Assessing T Cell Bioenergetic Poise and Spare Respiratory Capacity Using Extracellular Flux Analysis

Alfredo Caro-Maldonado, PhD
Seahorse Product Specialist

Agilent
Trusted Answers

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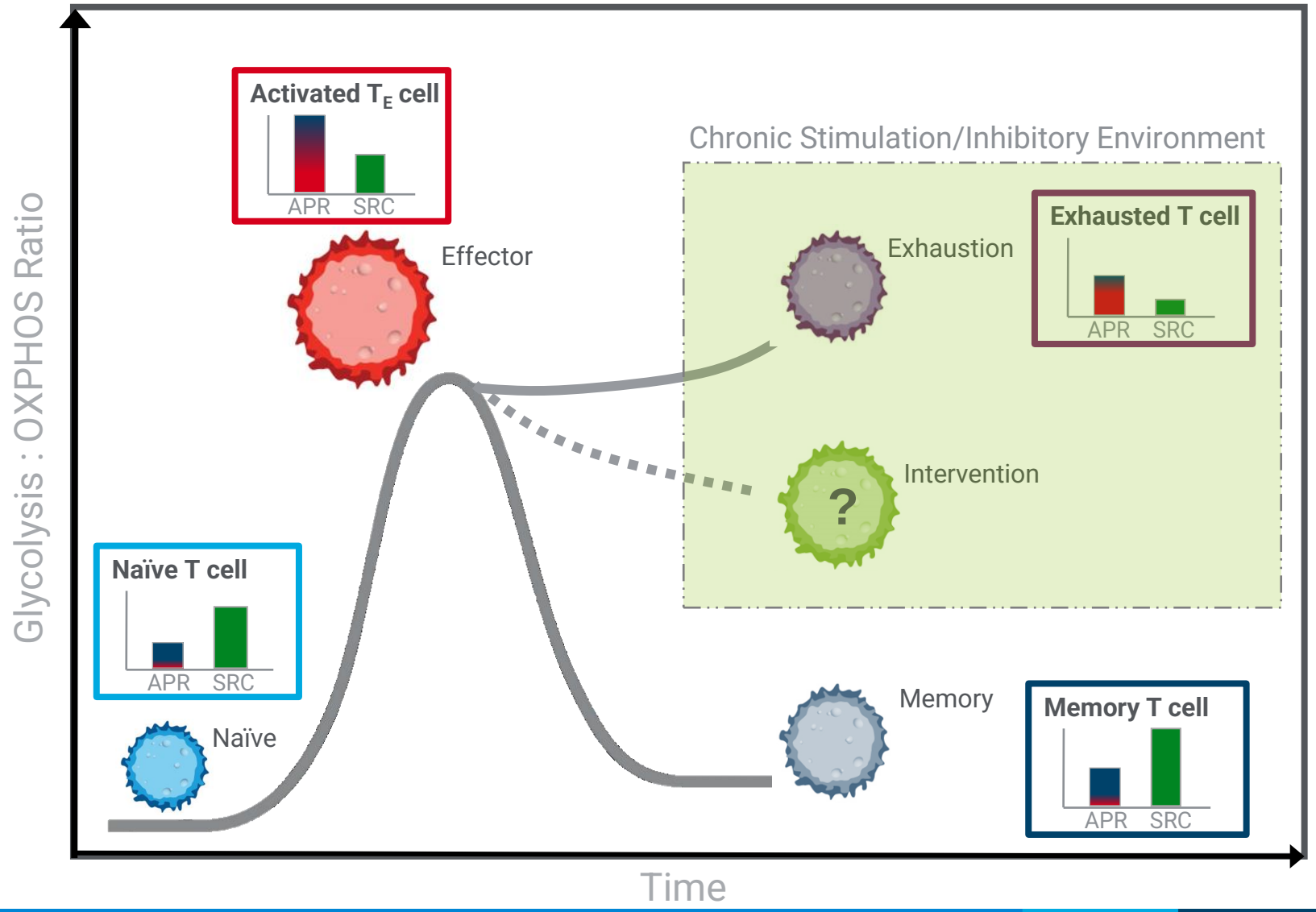
T Cell Immunity Fundamentals



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- Agilent Application Note:

Agilent Seahorse XF technology measures the rate change of the two key energy metabolism pathways

– A powerful technology for studying cellular metabolism and bioenergetics

Glycolysis

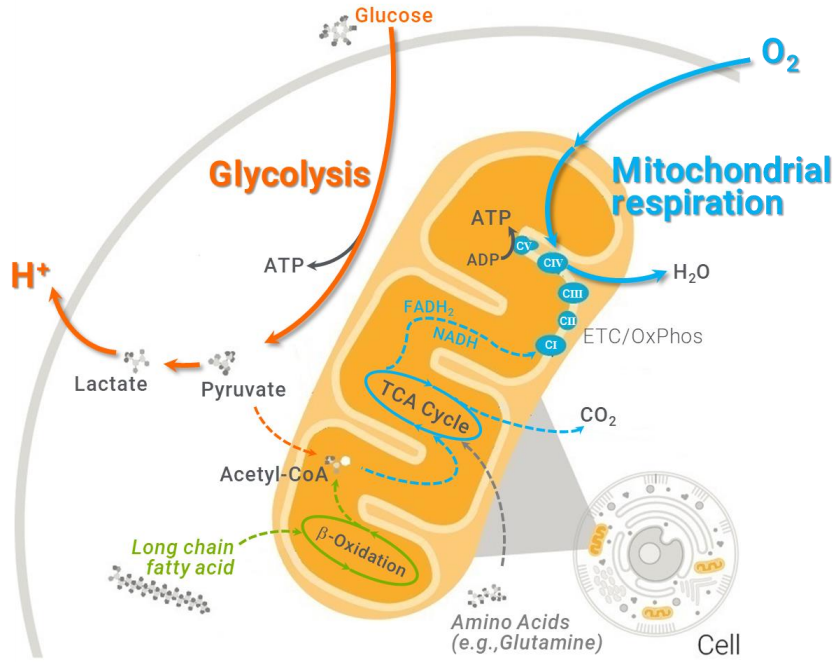
Anaerobic conversion of glucose to lactate

ECAR
(mpH/min)

Extra Cellular Acidification Rate

PER
(pmol [H^+]/min)

Proton Efflux Rate



Respiration

Oxidation of glucose, fatty acids, amino acids

OCR
pmol [O_2]/min

Oxygen Consumption Rate

Identify metabolic phenotype & liability

Assess cellular functions in real time

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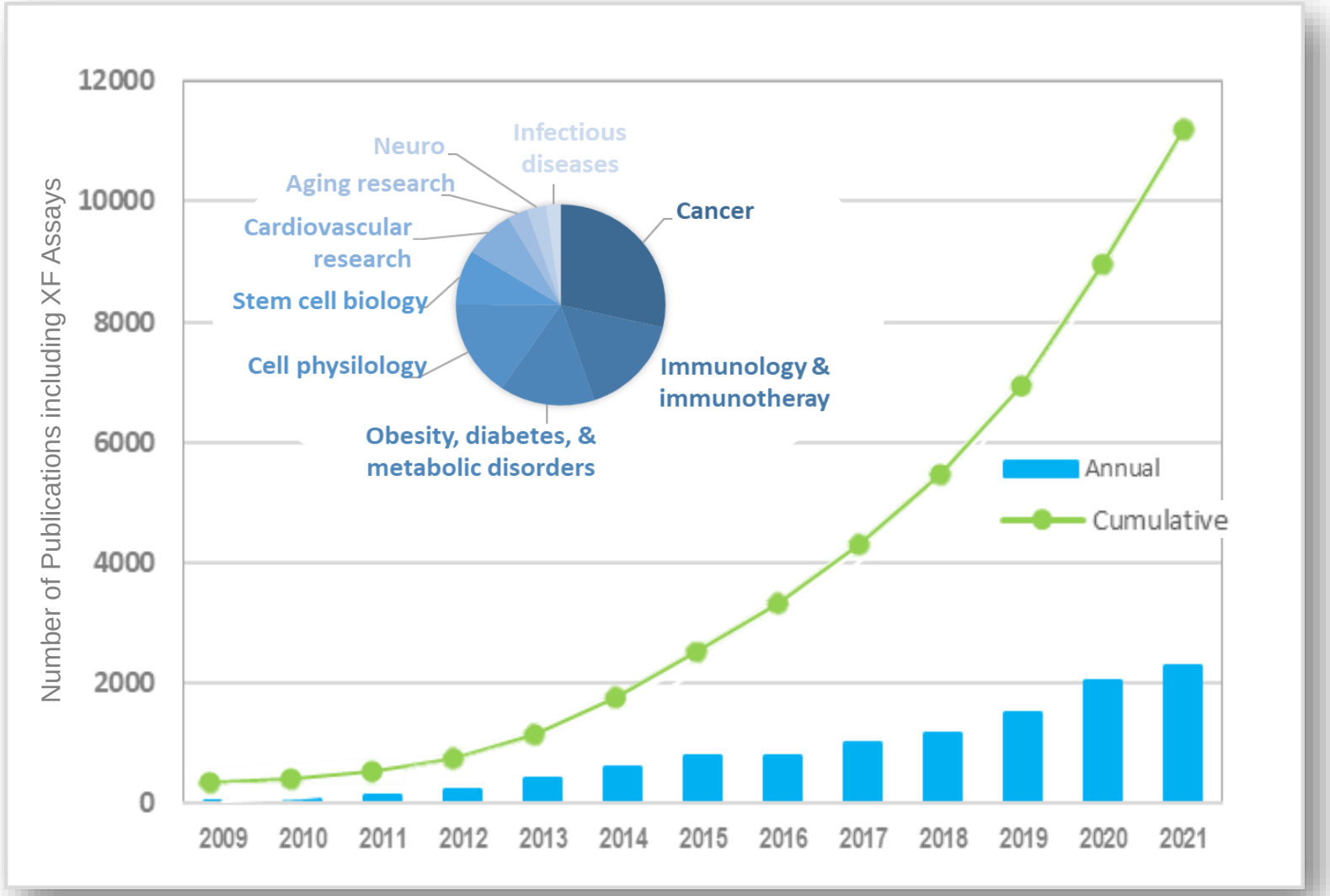
Continuing Interest in Using XF Technology to Answer Research Questions



