Determining the Metabolic Consequences of Pharmacological Modulation of T Cell Activation

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Introduction

Cellular metabolism is a primary regulator of immune cell fate and function.

T cell activation is correlated with metabolic shifts, especially in glycolysis, providing increased metabolic flux to support high rates of cellular proliferation[1,2].

XF Hu T Cell Activation Assay Kit[3] provides a rapid method to measure early activation-associated metabolic responses in human T cells, offering important insights into both the early dynamics of activation and its broader metabolic underpinning.

The assay employs a soluble CD3/CD28 activator, facilitating investigation of immune or metabolic modulators on T cell activation by providing a reliable and convenient workflow.

To exemplify the utility of the XF Hu T Cell Activation Assay in assessing the pharmacological modulation of activation, the effects of different tyrosine kinases on primary T cell activation were measured.

Experimental

XF Hu T Cell Activation Assay Workflow

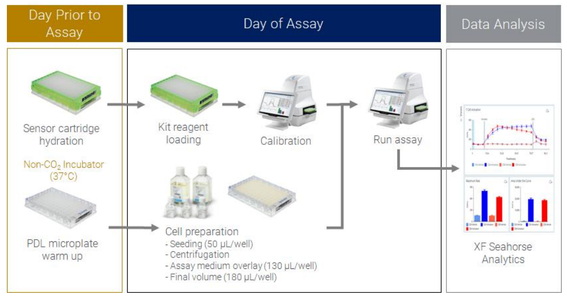

Figure 1. Workflow summary XF Hu T Cell Activation Assay.

XF Hu T Cell Activation Assay Designs

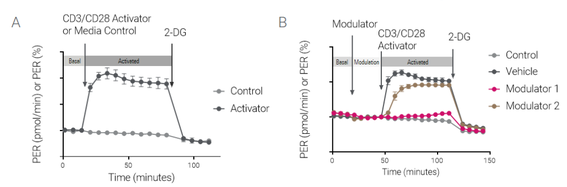

Figure 2. Agilent Seahorse XF Hu T Cell Activation Assays measuring the proton efflux rate (PER) upon the injection of a soluble CD3/CD28 activator in the standard assay design (A) and the modulation assay design (B). The glycolytic rate can be analyzed by the absolute PER (pmol/min) or by the relative increase to the baseline, PER (%).

Data analysis strategy of XF Hu T Cell Activation Assay

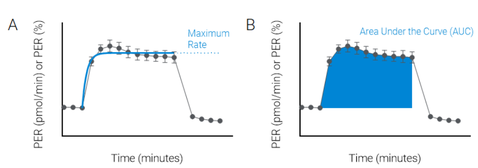

Figure 3. Data analysis of Agilent Seahorse XF Hu T Cell Activation Assay using XF Seahorse Analytics. (A) and (B) are schematic illustrations for the methods calculating the maximum rate and the AUC, respectively.

Results and Discussion

XF Hu T Cell Activation Assay can rapidly measure T cell activation potential

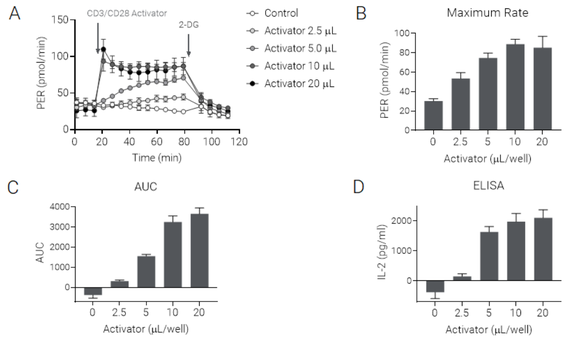

Figure 4. Activation of naïve CD4+ T cells by different amounts of the CD3/CD28 activator. The total injection volume of activator was fixed as 20 µL/well and the dose of activator was adjusted by diluting the indicated amount of activator in XF assay medium prior to loading in the injection port. (A) Assay kinetics exhibiting real-time changes in PER upon injection of the activator at different doses. (B) and (C) show maximum PER and AUC values at increasing activator doses. (D) IL-2 production at different activator doses as measured by ELISA with cells collected 72 hours post-activation.

