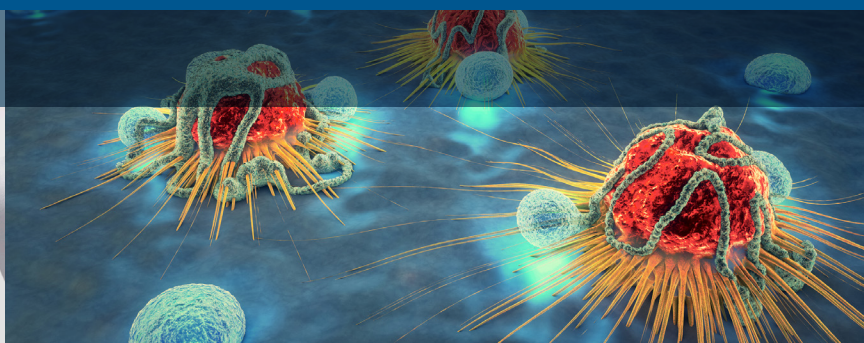
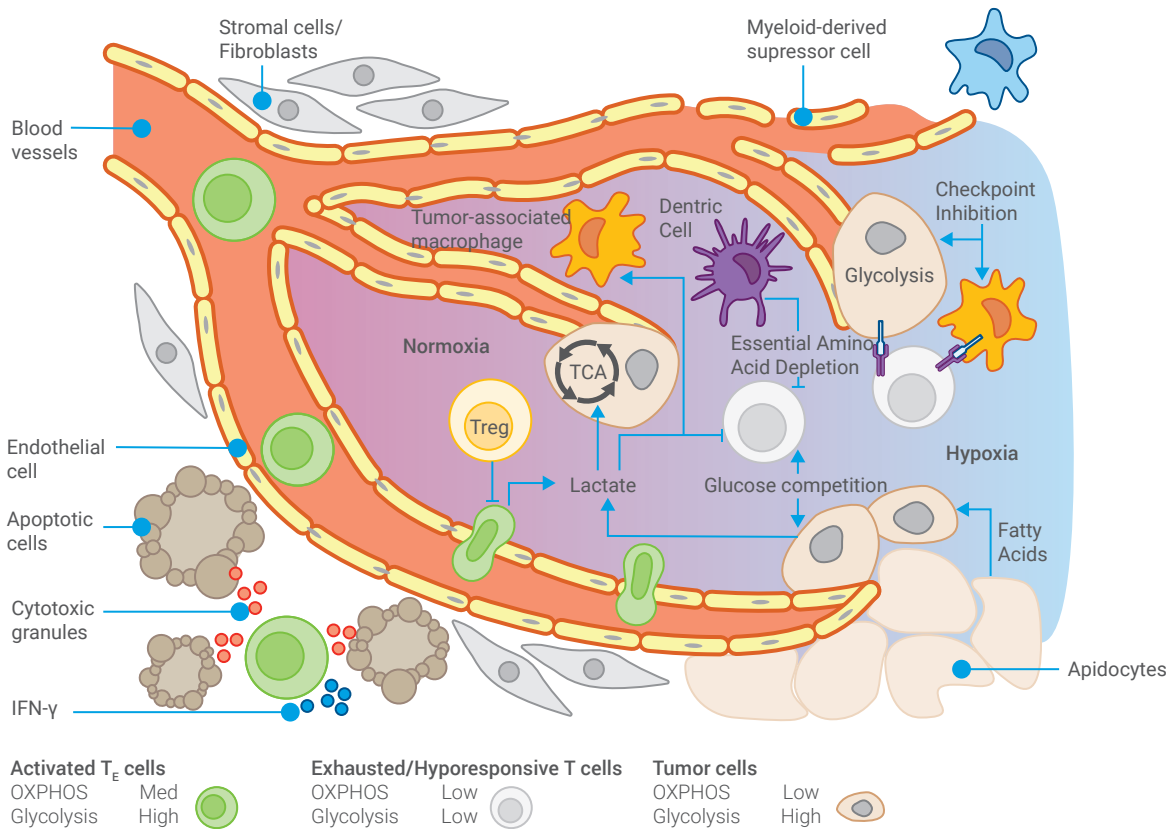