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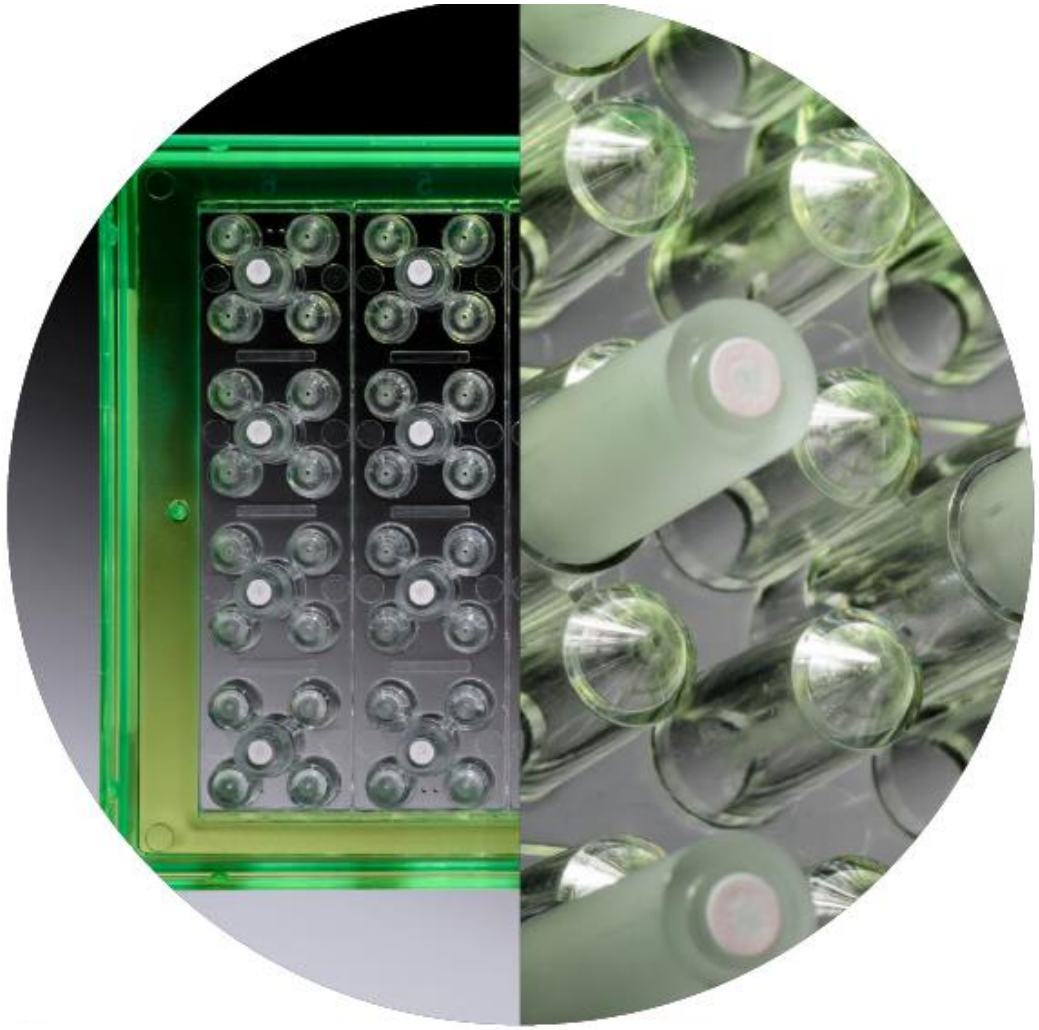
XF Technology Empowers You to Answer Your Questions About Cellular Function in Real-Time

Real-time kinetic measurements

Advanced software & multiparameter analytics

Live cell Samples



Label-free H^+ & O_2 sensor

Integrated injection ports
for real-time compound testing

Ready-to-use validated kits & reagents

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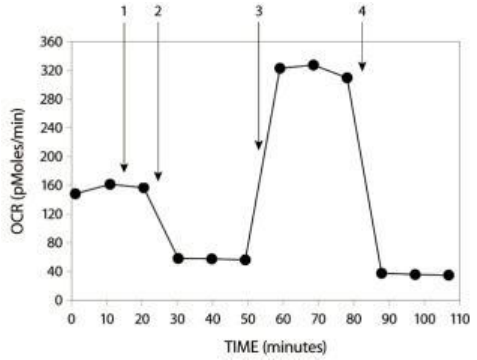
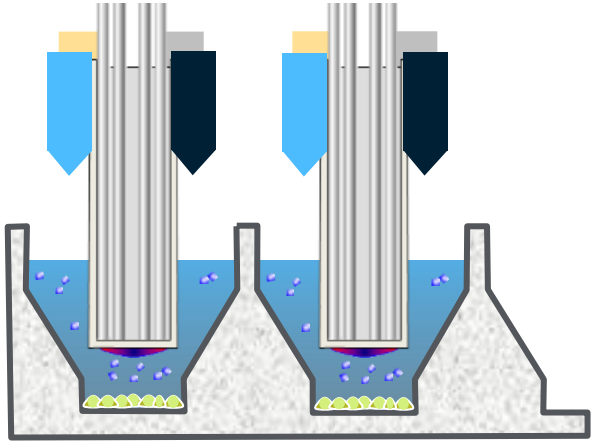
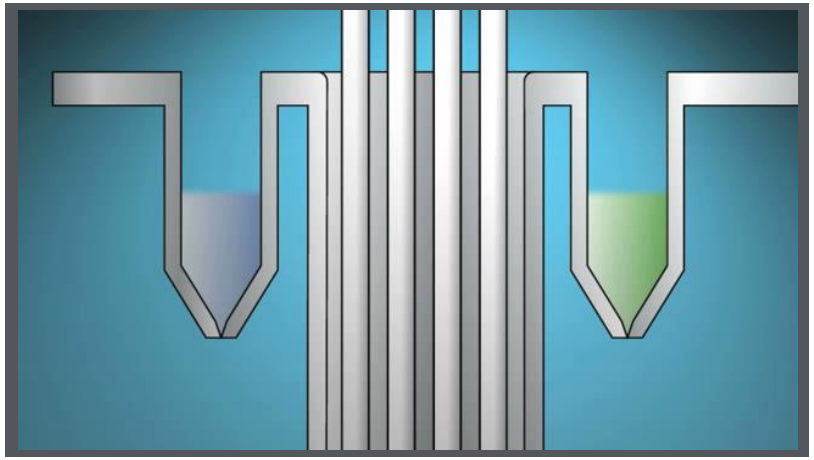
Automated compound injections enable kinetic, functional data

- 4 compound injection ports per well
- Add inhibitors, stimulants, substrates, etc.
- Injections are defined by the user