Effective immune modulator dosing can be measured by using metrics calculated from T cell activation kinetic data

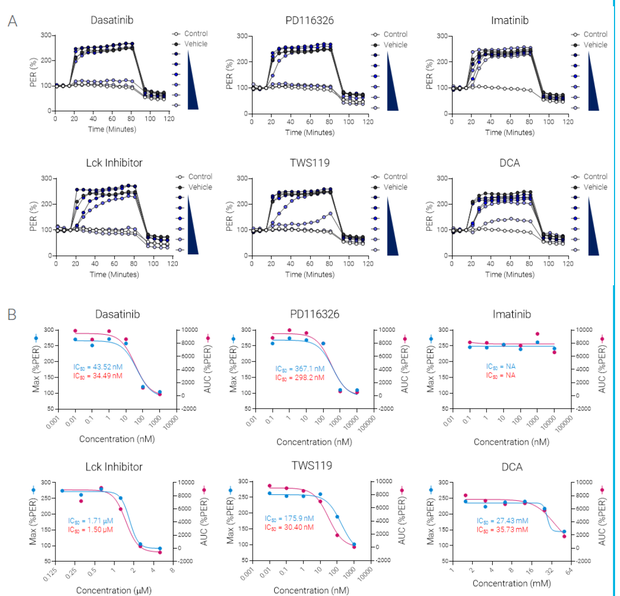

Figure 5. Modulator efficacy test using XF Hu T Cell Activation Assay Kit. Pre-activated CD4+ T cells were pretreated with different inhibitors at various concentrations for 30 min prior to the T Cell Activation Assay. (A) The kinetic graphs detected dose-dependent suppression of PER increase induced by CD3/CD28 Activator showing kinetic response variation depending on the inhibitor type. (B) The % maximum PER and % AUC values were calculated in Seahorse Analytics and plotted against the drug concentrations using GraphPad Prizm to assess IC_{50} values.

Acute effect of modulator can be evaluated by *in-situ* modulator injection

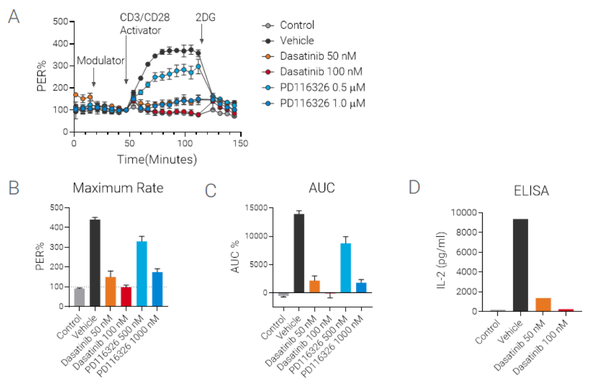

Figure 6. Evaluation of the acute effect of dasatinib and PD116326 on naïve CD4+ T cell activation by using modulation assay protocol. Dasatinib and PD116326 were injected at the concentration indicated prior to the activator injection. The concentrations of dasatinib and PD116326 were determined based on the IC_{50} value obtained from the data in Figure 5. (A) Kinetic graph of real-time changes in % PER; (B) % Maximum rates calculated; (C) % AUC calculated; (D) IL-2 production measured by ELISA using culture media collected at 72 hours post-cell activation.