Agilent Seahorse XF Live-Cell Metabolism Solutions for Immunology Research

Immunometabolism solutions for live-cell research



Direct the Immune System by Modulating the Metabolic Pathways that Regulate Cell Fate and Function



The Tumor Microenvironment*. Agilent Seahorse XF assays reveal an early window into the upstream drivers of immune responses

Immune cells are highly integrated, relying on a cascade of signaling, networks, and checkpoints to regulate cellular processes. Conventional analytical approaches such as immunophenotyping, cytokine assays, and pathway profiling provide a comprehensive view of immune cell fate and function after the immune cell is committed to a particular outcome. However, pathway intervention and modulation to control immune cell function requires an understanding of the upstream drivers of immune cell processes. Metabolic programs are upstream determinants of immune cell outcomes.

Agilent Seahorse XF technology provides kinetic, functional metabolic measurements for a more direct measure of immune cell processes as they are happening, in real time. To fully understand and control the dynamic nature of immune cell biology, real-time activation analysis is needed to uncover the drivers of activation and provide an opportunity to intervene. Metabolic analysis not only delivers real-time activation kinetics but provides a rich set of pathway targets to modulate immune cell biology.

Adapted from Buck et al. (2017) Cell . Metabolic instruction of immunity, 169(4), 570-86.

Agilent Seahorse XF Cell Analysis Solutions for Immunology Research

Generate time-resolved, real-time functional data

The Agilent Seahorse XF Analyzer provides real-time monitoring of energy metabolism for insights into the immediate-to-early functions of immune cells, and a more complete understanding of immune cell biology.

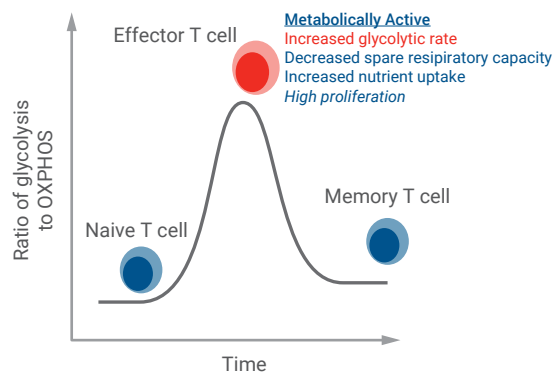


Agilent XF Cell Analysis features:

- Live cell analysis
- Real-time kinetics
- Label-free
- Dynamic injection ports
- Simultaneous measurement of oxygen consumption and glycolytic rates
- An early window into activation
- Quantitative glycolytic and ATP production rates

Immunology researchers are using Agilent Seahorse XF technology to investigate:

- Immune cell life cycle, lineage, and checkpoints
- Real-time activation
- Metabolic requirements and fuels
- Metabolic signaling and pathways
- Modulation to control outcome



Metabolic switching controls immune cell activation; increased glycolysis supports effector T cell function.

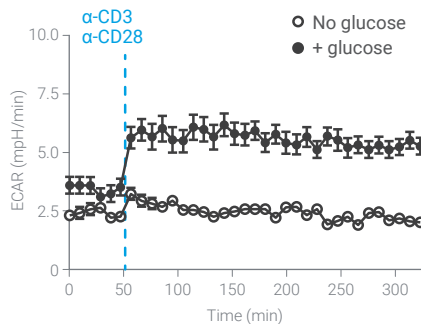
Any Change in Immune Cell Function Requires a Change in Energy Production

Metabolic phenotyping provides a window into immune cell function

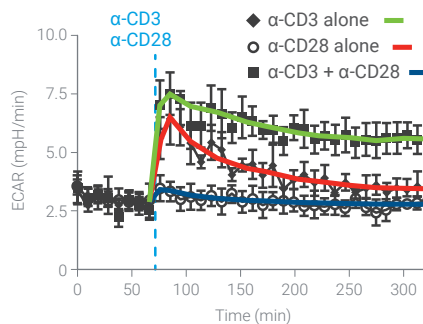
Activation, amplification, engagement of effector functions, and return to homeostasis are all crucial aspects of the immune cell life cycle—with lineage and immune cell identity constraining the ability of immune cells to function in these roles. Agilent cell analysis provides real-time functional measurements for a comprehensive picture of immune cell function and insights into pathways and targets to modulate immune cell responses.