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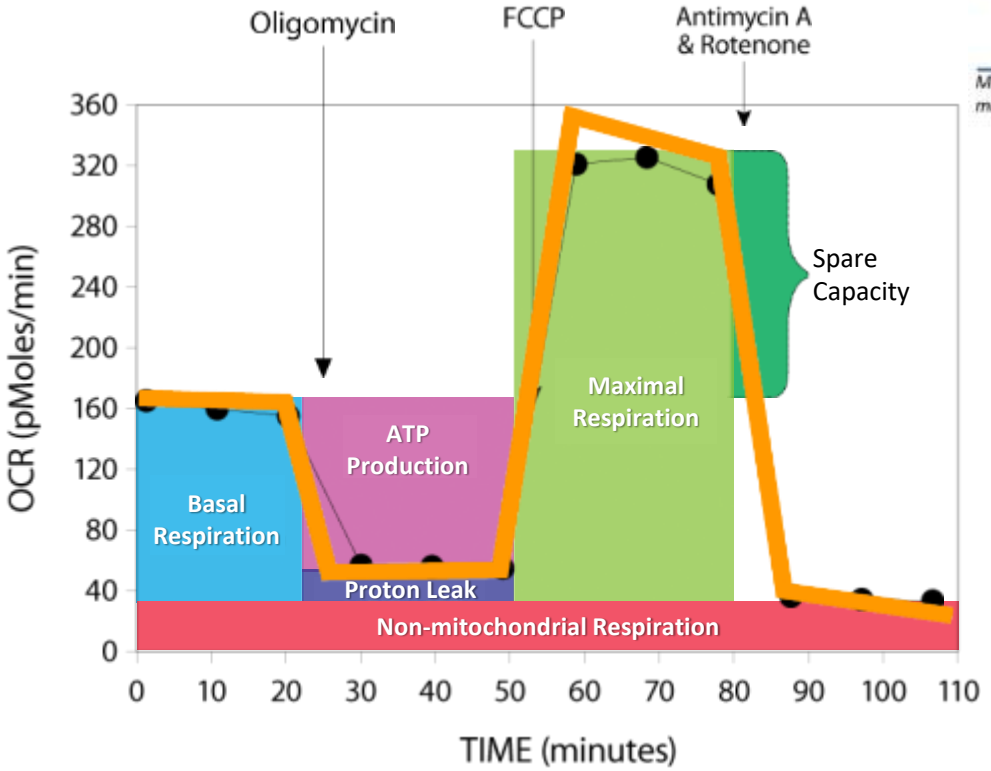
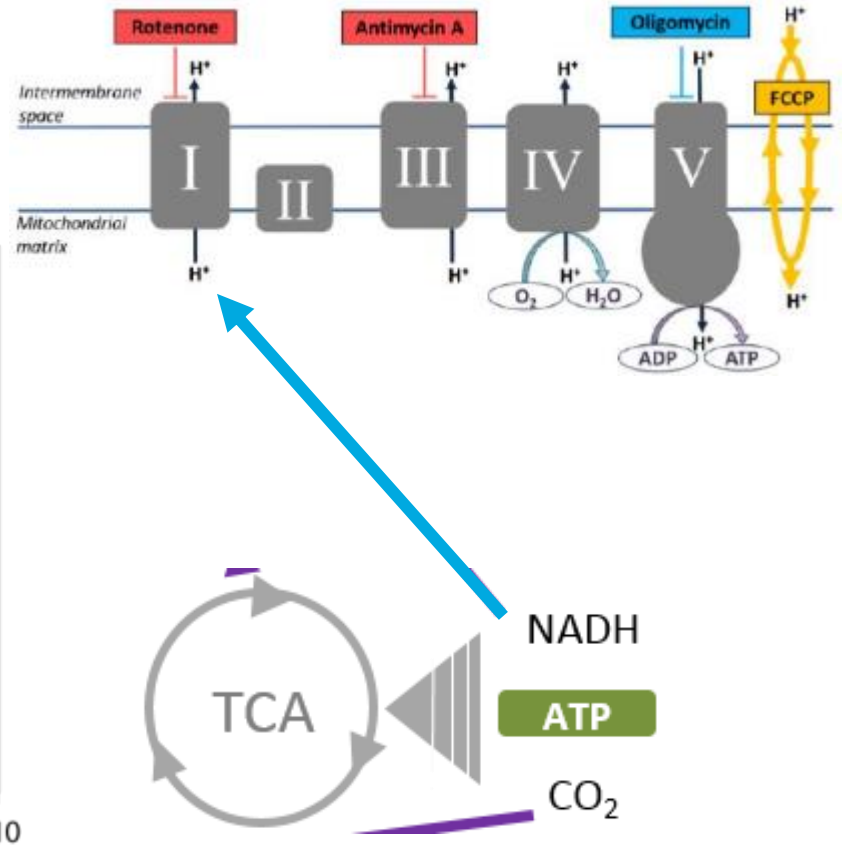
XF Cell Mito Stress Test



XF Cell Mito Stress Test Profile

Mitochondrial spare capacity enables cells to respond to acute stress



Notes

- The reduction of the proton gradient by FCCP can interfere with substrate transport processes
- **Optimal FCCP concentration must be titrated**

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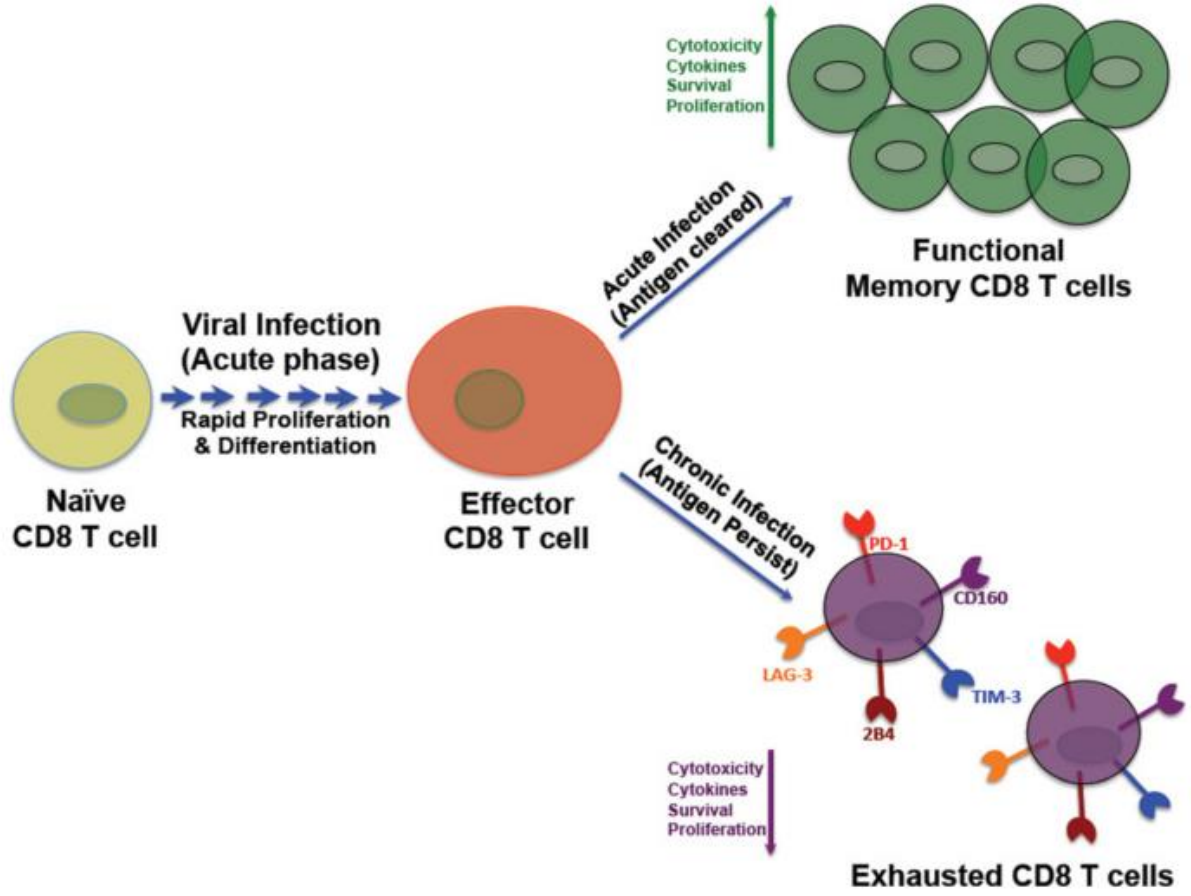
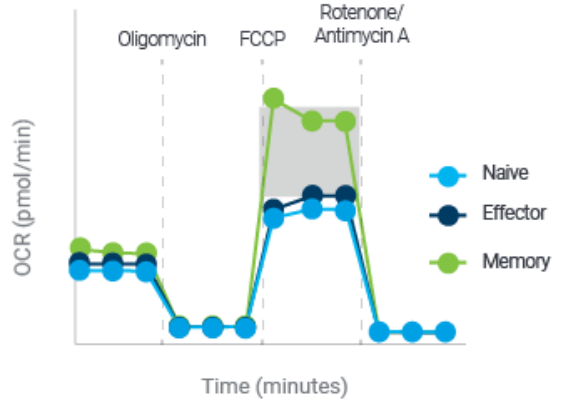
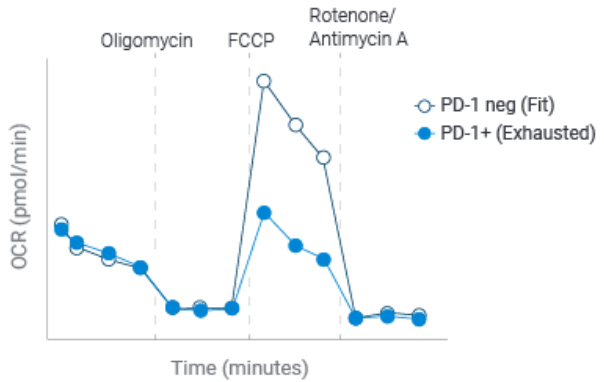
Fighting Infection with Immune Response

