration is wider than the optimal FCCP range. In all cases, the maximal OCR obtained when using 2.5 µM BAM15 was at least 90% of the maximal OCR obtained at any uncoupler concentration for the same donor, and consistently higher than the value obtained with the optimal FCCP concentration. This demonstrates that the BAM15 reagent uncoupler performance is superior to FCCP when used with NK cells (Figure 4). It also suggests that there is minimal need to optimize uncoupler concentrations for each sample when BAM15 is used.

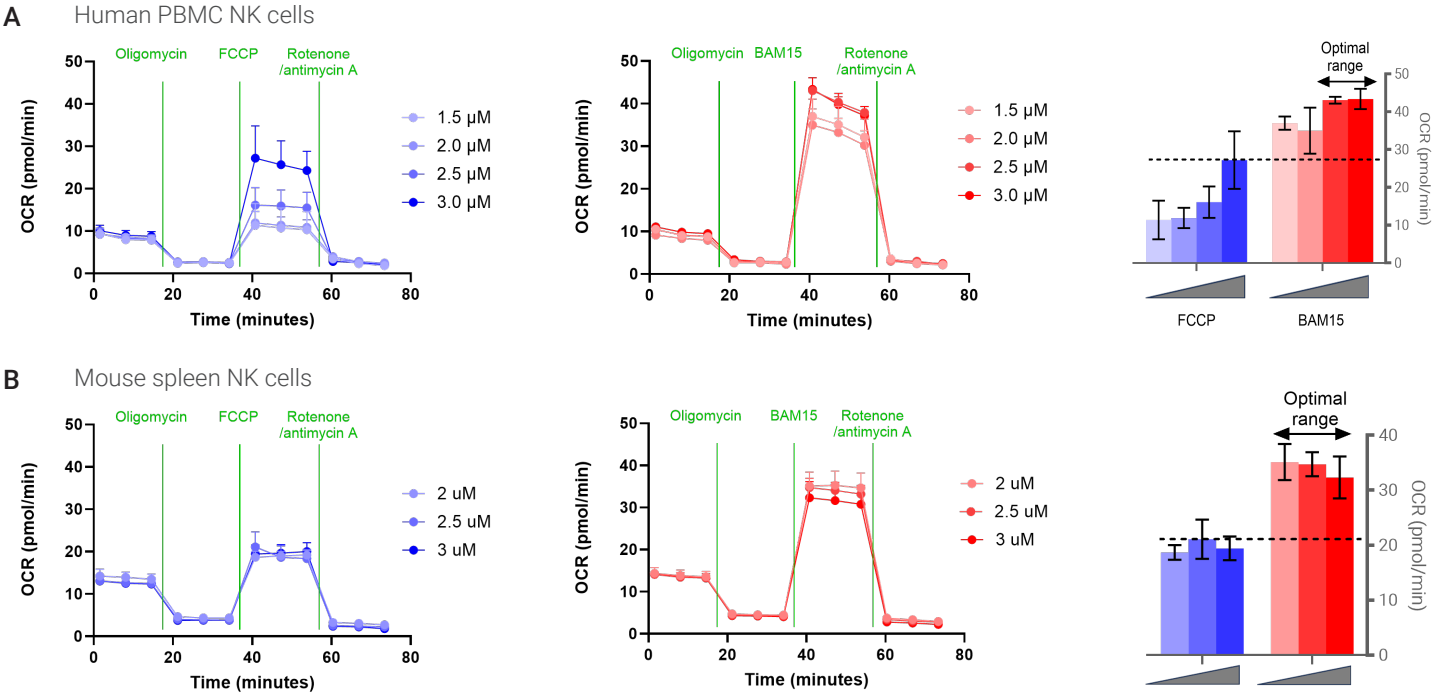

Figure 3. Comparison of maximal OCR measurements from FCCP (blue) and BAM15 (red) titration experiments. The figure shows representative data of OCR kinetic traces and maximal OCR obtained at the different uncoupler concentrations using (A) human pbNK cells and (B) isolated NK cells from the spleen of nonimmunized C57BL/6 mice. The dotted line in the bar graph indicates the maximal OCR obtained with FCCP at any of the concentrations tested.

