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Simplifying Progress

Validation of Novel Continuous Live-Cell Assays for Immune Cell Activation and Killing of Blood Cell Cancers

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Overview

The blood cancers leukemia, lymphoma and myeloma are expected to cause the deaths of an estimated 56,840 people in the US in 2020. New immunological approaches afford great promise for improved therapies. Here, we describe novel high throughput live-cell image-based assays for immune cell activation and killing of blood cancer cells that are geared towards screening for new treatments for these malignancies. The data examples illustrate how continuous live-cell analysis can be used with suspension cells, to provide both additional biological insight (full time-courses, clustering information, morphology) and enhanced productivity (automation, miniaturization) compared to other techniques. We believe this approach will be a valuable addition to the technology toolbox for academic and industrial immuno-oncology researchers.

Continuous Live-Cell Analysis: Methodology

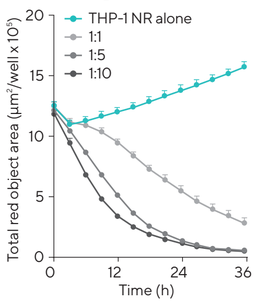
Incucyte® S3 Live-Cell Analysis System
A flexible assay platform that sits inside a standard tissue culture incubator. Incucyte® automatically and continuously acquires and analyzes HD phase and fluorescent images of living cells cultured in microplates, dishes, or flasks.

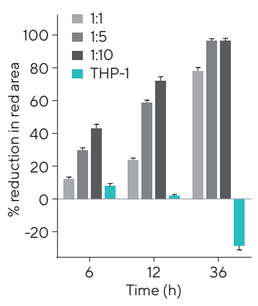
Incucyte® Software
Fast, flexible and powerful control hub for continuous live-cell analysis comprising image acquisition, processing and date visualization.

Incucyte® Reagents and Consumables
A suite of non-perturbing cell labeling and reporter reagents. Includes nuclear-targeted GFP and RFPs for cell counting, no-wash Caspase 3/7 substrate for apoptosis and cell kits for angiogenesis.

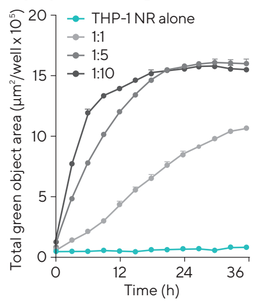
Immune Cell Killing of Leukemic Monocytes

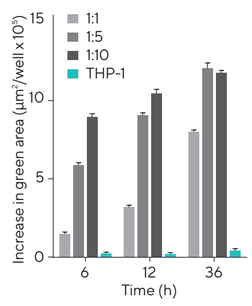
Viable Target Cells

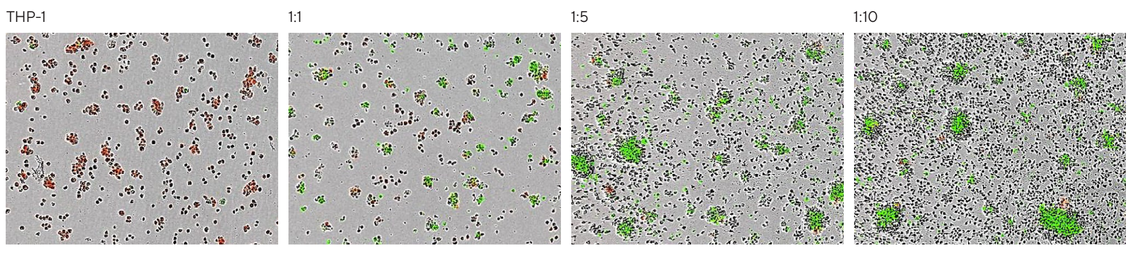




Apoptotic Target Cells







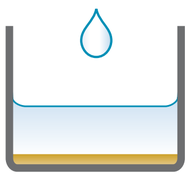
- THP-1 Nuclight® Red labeled monocytes (10 K/well) were seeded on 96-well PLO-coated flat-bottom plates in the absence or presence of pre-activated PBMCs (IL-2/α-CD-3 10 ng/mL, 4 days) and Incucyte® Annexin V green reagent
- Immune cell killing assay—note the time-dependent decrease in the number of red target cells and an increase in the Annexin V signal indicating cell death with increasing effector cell number
- Images show the aggregation of effector and target cells associated with cell death (increase in green fluorescence)

Non-Adherent Cell Assay Methodology

1. Coat wells

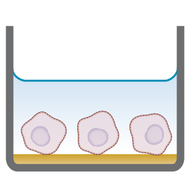
Add plate coating
Coat wells of plate (50 µL/well) with your choice of plate coating.

2. Add treatments



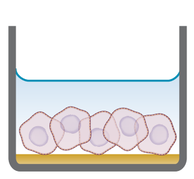
Add test agents
Add desired treatments (100 µL/well) at 2X final assay concentration.