Phenotyping immune response to acute vs. chronic infection





van der Windt *et al. (2011) Immunity, 36(1): 3-5.*



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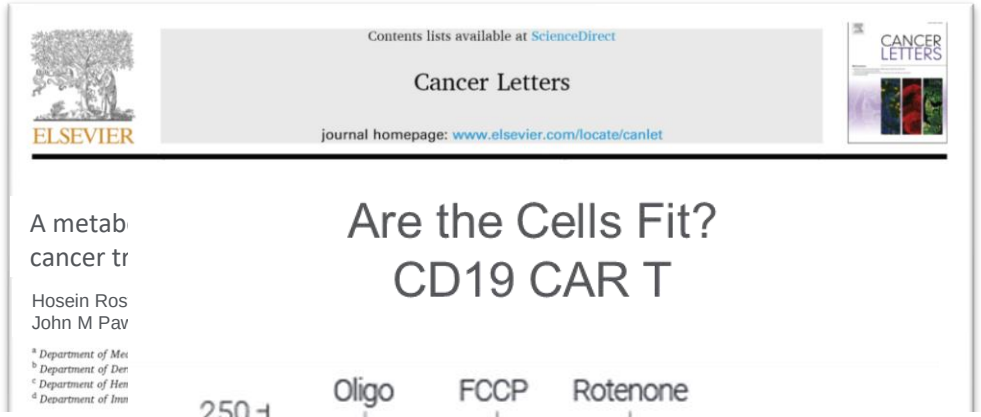
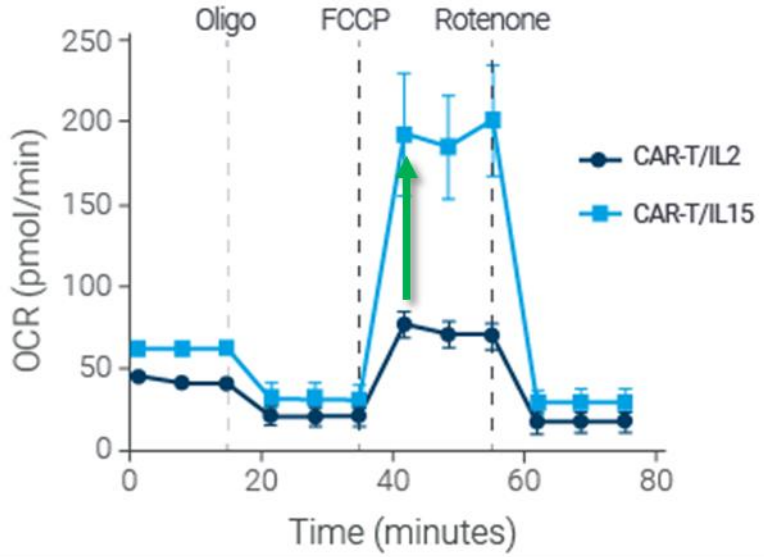
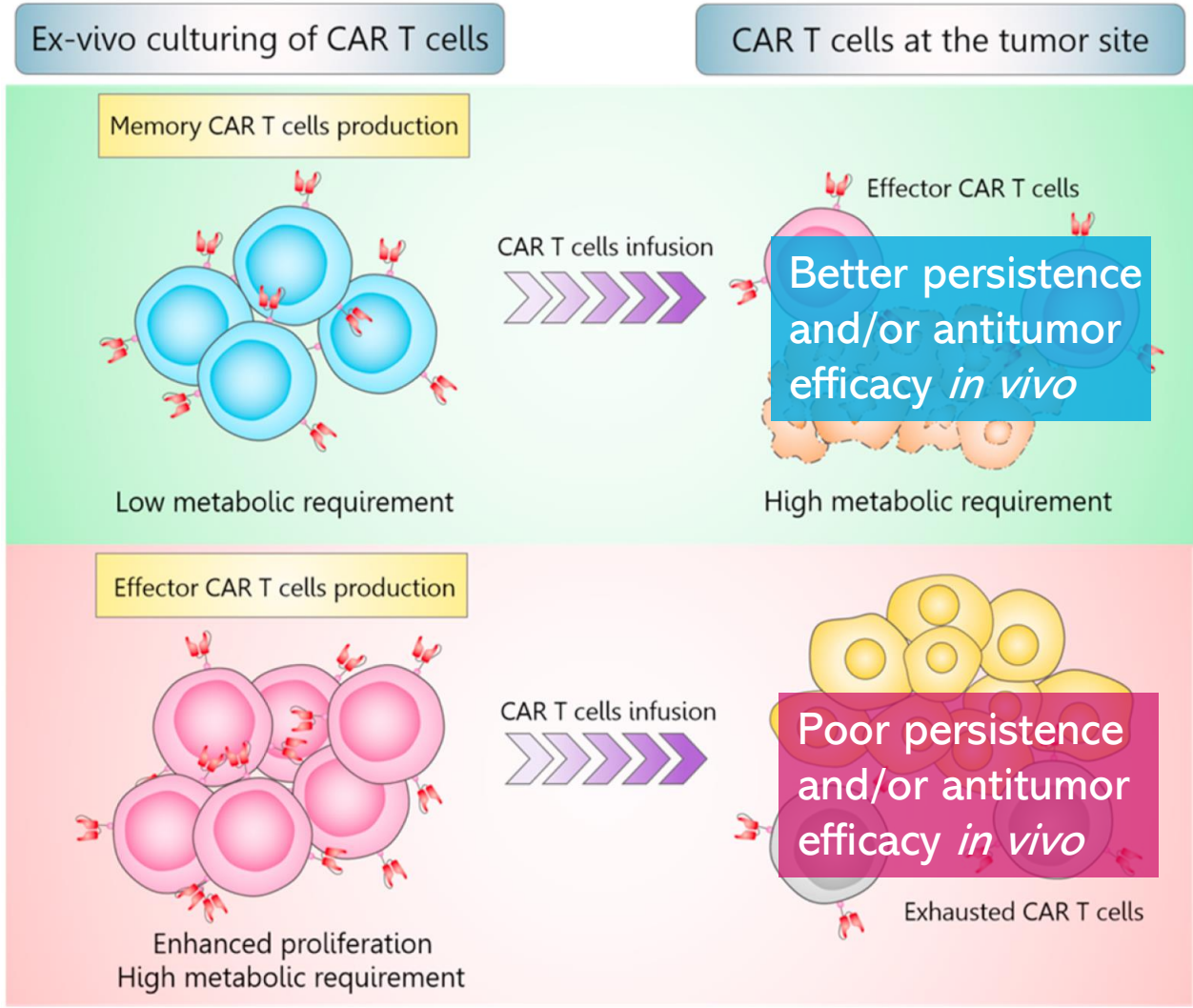
Metabolic Measurements Matter to Cell Therapy Development

Cancer Letters 500 (2021): 107-118





Alizadeh, D., et al. (2019). IL15 Enhances CAR-T Cell Antitumor Activity by Reducing mTORC1 Activity and Preserving Their Stem Cell Memory Phenotype. *Cancer Immunol Res*, 7(5), 759-772. doi:10.1158/2326-6066.CIR-18-0466



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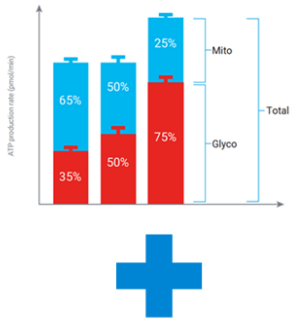
Challenges for robust immunemetabolism experiments

- FCCP for maximal respiration (reproducibility, variability, titration, toxicity, ATP production, etc.)
- Sensitivity of the instrument

XF Real-Time ATP Rate Assay



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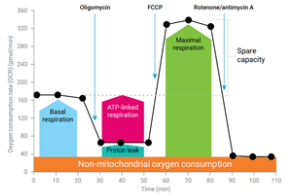
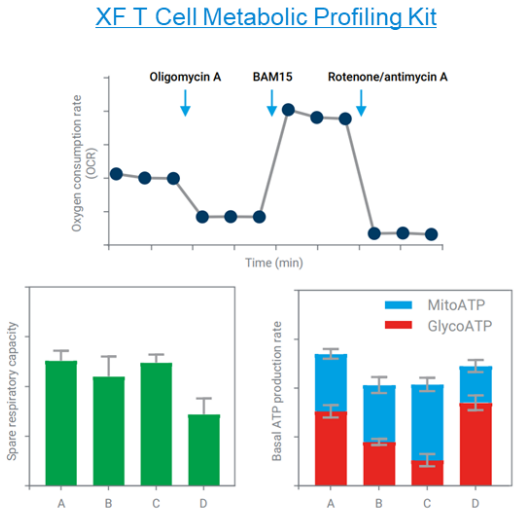
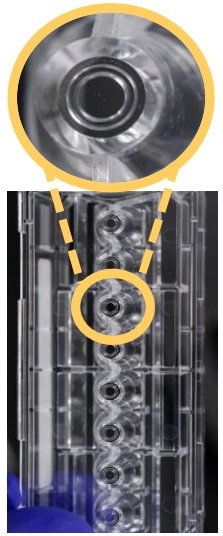


XF T Cell Metabolic Profiling Kit





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FCCP cytotoxicy:

- plasma membrane depolarization
- shift in the oxygen sensitivity of the ETC
- hydrolysis of cellular ATP resulting from oxygen depletion

BAM15

Mol Metab. 2013 Nov 28;3(2):114-23. doi: 10.1016/j.molmet.2013.11.005. eCollection 2014 Apr.