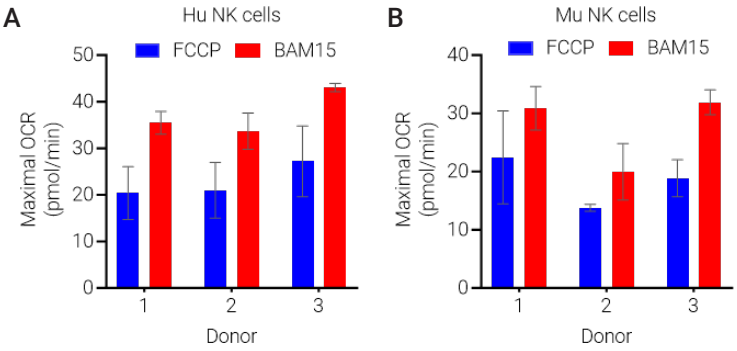

Figure 4. Comparison of the maximal OCR obtained at the optimal FCCP concentration vs the OCR obtained at 2.5 µM of BAM15 in (A) human and (B) mouse NK cells. Data represent the mean ± SD of three replicate wells.

Application of XF NK Cell Metabolic Profiling assay for monitoring changes in metabolic signature during NK cell expansion

Glycolysis and OXPHOS play critical roles in several aspects of NK cell function. Both metabolic pathways differentially support degranulation, Fas ligand (FasL) expression, and interferon-gamma production, impacting NK cell cytotoxicity. For example, previous studies have suggested that glycolysis is critical for NK cell cytotoxicity, degranulation, and Fas ligand expression, while OXPHOS may have a more predominant role in supporting NK cell production of interferon-gamma.[10] Moreover, glycolysis and OXPHOS can influence each other and cooperate to support NK cell function. For instance, glycolysis can provide intermediates for the citrate-malate shuttle (CMS), which transfers reducing equivalents from the cytosol to mitochondria to fuel OXPHOS.[11]

Due to the essential role of both bioenergetic pathways in supporting NK cell activation, proliferation, and effector functions, modulation of NK cell metabolism could be a potential strategy to improve the potency of NK cell-based immunotherapy for cancer. Early work has shown that basal energy production, glycolytic capacity, and overall metabolic poise are critical parameters to modulate if seeking to improve the effectiveness of NK cell adoptive transfer approaches for the treatment of cancer.[10] The ability to monitor the phenotypes of cells and correlate phenotype to function and outcomes is critical for enhancing NK cell fitness and antitumor function.

The development of optimized in-vitro expansion protocols requires the ability to increase cell yields while also supporting NK cell function and avoid exhaustion states. Recent studies showed that metabolic reprogramming of NK cells during expansion leads to the development of NK cells with metabolic flexibility and sustained tumor killing in the nutrient-deprived tumor microenvironment.[12] The presence of different combinations of cytokines during expansion have shown to affect NK cell metabolism, while improving NK cell fitness. For example, stimulation of human NK cells with IL-2 or IL-12/15 upregulates both glycolysis and OXPHOS, and elevated levels of OXPHOS were essential for NK cell effector cytotoxicity and IFN-γ production.[10] IL-12/15/18-preactivated NK cells, also known as cytokine-induced memory-like (CIML) NK cells, have been revealed as a powerful tool in cancer immunotherapy due to their persistence in the host. They also have increased effector functions and metabolic shifts towards glycolysis.[13] Another approach for NK cell-derived cancer therapies is the use of patient-derived PBMCs to generate large-scale expansion of autologous NK cells by stimulating with IL-2/15/18. These cells showed prolonged persistence, high cytotoxicity against K562 cells, and high levels of activating molecules, such as CD16 and NKG2D.[14] IL-15 exposure alone has been shown to improve metabolic fitness, including the NK cells' ability to use nutrients. Sequentially, using feeder cells expressing IL-21, followed by feeder cells expressing IL-15, supports higher functionality and in vitro tumor control.[15] Specific agents (for example, different cytokines as well as modifications or combinations) during ex vivo expansion may offer a safe yet powerful approach to creating NK cell populations with increased resistance to the adverse metabolic and immunological conditions of the tumor microenvironment (TME), such as cytokine-induced memory-like NK cells that can result in improved efficacy.[16] However, continuous ex vivo stimulation renders NK cells cytokine-addicted and leads to decreased persistence when these cells are infused in the absence of in vivo cytokine support.[17] In addition, cytokine overstimulation of NK cells can compromise NK cell function, inducing metabolic exhaustion.[2] Monitoring NK cell metabolic profiling during expansion is critical to optimize NK cell fitness and may contribute to predicting their functional fate.