Real-time analysis of immune cell activation opens an early window into metabolic requirements, signaling, pathways, and checkpoints

Metabolic Requirements Exogenous glucose



Signaling Requirements CD28



Pathway Requirements mTOR

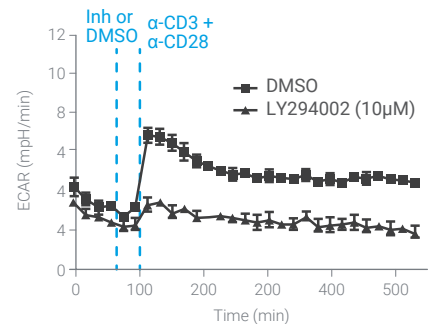


Figure 1. The Agilent Seahorse XF real-time immune cell activation assay reveals metabolic and signaling requirements critical for T cell activation. Adapted from Gubser, P. M., et al. (2013). *Nat Immunol.* 14 (10): 1064-72.

Measure metabolism as an indicator of immune cell type, function, and lineage commitment

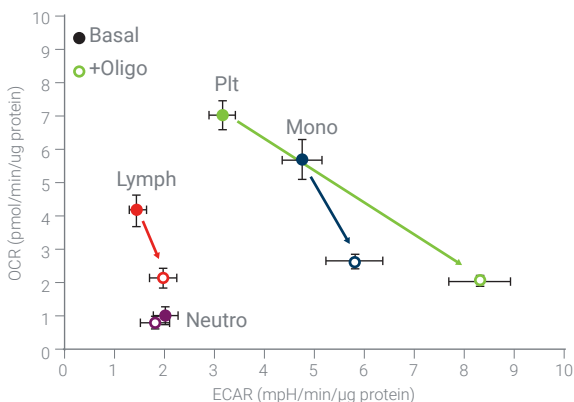


Figure 2. Simultaneous measurement of oxygen consumption and extracellular acidosis rates using the Agilent Seahorse XF Analyzer shows that distinct immune cells have a distinct metabolic phenotype. Adapted from Kramar et al. (2014). *Redox Biol.* 2:206-10.

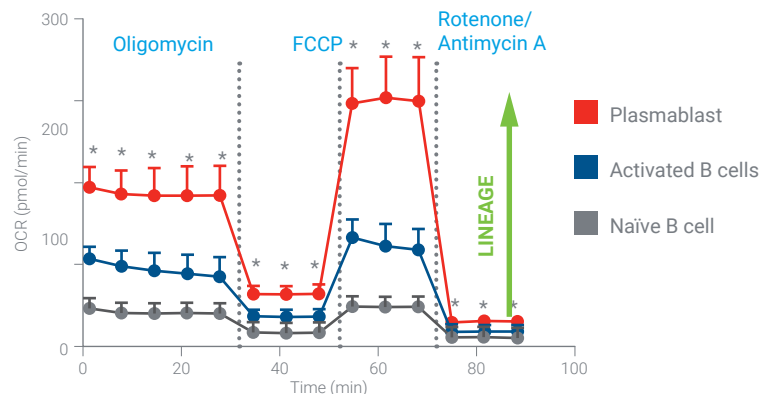


Figure 3. The Agilent Seahorse XF Cell Mito Stress Test shows that energy requirements change with lineage commitment. Adapted from Price et al. (2018). *Cell Rep.* 23(11): 3152-9.

Metabolic Analysis Reveals a Rich Set of Pathway Targets to Advance New Therapeutic Opportunities in Immuno-Oncology

Discover strategies to perturbate pathways and control immune cell response to advance therapeutic opportunities

The goal of immune cell-based therapy is to enhance the performance of native immune cells by modifying and perturbing immune cell biology and the associated signaling and feedbacks. XF analysis provides critical measurements of live cells in real time revealing the functional outcome of modulation strategies. Discover how modulation of immune cell responses via signaling, checkpoint blockade, or pathway perturbation is "functionalized" through alterations in metabolic programming.

