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 leukaemia, lymphoma and myeloma are expected to cause the deaths of >55,000 people in the US in 2017. 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-course, clustering information, morphology) and enhanced productivity (automation, miniaturisation) compared to other techniques.
- We believe this approach will be a valuable addition to the technology toolbox for academic and industrial immuno-oncology researchers.

Immune cell killing of leukemic monocytes

Viable Target Cells

Apoptotic Target Cells

Antibody-dependent cell-mediated cytotoxicity of B cells

L-kynurenine inhibits T-cell proliferation & clustering

- CD20-positive Ramos B lymphocytes (10K/well), expressing NucLight Red, were seeded on 96-well PLO-coated flat-bottom plates in the absence or presence of pre-activated PBMCs (IL-2/CD-3 10ng/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).

Continuous Live-Cell Analysis: Methodology

IncuCyte® S3 Live-Cell Analysis System

A flexible assay platform that can include a standard tissue culture incubator. IncuCyte® automatically and continuously acquires and analyzes (20) phase and fluorescent images of cells cultured on microplates, dishes, or flasks.

IncuCyte® Software

First, flexible, and powerful control hub for continuous live-cell analysis comprising image acquisition, processing, and data visualization.

IncuCyte® Reagents & Consumables

A suite of non-fluorescent cell labeling and reporter reagents. Includes nuclear-targeted GFP and MPP for cell counting, co-wash compatible substrates for cell culture, and cell lines for applications.

Plate-coatings: impact on distribution of cells

No coating

Poly-L-ornithine

Laminin

Matrigel®

FBS

- Coating of plates with Poly-L-ornithine (PL0) 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

% Confluence as a measure of cell proliferation

- 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.

T-cell activation is stimulus & concentration-dependent

- 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 96h) before proliferation and the concomitant clustering of the PBMCs.

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