The XF NK Cell Metabolic Profiling assay delivers a complete characterization of the metabolic profile of NK cells, including the rates of glycolytic and mitochondrial adenosine triphosphate (ATP) production, metabolic poise, compensatory glycolysis, and maximal mitochondrial bioenergetic capacity, all parameters associated with increased NK cell function.

The XF NK Cell Metabolic Profiling assay uses reagents previously validated for NK cell metabolic interrogation and data analysis tools (Agilent Seahorse Analytics software) that rapidly deliver the relevant parameters for NK cell metabolic characterization.

Here, the XF T Cell Metabolic Profiling kit, including NK analytics, was used to monitor the metabolic changes of NK cells over days of expansion. For this study, Hu pbNK cells from three different donors were expanded using a commercially available expansion kit (STEMCELL Technologies). The NK cell expansion yielded a 10-, 100-, or 16-fold increase in cells for donors 1 to 3, respectively, with an average viability of 70 to 80 % over 14 days of expansion. Samples of the expanded NK cells were collected at days 0, 7, 10, and 14, and the metabolic profile was analyzed using the XF NK Cell Metabolic Profiling assay.

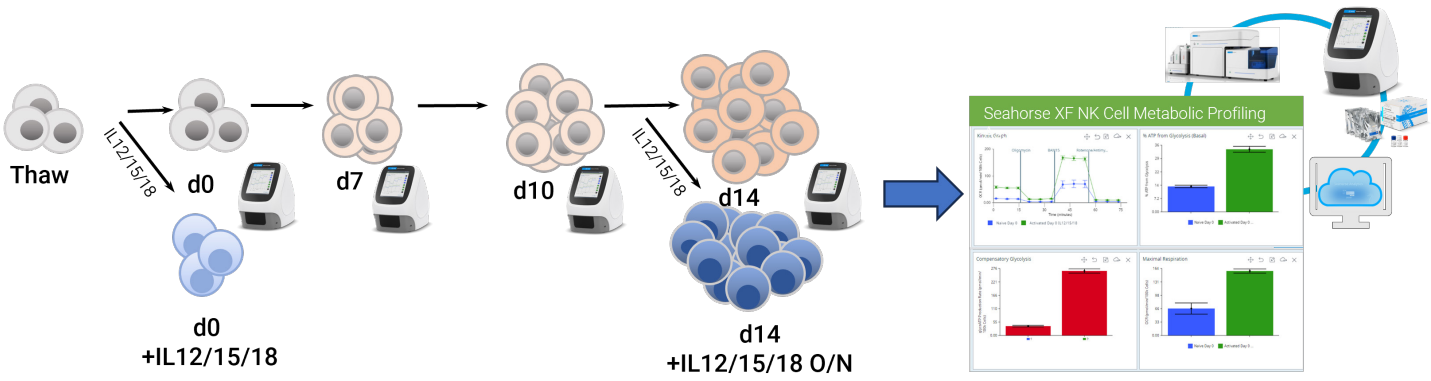

Figure 5. Schematic depicting an experimental design to evaluate human peripheral blood NK cells during expansion, using the ImmunoCult NK expansion kit (STEMCELL Technologies). An XF NK metabolic profiling assay was performed using the Agilent Seahorse XF T Cell Metabolic Profiling kit on the Agilent Seahorse XF HS Mini analyzer, with the HS Mini cell culture plate. Data were analyzed using the Agilent Seahorse Analytics (SHA) software, using dedicated analysis views for the NK Cell Metabolic profile. Flow analysis of NK cells was performed using the Agilent NovoCyte Advanteon flow cytometer before and after stimulation with IL12/15/18 before and after NK cell expansion.

The study found that the ATP production rate of NK cells increases with expansion, accompanied by a shift in the metabolic poise. NK cells at day 0 have low metabolic demand, measured as the rate of ATP production, and an oxidative metabolic poise with over 80% of ATP produced through the mitochondrial OXPHOS pathway (Figure 6A). During expansion, the activity of both bioenergetic pathways increases, but there is a more pronounced increase in the glycolytic activity, representing 50 to 70% of the total ATP production, vs 10 to 15% observed at day 0 (Figure 6B). At day 10, the cells reach the highest basal metabolic demand and the mitochondrial ATP production rate significantly increases to meet this demand. By day 14, the basal metabolic demand is reduced again with 30 to 40% of ATP production coming from the glycolytic pathway. Both glycolysis and OXPHOS activity are increased during the expansion of NK cells, but with a switch in the metabolic profile from a highly aerobic (OXPHOS-dependent) phenotype to a more glycolytic phenotype.

Overnight stimulation of NK cells with cytokines IL12/15/18 at day 14 resulted in a consistent increase in basal ATP from glycolysis in all donors (Figure 6). Although the absolute metabolic rates varied between the different donors, there are consistent trends in metabolic changes during cell expansion.

Another parameter delivered from the metabolic profiling of NK cell is compensatory glycolysis, which represents the ability of the cells to increase glycolytic ATP production when mitochondrial activity is inhibited. This parameter has been previously correlated with the increased metabolic potential of NK cells.[16] Donors 1 and 3 had increases in compensatory glycolysis and maximal respiration, but not donor 2 (Figures 6C and 6D).