3. Plate cells



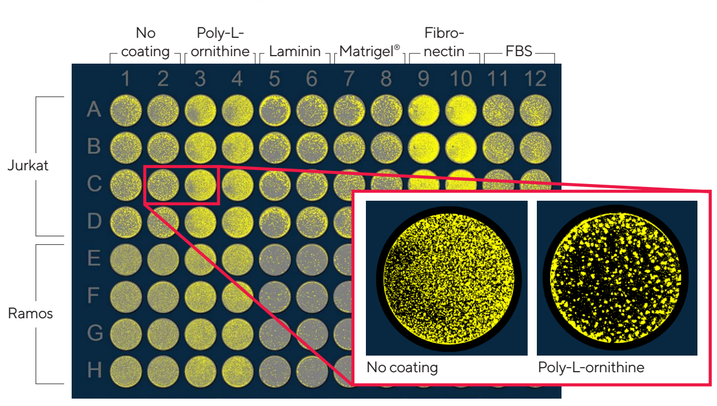
Seed cells
Seed cells (100 µL/well, 5,000–50,000 cells/well) into the 96-well plate.

4. Live-cell imaging

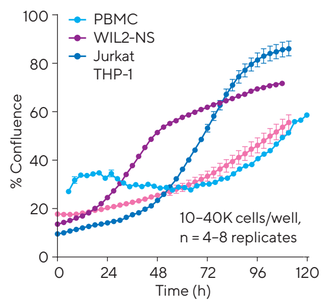


Automated imaging and quantitative analysis
Capture images every 1 to 4 hours (10X or 4X) in an Incucyte® system. Analyze using integrated software.

Plate-Coatings: Impact on Distribution of Cells

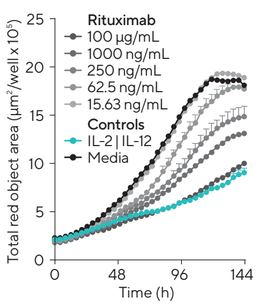


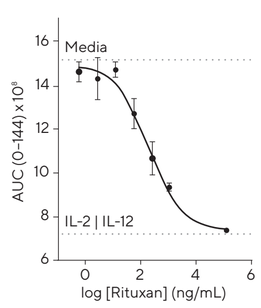
PLO-Coated

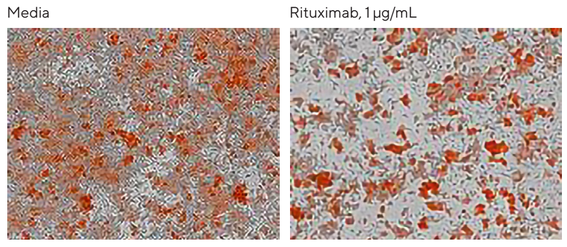


- Coating of plates with Poly-L-ornithine (PLO) facilitates uniform distribution of cells within each well
- Fibronectin is also suitable for most cell types but may enhance cell proliferation per se
- Yellow = phase confluence mask

Antibody-Dependent Cell-Mediated Cytotoxicity of B Cells





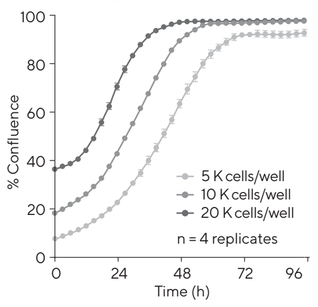


Images taken at 120 h

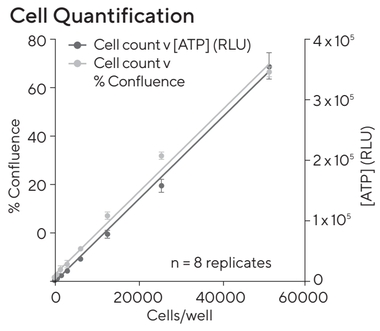
- CD20-positive Ramos B lymphocytes (10K/well), expressing Nuclight® Red, were seeded on 96-well PLO-coated flat bottom plates in combination with PBMCs (1:10 Target:Effector ratio)
- Rituximab induced a concentration-dependent ADCC (reduction in red fluorescence); IC_{50} 0.22 µg/mL
- IL-2 | IL-12 activation of the NK cell population was used as a positive control

% Confluence as a Measure of Cell Proliferation

Growth Curves



Cell Quantification

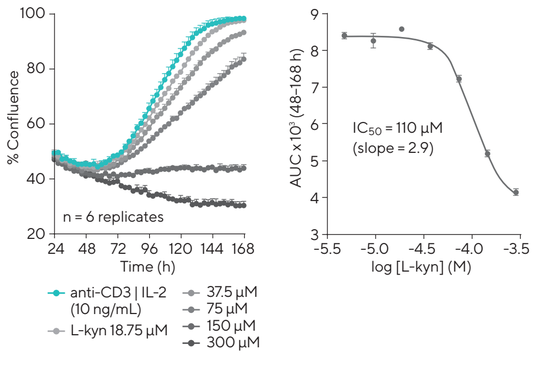


- Cell proliferation, quantified as % confluence with Incucyte® correlates with direct cell counting (Coulter counter, Scepter™) and ATP bioluminescence
- WIL2-NS cells were plated at the indicated densities on PLO-coated 96-well plates