Identification of a novel mitochondrial uncoupler that does not depolarize the plasma membrane

Brandon M Kenwood [1], Janelle L Weaver [1], Amandeep Bajwa [2], Ivan K Poon [3], Frances L Byrne [1], Beverley A Murrow [1], Joseph A Calderone [4], Liping Huang [2], Ajit S Divakaruni [5], Jose L Tomsig [1], Kohki Okabe [6], Ryan H Lo [7], G Cameron Coleman [1], Linda Columbus [7], Zhen Yan [8], Jeffrey J Saucerman [9], Jeffrey S Smith [10], Jeffrey W Holmes [9], Kevin R Lynch [1], Kodi S Ravichandran [3], Seiichi Uchiyama [6], Webster L Santos [4], George W Rogers [11], Mark D Okusa [2], Douglas A Bayliss [1], Kyle L Hoehn [12]

Affiliations + expand

PMID: 24634817 PMCID: PMC3953706 DOI: 10.1016/j.molmet.2013.11.005

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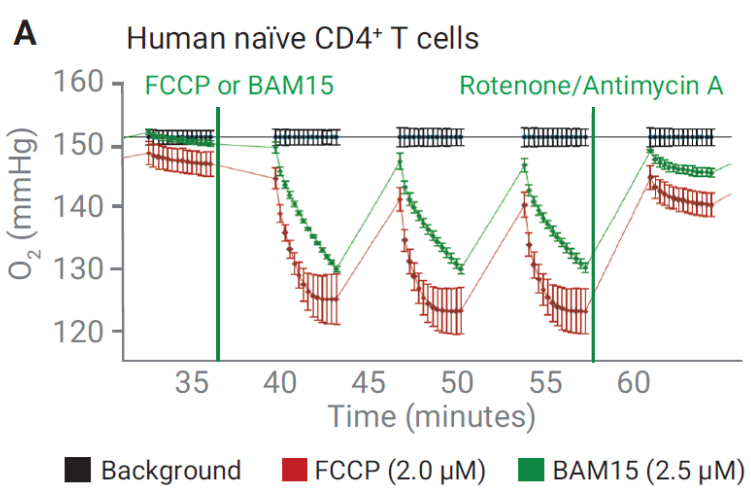
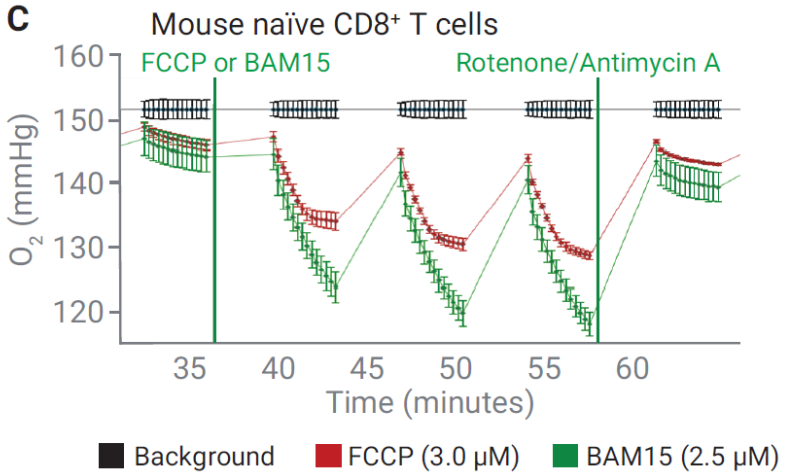
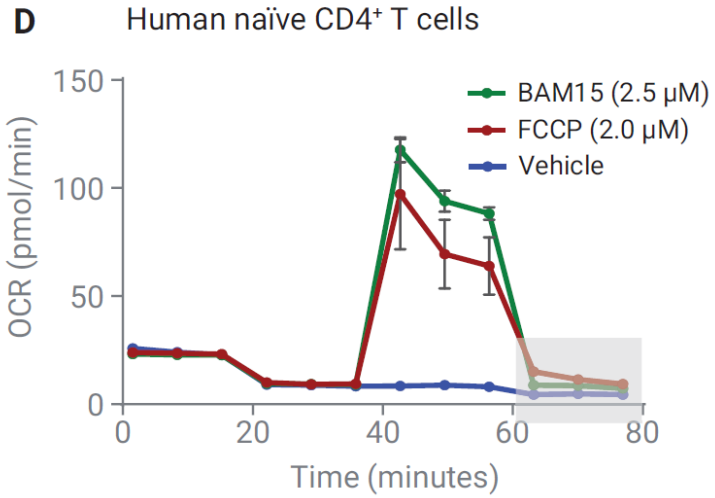
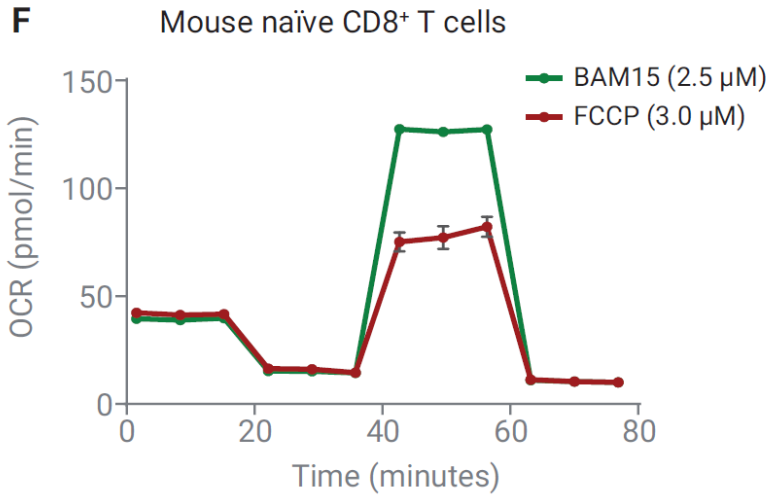
Comparison of Oxygen consumption measurements using uncoupler FCCP and BAM15.

Citotoxicity
ATP production









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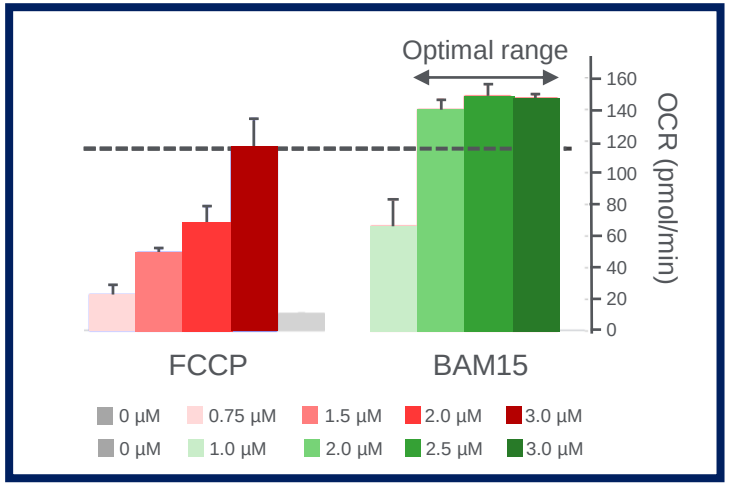
Example Data: Performance of BAM15 vs FCCP in Human Naïve CD8+



Titration experiments show:

- BAM15 has wider concentration range to obtain maximal OCR

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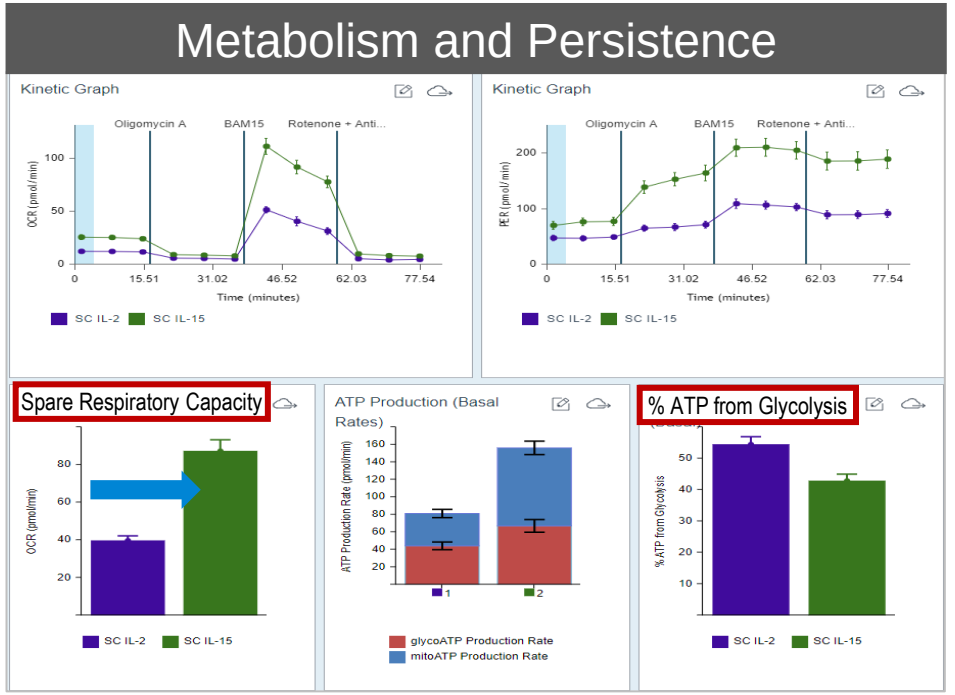
XF T Cell Persistence Assay and Fitness Assay



- Key parameters:
  - % ATP from glycolysis
    (calculated from basal glycoATP & mitoATP)
  - Spare respiratory capacity (SRC)