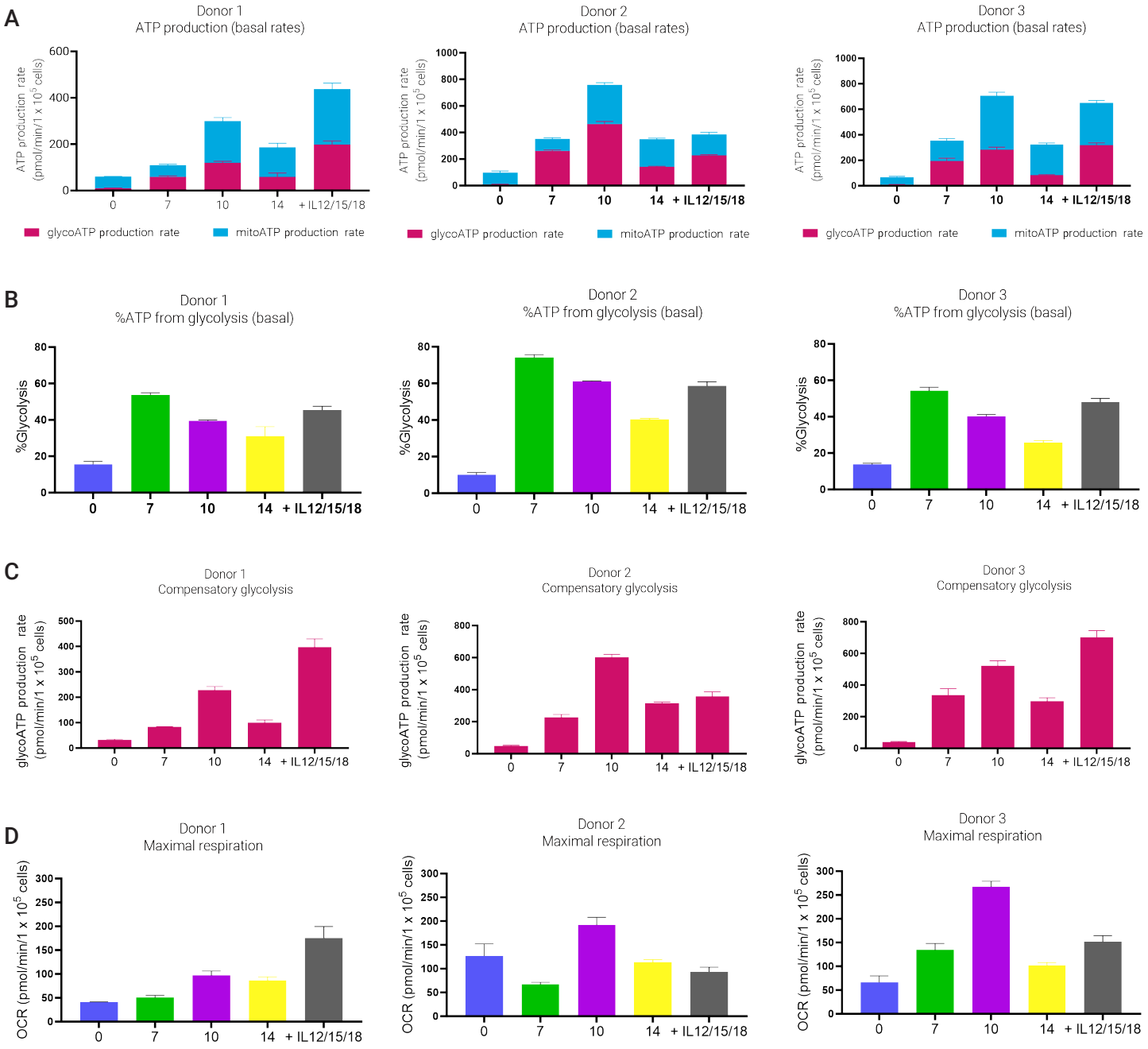

Figure 6. Metabolic measurements over days of expansion and subsequent activation of human NK cells from three different donors. (A) ATP production rate, (B) %ATP from glycolysis, (C) compensatory glycolysis, and (D) maximal respiration rate.

The level of response from NK cells to activation varied with the donor, indicating differences in maturation and fitness. If we compare individual donors at day 0 (pre-expansion) and day 14 (postexpansion), there is higher metabolic demand in the expanded cells, with higher rates of both glycolysis and mitochondrial ATP, as mentioned in Figure 7.

In addition, for both expanded and nonexpanded cell populations (days 0 and 14), overnight stimulation with IL-12/15/18 resulted in an increase in the percentage of ATP from glycolysis. The overall metabolic capacity of NK cells, measured as compensatory glycolysis and maximal respiration, also increased. However, expanded cells reached a higher metabolic activity, with higher compensatory glycolysis and maximal respiration. This illustrates the increased metabolic fitness of these cells, with higher potential to meet extra metabolic demands.

Since the NKG2D-NKG2D ligand axis represents a critical activating pathway for human NK cell-mediated recognition of tumor cells and virus-infected cells[18], we examined the expression of the activation receptor in the different cell populations. At day 14 of expansion, 68% of NK cells express the NKG2D activation receptor vs only 4% at day 0, indicating that cells are primed during the expansion (Figure 8A).

A short, 18-hour overnight stimulation with IL12/15/18 did not change the level of this receptor. Both the pre- and postexpansion of NK cells were able to produce INF-γ in response to IL12/15/18 stimulation. The percentage of cells expressing INF-γ increased in the IL12/15/18-treated NK cells, from 0.6 to 67% during pre-expansion and 1.6 to 94% in postexpansion (Figure 8B).