XF analysis advances adoptive immunotherapy research by revealing a critical metabolic link between CAR-T coreceptors and effector memory cell response

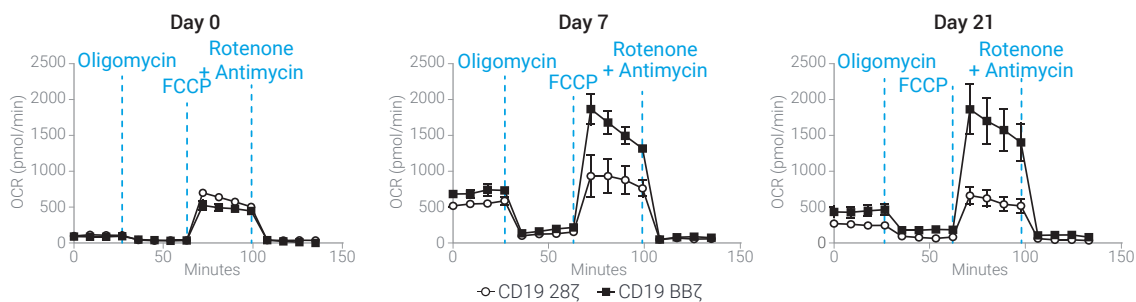
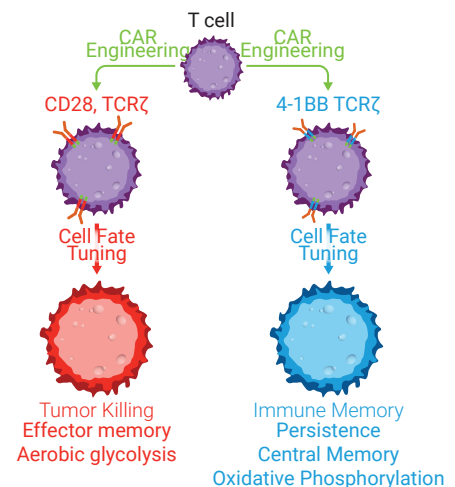


Figure 4. Distinct signaling coreceptors in CAR-T Cells (CD28 vs. 4-1BB) impact cellular metabolism to drive effector and memory cell functions, translating into a better understanding of the efficacy in CAR-T cell therapies. Adapted from Kawalekar 2016 Immunity.

Monitor immune cell responses using time-resolved, real-time functional data to exploit checkpoint blockade

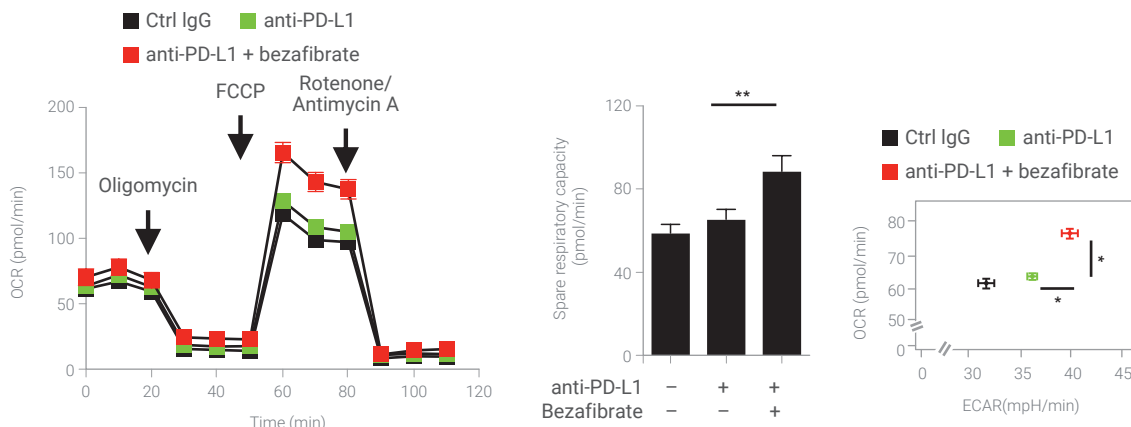


Figure 5. Via a combination immunotherapy strategy, bezafibrate enhances the effectiveness of PD-1 blockade in antitumor immunity by activating mitochondrial respiration of killer T cells to promote survival and proliferation and improve effector function. Adapted from Chowdhury 2018 Cancer Immun Res. DOI: 10.1158/2326-6066.

Real-Time, Quantitative Measurements Provide a Comprehensive Picture of Immune Cell Function

Functional XF measurements provide a window into the critical factors affecting immune cell response, fate, and function

Metabolic measurements provide a sensitive measure of how immune cells respond under various conditions to meet the integrated and multifaceted demands associated with immune responses. Researchers are applying XF technology to investigate questions relating to inflammation, autoimmunity, and immunosuppression; along with substrate dependencies and the immunosuppressive tumor microenvironment.