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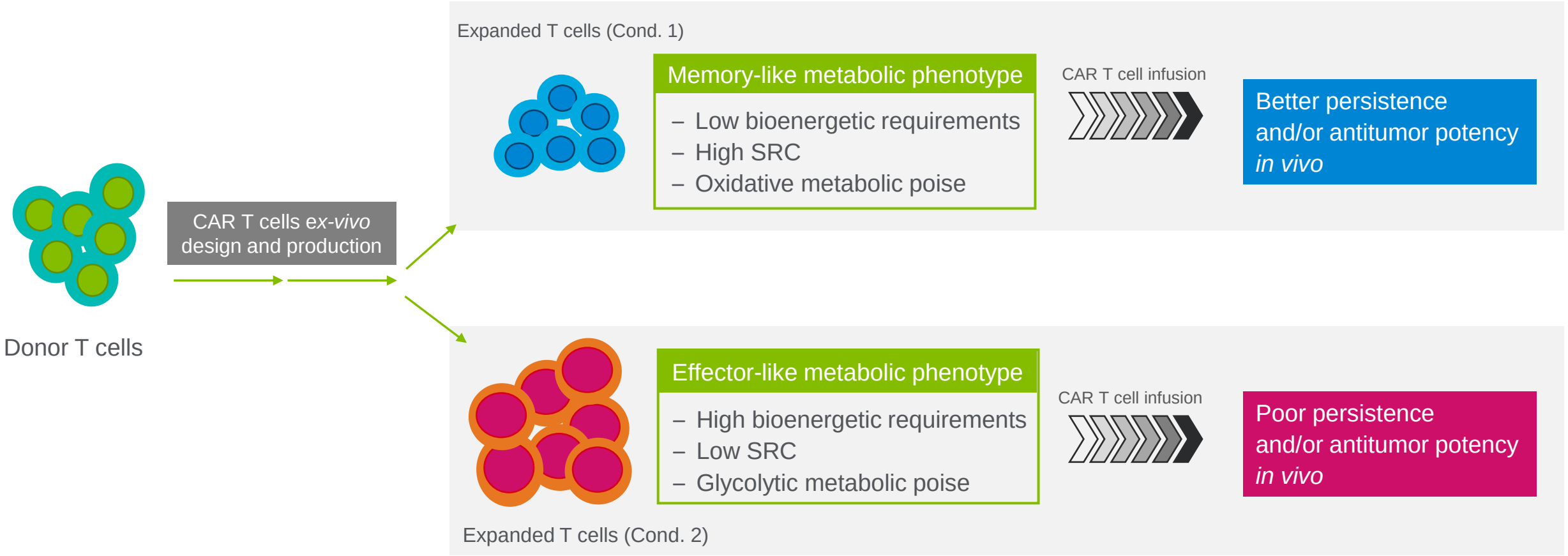
Example Application: Using XF T Cell Persistence Assay with XF T Cell Metabolic Profiling Kit to dive a phenotype that correlates with better persistence during T cell expansion



Modified from Rostamian et al. (2021) . Cancer Letters 500:107-118

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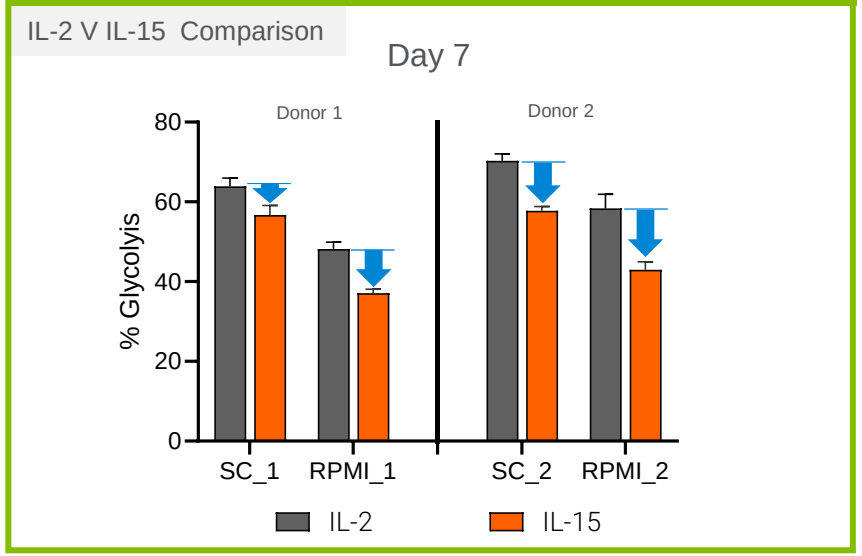
Application of XF T Cell Metabolic Profiling Kit in Optimizing T Cell Persistence for Cell Therapy Product Development

Day 7: T cells expanded in IL-15 are more oxidative than those expanded in IL-2 (lower % glycolysis)



You can use an XF persistence assay to optimize T cell manufacturing/production conditions driving a phenotype that correlates with persistence

Cells were activated with CD3/CD28 beads and expanded during indicated time (2 independent experiments with samples from different donors) in culture media supplemented w/ IL-2 or IL-15: SC: Immunocult XF medium (Stem Cell Technologies) RPMI: RPMI Medium + 10% FBS (Gibco)

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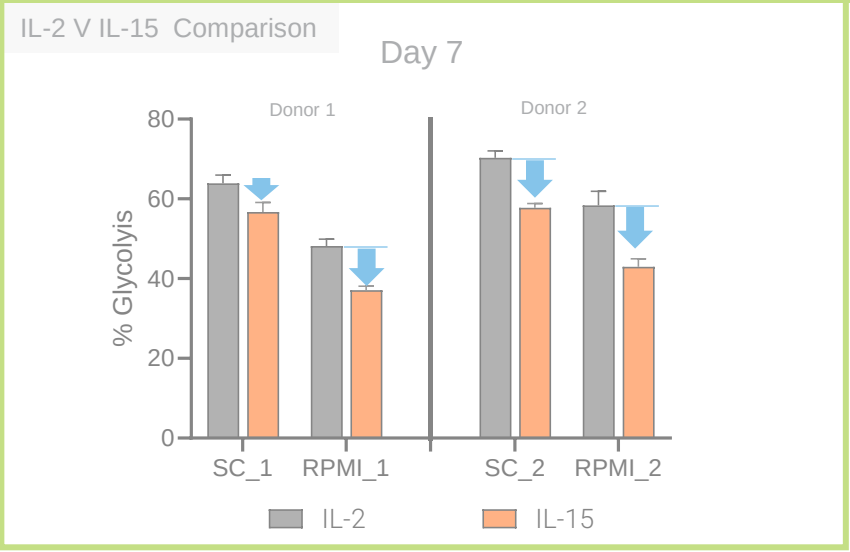
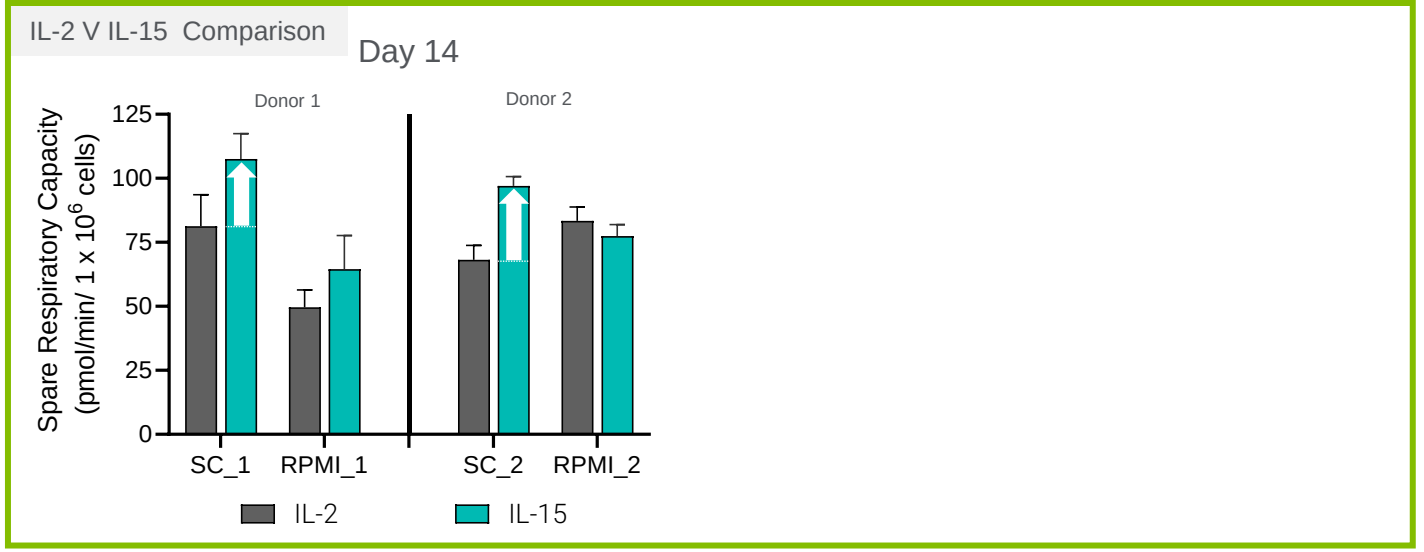


Day 14-22: T cells expanded in IL-15 have higher **SRC** than those expanded in IL-2



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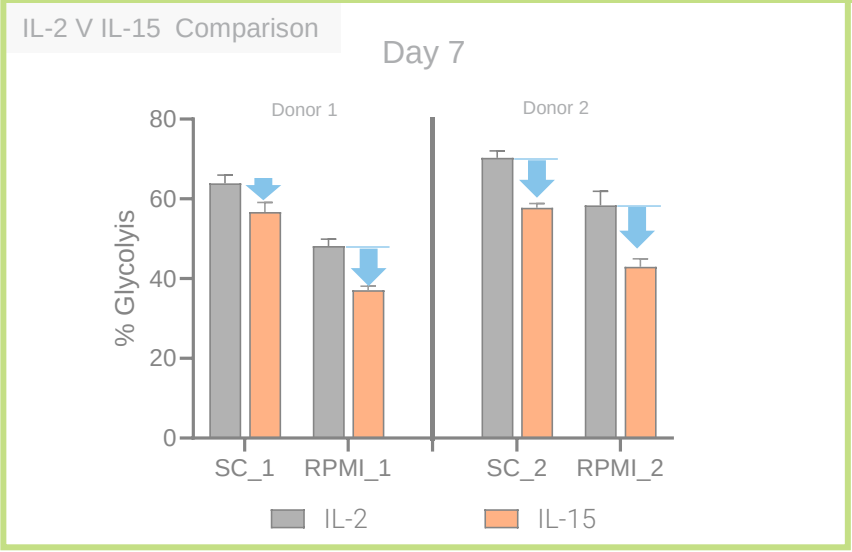