In summary, postexpansion cells have a higher basal metabolic rate, and a more glycolytic phenotype. They also have increased metabolic capacity, measured by higher maximal respiration, and compensatory glycolysis, indicating higher bioenergetic reserves to meet energy demand under stressed conditions. In addition, these expanded NK cells show a higher expression of activation receptors. When stimulated with cytokines overnight, both expanded and nonexpanded cells showed further increased metabolic rate compensatory glycolysis, together with an increase in INF-γ production. The results obtained are consistent with previous studies showing that expansion protocols can lead to NK cells that have increased metabolic fitness, increased cytotoxicity, upregulated activation receptors, and increases in glucose and other nutrient transporters.[12–16]

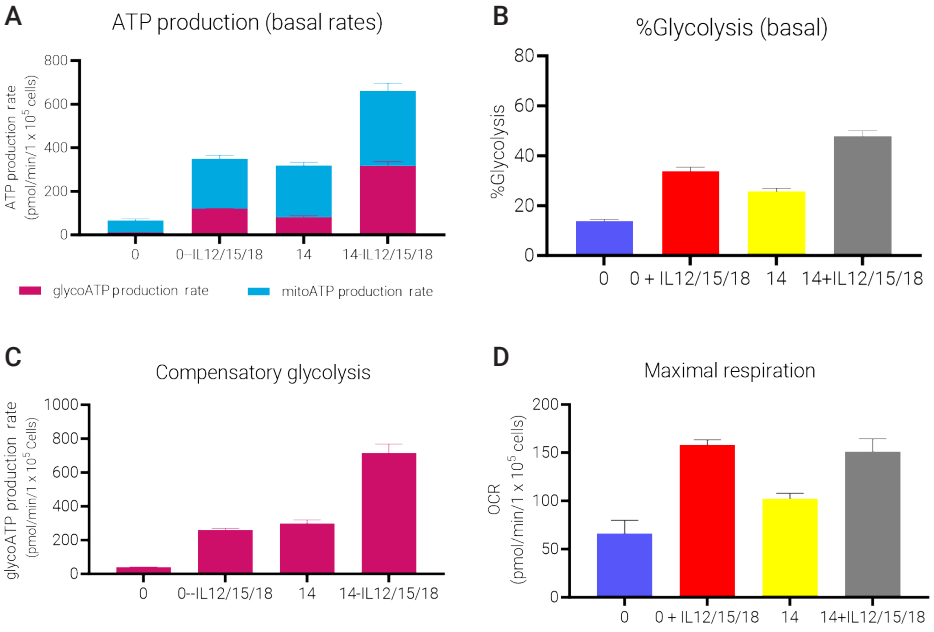

Figure 7. NK metabolic profile of donor 3 on day 0, day 0 post-activation with IL12/15/18, day 14 of expansion, and day 14 post-activation with IL12/15/18. (A) ATP production rate, (B) %ATP from glycolysis, (C) compensatory glycolysis, and (D) maximal respiration rate.