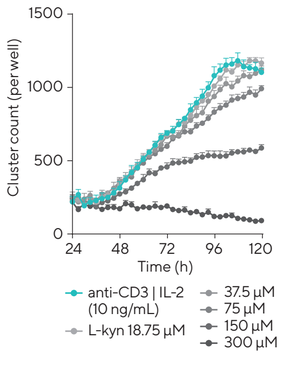
L-Kynurenine Inhibits T Cell Proliferation and Clustering

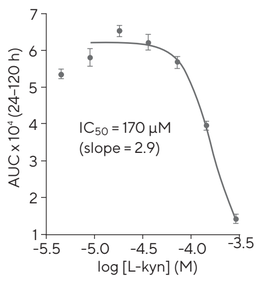
- L-kynurenine (L-kyn) is a metabolite formed from the catabolism of L-tryptophan by the enzymes IDO and TDO
- Some cancers increase L-kyn production in a bid to block antigen-driven T cell proliferation and induce T cell death, thus allowing cancer cells to escape immune surveillance
- Inhibitors of IDO and/or TDO are, therefore, promising therapeutic targets for the treatment of cancer

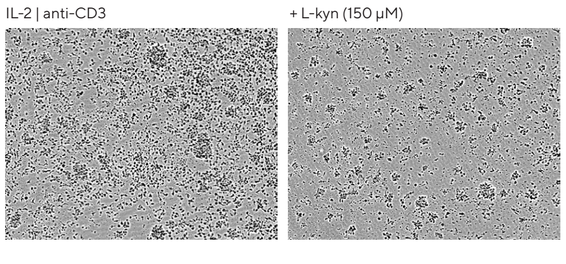
Proliferation



Cluster Formation



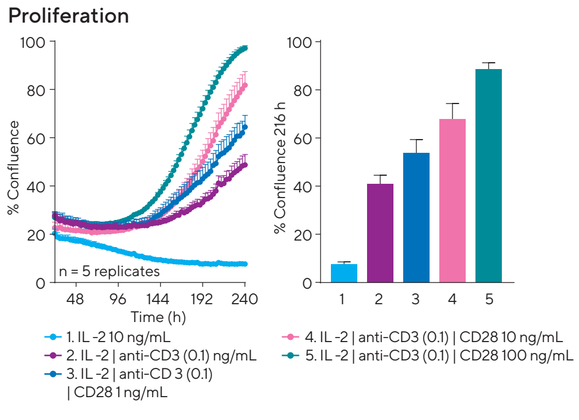




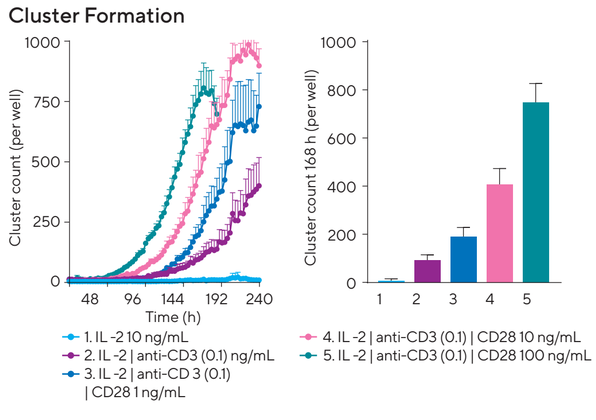
- Note the clear concentration-related inhibition of IL-2 | anti-CD3 activated T cell proliferation and clustering with the addition of exogenous L-kyn, over time.
- Time-course profiles enabled area under curve (AUC) analysis and generation of concentration-response curves from which IC_{50} values for inhibition of proliferation and clustering were determined.

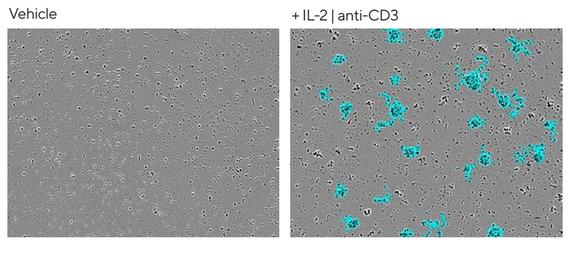
T Cell Activation is Stimulus and Concentration-Dependent

Proliferation



Cluster Formation





- PBMCs treated with combinations of IL-2 (10 ng/mL), anti-CD3 (0.1 ng/mL), and/or anti-CD28 (1–100 ng/mL)
- Note the lag period (approx. 96 h) before proliferation and the concomitant clustering of the PBMCs