Modulator effect on energy metabolism can be assessed by XF Real-time ATP Rate Assay[4]

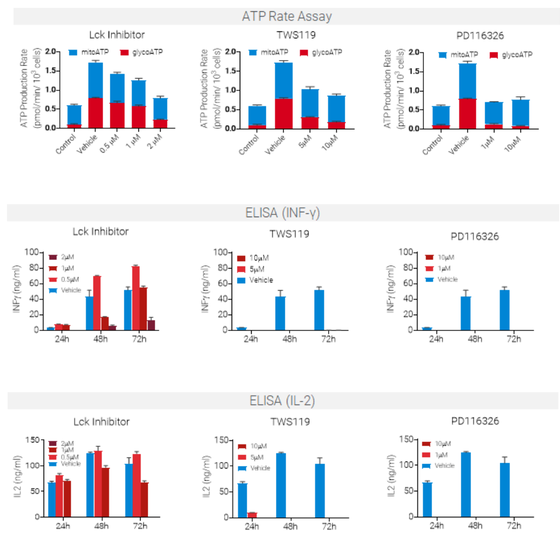

Figure 7. Naïve CD4+ T cells were pretreated with inhibitors and activated by beads conjugated with CD3/CD28 antibodies in Seahorse XF RPMI Medium, pH 7.4 containing 10 mM glucose,1 mM pyruvate and 2 mM glutamine (200K cells/well). After 2 h of beads injection, ATP production rate was measured using the Seahorse XF Real-Time ATP Rate Assay (A). Accumulation of IFN-γ (B) and IL-2 (C) were measured in the extracellular medium after 24 to 72 hours of activation.

Conclusions

By monitoring increases in PER, the XF platform enables a rapid method to visualize T cells activation in real-time.

The XF Hu T Cell Activation Assay Kit facilitates such measurements, allowing early detection of T cell activation, a real-time interrogation of activation kinetics, and an assessment of pharmacological or genetic activation modulation.

The assay employs a soluble CD3/CD28 activator and ready-to-use PDL-coated XF Cell Culture Microplates to maximize assay performance and provide a simple and convenient workflow.

Maximum PER provides an intuitive assessment of T cell activation potential, while AUC assessment enables interrogation of more complex kinetics.

Using the Standard assay design, these parameters can be used to generate quantitative dose response metrics useful for *in vitro* assessments of immune modulators as shown using Lck1 and Bcr-Abl kinase inhibitors.

Data is also presented illustrating how *in-situ* injections are utilized within the Modulation assay to conveniently reveal the immediate impact dasatinib in real-time, and these observations correlate with orthogonal ELISA measurements. This is particularly interesting as recent reports suggest dasatinib as a potential CAR-T cell activation modulator[5].

Additional metabolic interrogations including XF Real-time ATP Rate Assay can also be incorporated to determine the broader metabolic consequences of perturbed activation.

These data therefore illustrate how the XF Hu T Cell Activation Assay can provide additional insights into both chronic and acute immune modulators, demonstrating its utility in cellular and molecular immunotherapy discovery for various diseases including cancer, immune dysfunction, and metabolic diseases.

Materials

- **T cells:** Naïve CD4+ or CD8+ T cells (StemCell Technologies and Hemacare Corporation) and pre-activated cells cultured in ImmunoCult-XF T Cell Expansion Medium supplemented with IL-2 (StemCell Technologies) for 12 to16 days after the activation were used.
- **Kits:** XF Hu T Cell Activation Kit (Agilent Technologies).
  XF Real-Time ATP Rate Assay Kit (Agilent Technologies).
- **Plate**: XFe96 PDL Cell Culture Microplates (Agilent Technologies)
- **Modulators:**

Test Compound	Target
Dasatinib	Bcr-Abl kinase
PD116326	Bcr-Abl kinase
Lck Inhibitor (CAS No. 213743-31-8)	Lck1 kinase

Test Compound	Target
Imatinib	Bcr-Abl kinase
TWS119	GSK-3β
DCA	Pyruvate dehydrogenase

References

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3. Agilent Seahorse XF Hu T Cell Activation Assay Kit User Guide. Agilent Technologies technical overview, publication number: 5994-1811EN
4. Romero, N. *et al.* Quantifying Cellular ATP Production Rate Using Agilent Seahorse XF Technology. Agilent Technologies white paper, publication number: 5991-9303 EN.
5. Mestermann, K. *et al*. The Tyrosine Kinase Inhibitor Dasatinib Acts as a Pharmacologic on/off Switch for CAR T Cells. *Sci. Transl. Med*. 2019, *11*, eaau5907

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