Metabolic phenotyping as a proxy for the inflammatory response associated with obesity and metabolic syndrome

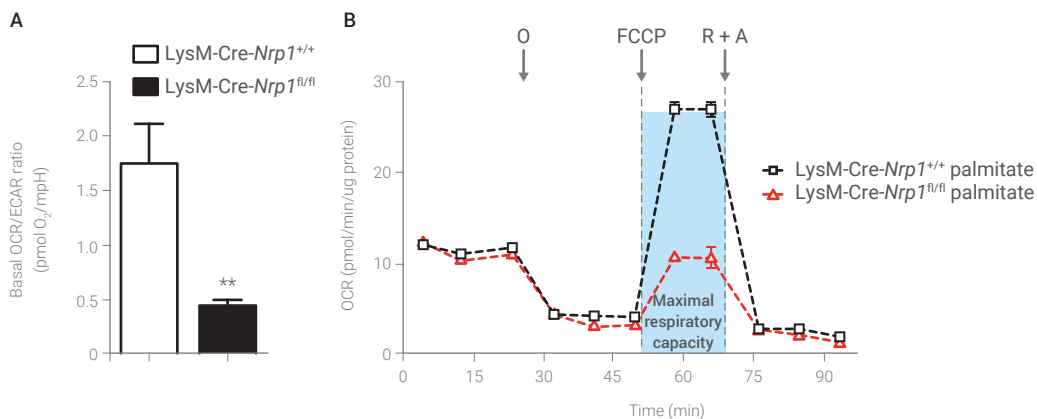


Figure 6. Agilent Seahorse XF technology shows that ablation of NRP1 in macrophages results in increased reliance on glycolysis (6A) while also compromising lipid uptake (6B), supporting the role of NRP1 in protecting against obesity and inflammatory glycolytic metabolism. Adapted from Wilson et al., 2018 *Sci Immunol.* 2018. 3 (21).

XF analysis provides insight into essential pathways, signaling, and nutrient requirements for anti-tumor immune responses relevant to the tumor microenvironment

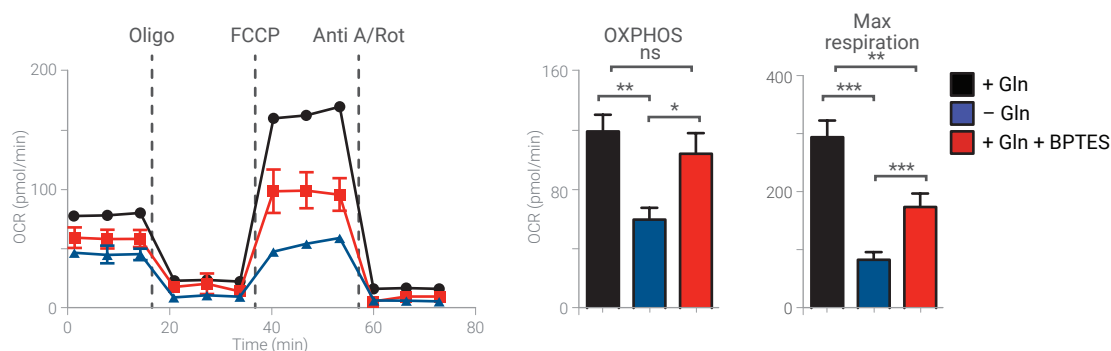


Figure 7. Agilent Seahorse XF resolved the mechanism of the cMyc expression in natural killer response by showing that glutamine withdrawal, but not the inhibition of glutaminolysis (via BPTES) results in impaired IL-2/IL-12 cytokine-stimulated NK cell response. Adapted from Loftus 2018 *Immunity.* Loftus, R. M., et al. 2018. *Nat Commun.* 2018. 9 (1): 2341.