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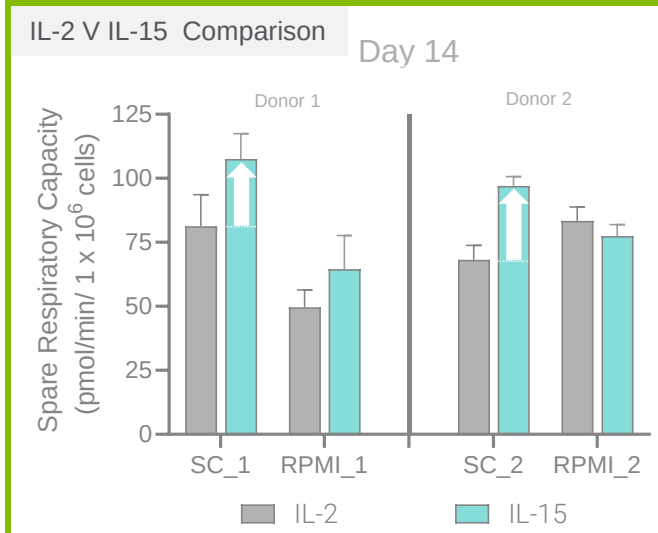

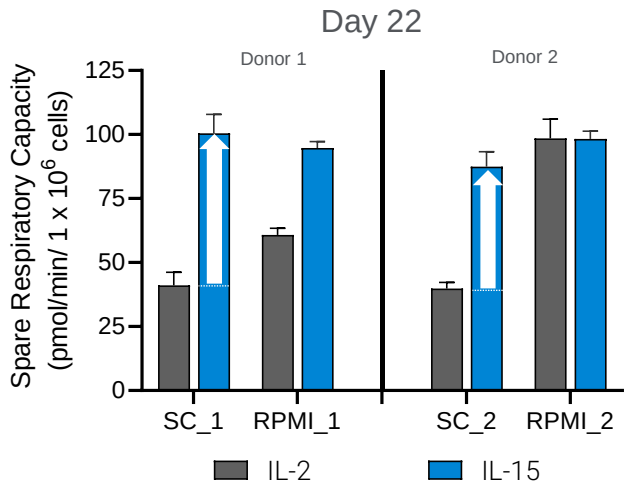

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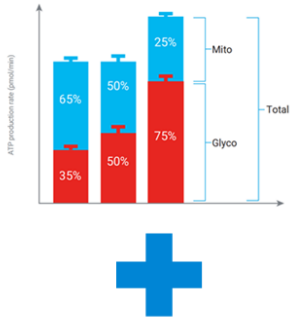
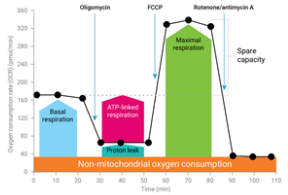
Challenges for robust immune cells experiments

- FCCP for maximal respiration (reproducibility, variability, titration, toxicity, ATP production, etc.)
- Sensitivity of the instrument

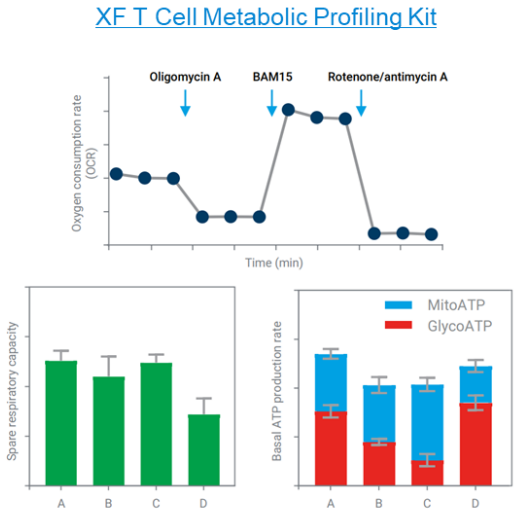
XF Real-Time ATP Rate Assay

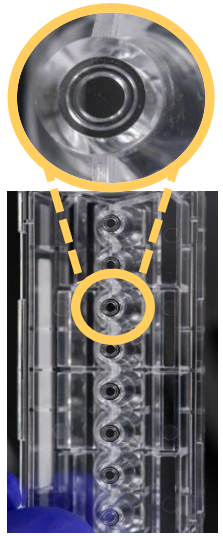


XF Cell Mito Stress Test



XF T Cell Metabolic Profiling Kit





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Seahorse XF Pro – New Features

System Performance Improvements

- 2x improvement in OCR precision to 10% CVs
- Sensitivity >2x lower for OCR than with XFe96
- Enables repeatability 20% inst.-to-inst. and plate to plate metrics

Manufacturability/Serviceability

- Reduced complexity of heater design
- Improved z-axis motion control modules
- Accessibility improvements implemented

Firmware-based Timing

- Calibration and data acquisition (DAQ) uses firmware-based timing for improved precision
- All timestamps are used to calculate OCR which improves precision

Improved Customer Experience

- Stall sensing feature added to improve reliability
- Heating complexity reduced to enable faster warm-up times

Increased Temperature Range

- New heating design increase temperature range to 28C – 40 C at room temperature. (8-20C above ambient to achieve 16-42C)
- Faster warm-up time achievable. No need to wait more than 24 hrs
- Control for edge effect and evaporation during assays

Mid-sized Plate Handler, Automation Ready

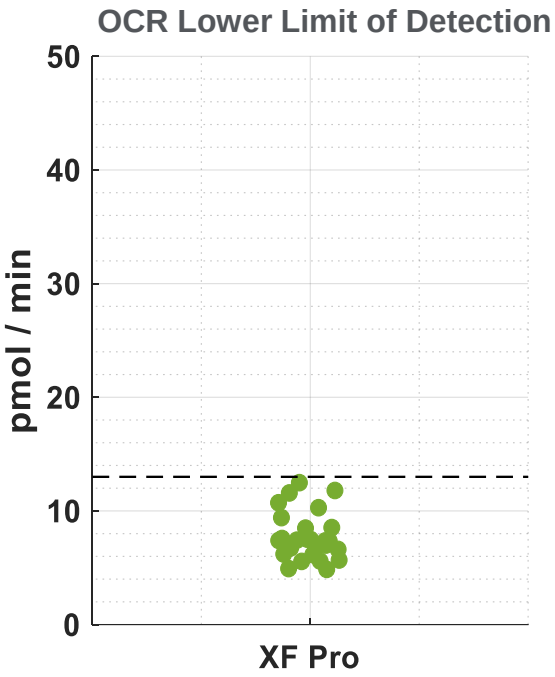
- X-axis mechanically elongated to enable compatibility with an automation solution
- Software drivers implemented

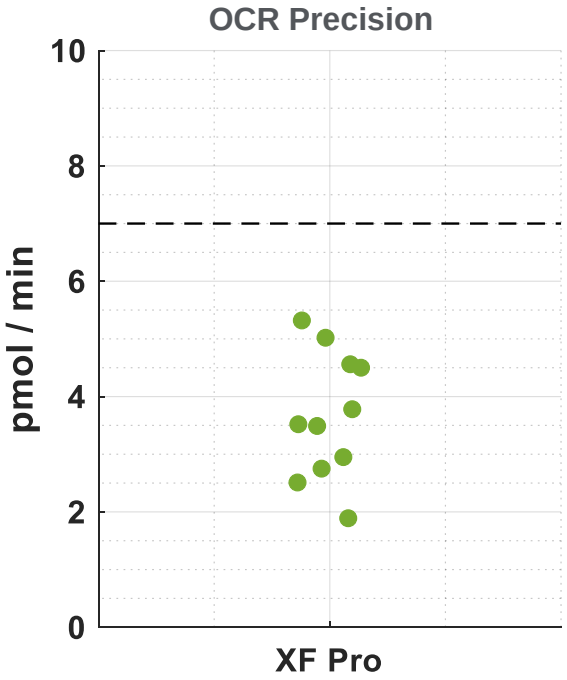
Key Specification Improvements

- Improved CVs, plate-to-plate, instrument-to-instrument repeatability!

Expanding Detection and Ensuring Confidence in Results

Verified Instrument Performance at Low OCR -
Gives confidence in measurements from low respiring cells.