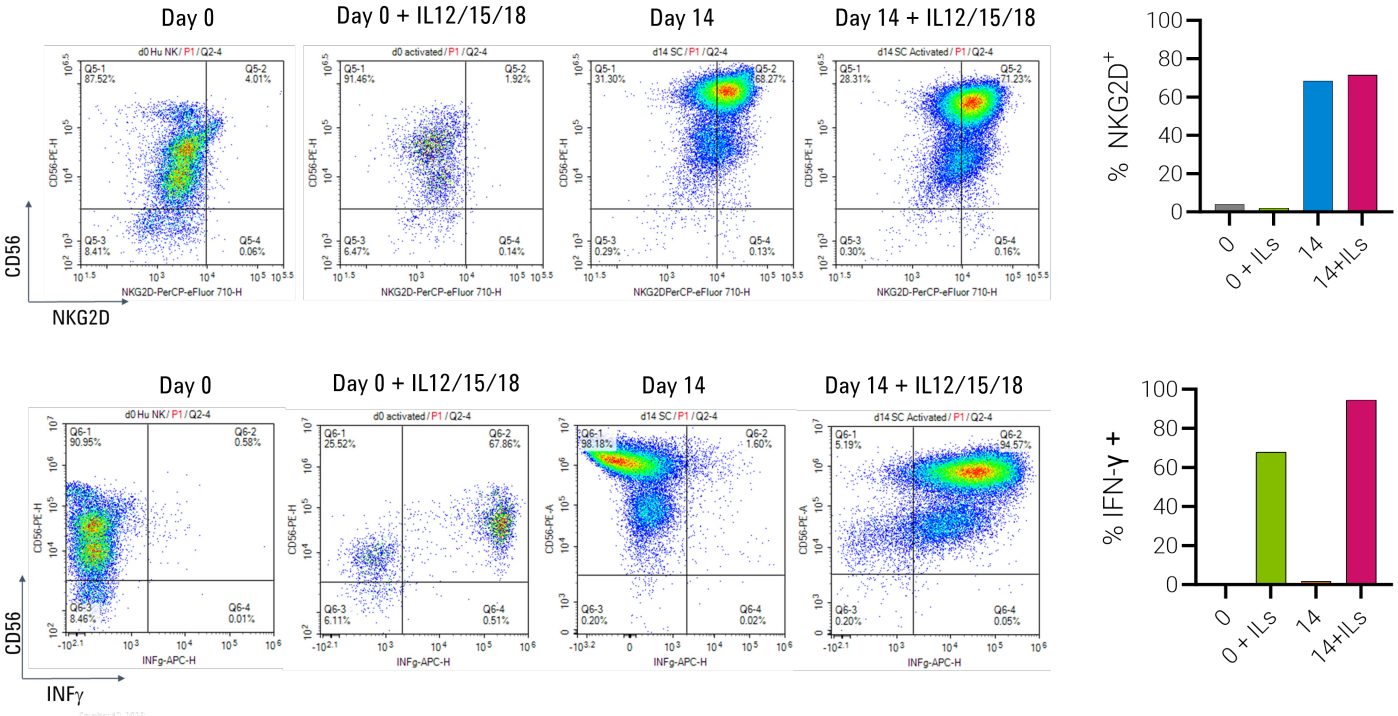

Figure 8. Flow cytometry analysis of NK CD3-CD56+ cells on day 0, day 0 post-activation with IL12/15/18 (ILs), day 14 of expansion, and day 14 postactivation with IL12/15/18. Percent cells expressing NKG2D and INFγ.

Conclusion

The Agilent Seahorse XF NK Cell Metabolic Profiling assay delivers a complete characterization of the metabolic profile of NK cells. The assay combines the use of the Agilent Seahorse XF HS Mini analyzer with the XF T Cell Metabolic Profile kit, providing an optimized uncoupler that allows for robust measurements of maximal respiration and percentage spare respiratory capacity (SRC) in NK cells, with minimal uncoupler concentration optimization. In addition, this assay allows users to obtain quantitative information about basal and compensatory glycolysis in the same cell sample. It also enables unique measurement of basal cell bioenergetic poise and demand, reducing the need to run multiple assays for a complete characterization of NK cell metabolic fitness.

Metabolic characterization of NK cells is a critical attribute that needs to be analyzed to improve the antitumor potency and persistance of NK cell products. A better understanding of how metabolism and the availability of metabolic fuels affect NK cell function is essential for accelerating the design and development of NK cell derived therapies. The XF NK Cell Metabolic Profiling assay delivers multiparametric outputs that provide detailed data on the metabolic attributes of NK cells from the same sample. It can be incorporated into studies as a routine assay to optimize the design and manufacturing of NK cell derived therapeutics.

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