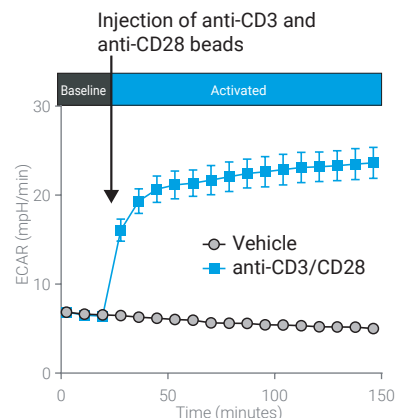
The Gold Standard Assays for Measuring Immunometabolism

Agilent Seahorse XF real-time immune cell activation assays deliver a rapid and robust indicator of activation

Immune cell activation is driven by a rapid switch towards growth-promoting pathways required for cellular proliferation. Researchers are using XF technology to measure immune cell activation in minutes, rather than the hours or days that would be required for this measure with typical markers.

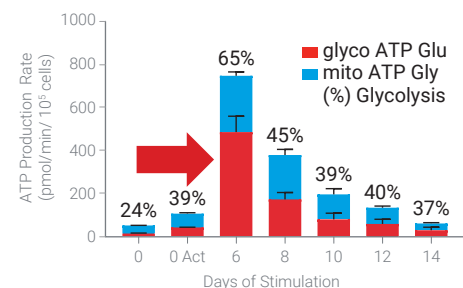
Immune cell specific:

- Agilent Seahorse XF real-time T cell activation assay
- Agilent Seahorse XF real-time Macrophage activation assay
- Agilent Seahorse XF real-time Neutrophil activation assay



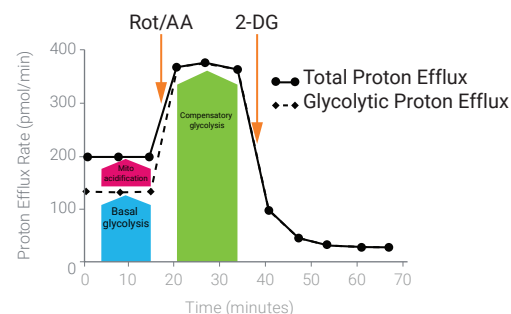
Discover a comprehensive picture of the drivers of immune cell fate and function

Agilent Seahorse XF Real-Time ATP Rate Assay Kit



Part number **103592-100** (XF/XFe)
and **103591-100** (XFp)

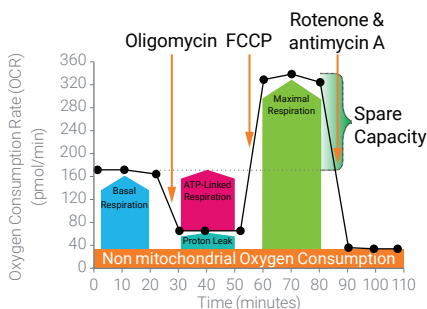
Agilent Seahorse XF Glycolytic Rate Assay Kit



Part number **103344-100** (XF/XFe)
and **103346-100** (XFp)

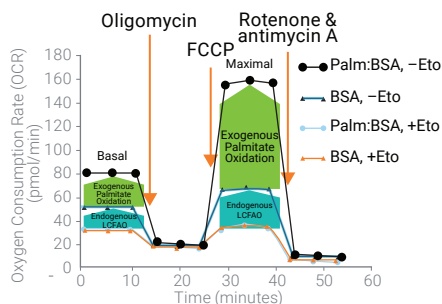
Assess fuel and substrate utilization using the Agilent Seahorse XF Cell Mito Stress Test kit and Palmitate-BSA FAO substrate

Agilent Seahorse XF Cell Mito Stress Test Kit



Part number **103015-100** (XF/XFe)
and **103010-100** (XFp)

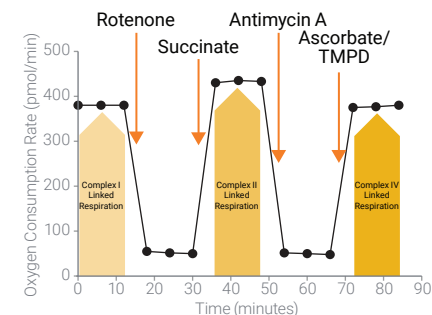
Agilent Seahorse XF Palmitate-BSA FAO Substrate



Part number **103015-100** (XF/XFe)
and **103010-100** (XFp)

Get isolated mitochondria data, without isolating mitochondria

Agilent Seahorse XF Plasma Membrane Permeabilizer



Part number **102504-100**

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