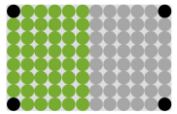
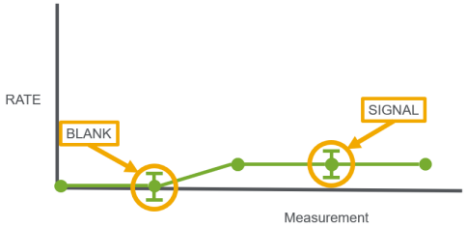
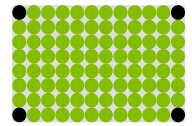


Plate Layout





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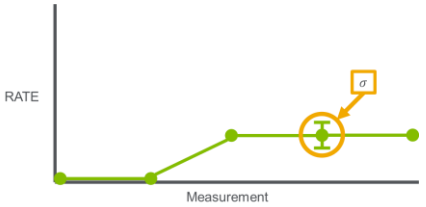

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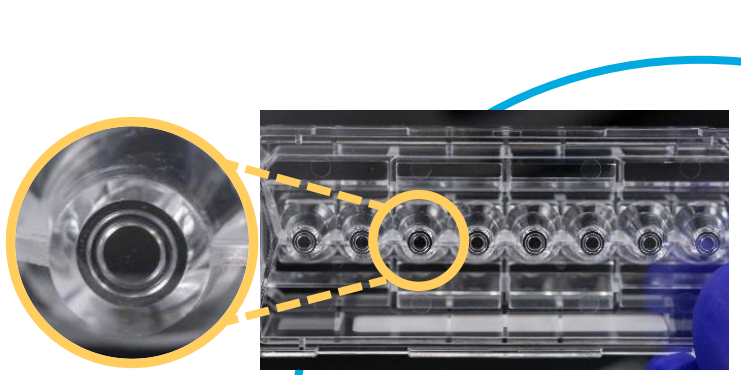
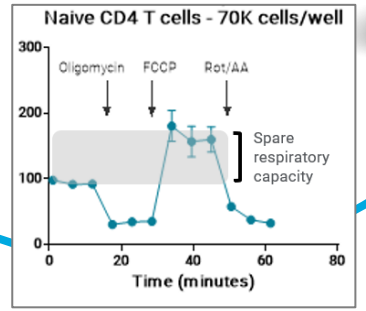
Seahorse XF HS Mini Solution

Greater Precision, Fewer Cells, Expanded Possibilities





- Limited & abundant amounts of biomaterial
- Enhanced data quality for cells that experience low levels respiration
- Compatible with both XF HS and XFp miniplates
- Compatible with all standard XF assays
- Powered by Windows **10**
- Seahorse Analytics for data analytics

More information about the XF HS mini analyzer

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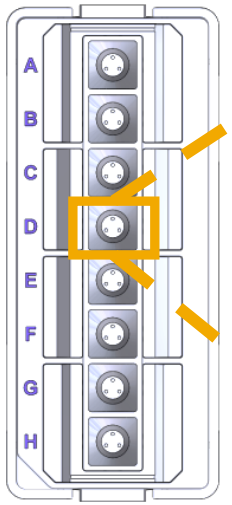
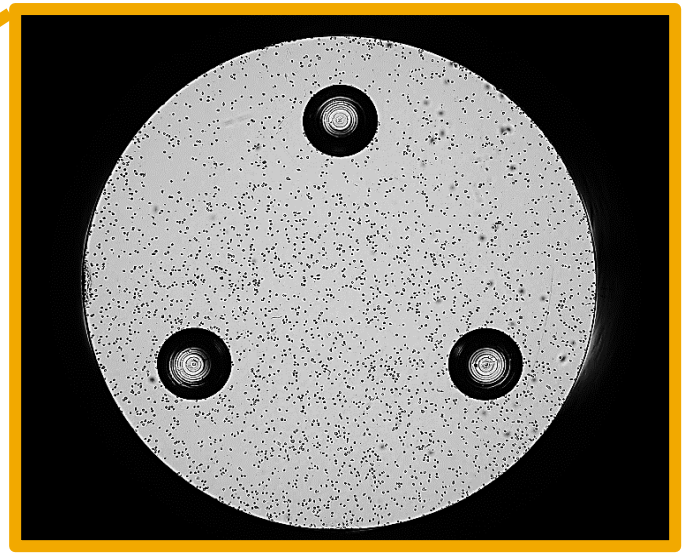
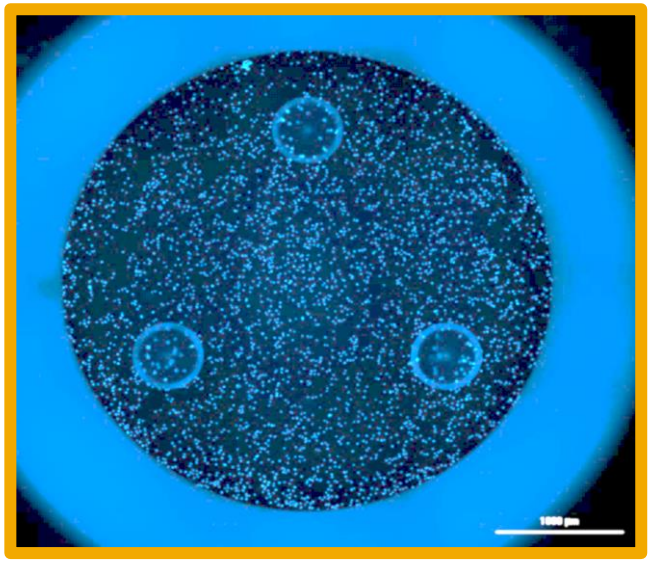
XFp miniplate

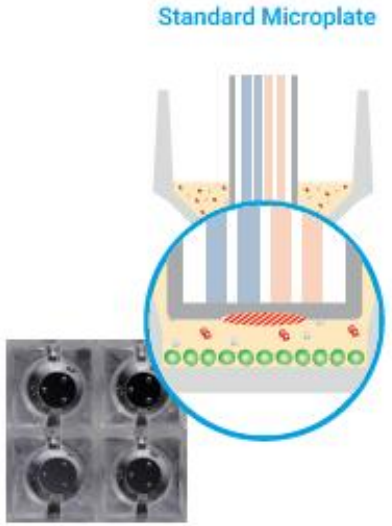


Brightfield

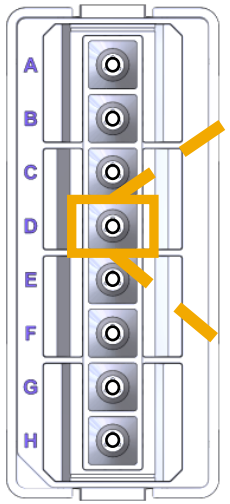


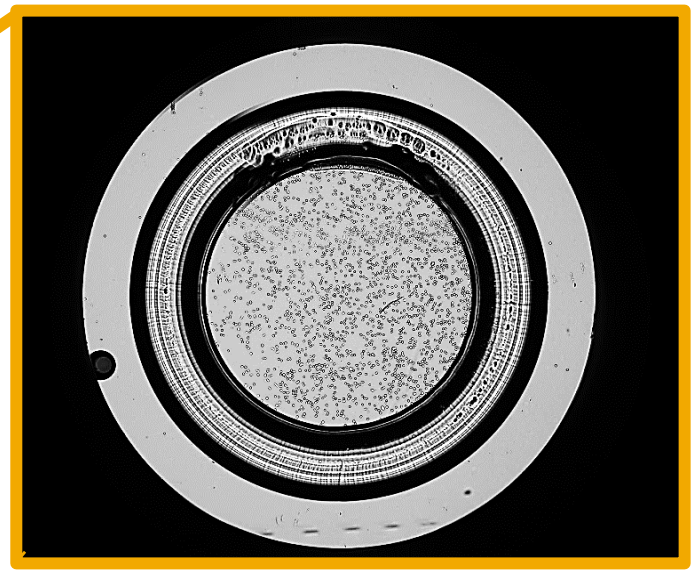
Fluorescence: Hoechst

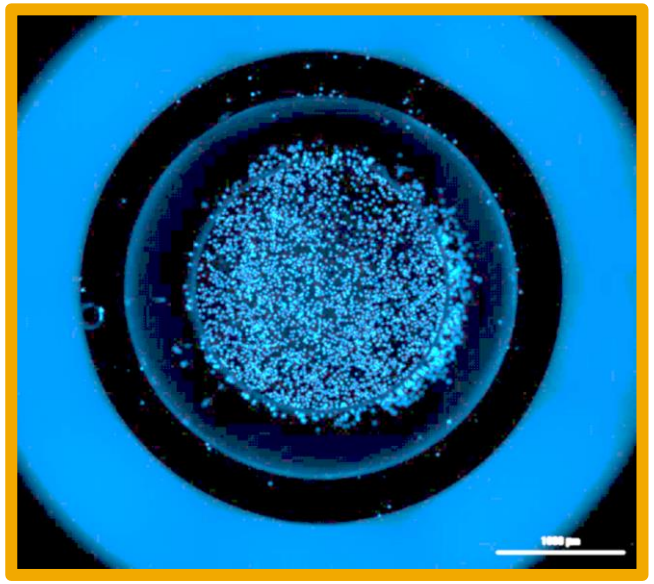


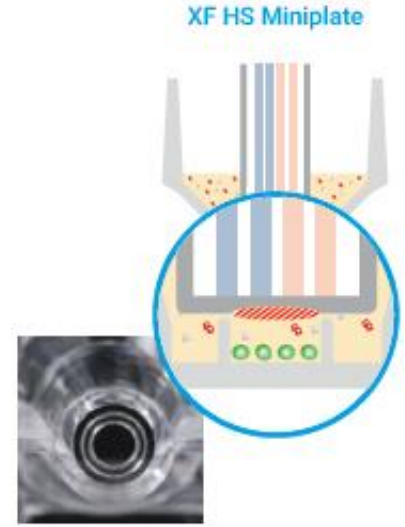


XF HS miniplate









THP-1 cells 5K per well density`

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Conclusions

- T Cell tumor persistence depends on their metabolic fitness
- BAM15 is a better uncoupler than FCCP in T cells
- XF Pro and HS improves sensitivity and reproducibility, optimal for lymphocytes.

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Visit my posters 11 and 213

THANK YOU!

Please, don’t hesitate in contacting me for anything

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