High Throughput Flow Screening Assays to Profile Cell-Mediated Killing

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Abstract

Cell-mediated killing is an immune response that involves the activation of cells such as phagocytes, natural killer cells (NK), or antigen-specific cytotoxic T-lymphocytes to induce death to pathogenic cells. Traditional assays for monitoring cell-mediated killing are only capable of homogenous live/dead readouts for an entire sample. IntelliCyt's iQue Screener is a high throughput suspension screening platform based on flow cytometry. The system can identify multiple cell types in solution and report on multiple cell killing readouts, including cell viability and apoptosis. Here we demonstrate high throughput flow assays for cell-mediated killing in examples using NK cells and chimeric antigen receptor (CAR) T-cells. For the NK cell assay, NK92 cells were utilized as effector cells and Jurkat cells were utilized as target cells. By labeling either target or effector cells with IntelliCyt's MultiCyt FL4 Cell Proliferation and Encoder dye, both cell populations can be simultaneously monitored. Viability was determined by cell membrane integrity and Caspase 3 activation for both Jurkat and NK92 cells, and the specificity of the killing response was demonstrated using inhibitors of cell killing activity. In the CAR T-cell assay, the efficacy of different CARs at targeting and killing a B-cell line (NALM-6) was profiled with multiplex assays. In addition to cell encoding and live/dead analysis, cytokine responses were evaluated using bead-based ELISA on the same analysis platform. These examples highlight the robustness and flexibility of the iQue Screener for performing multiplexed screening assays for cell mediated killing with greater contextual value.

Introduction to Cell Killing Assays

While immunotherapy approaches have seen tremendous success in the clinic, much work remains to be done to develop improved treatments for cancer. Harnessing the potential of the native immune system to target cancerous cells (Figure 1) is an area of research where the traditional assays utilized to study the response are limited. The "gold standard" chromium release assay utilizes a laborious protocol involving radioactive reagents, and only provides a homogenous assessment of viability for the entire sample.

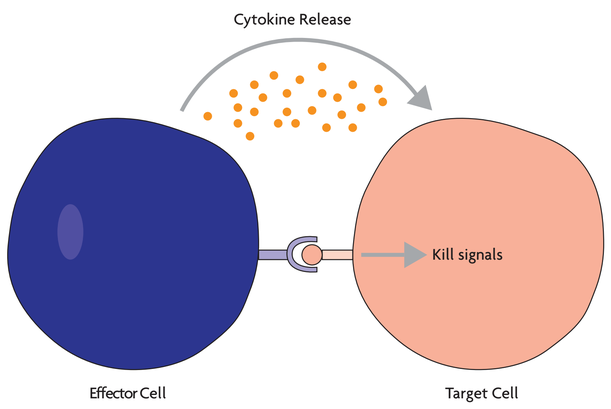

Figure 1. Schematic of the complex signaling involved in cell-to-cell killing. An effector cell capitalizes on multiple signaling mechanisms including recognition of cell surface receptors and secretion of cytokines to act upon a target cell, inducing death.

Because of the multifactorial nature of immune system responses to cancer, our high throughput discovery platform for immunotherapy is ideal because it enables:

- The ability to perform rapid, plate based experiments on cells in suspension, such as peripheral blood mononuclear cells (PBMC), isolated T and B cells, and other cells of the immune system.
- The simultaneous measurement of cells, cell sub-populations and secreted proteins in the same assay well provides high content information that can lead to a more complete profile of a treatment's activity.

In this study we describe the use of the iQue Screener and MultiCyt cell based reagents to develop a high throughput screening assay to assess cell mediated killing.

Cell Encoding to Differentiate Between Target and Effector Cells: Workflow

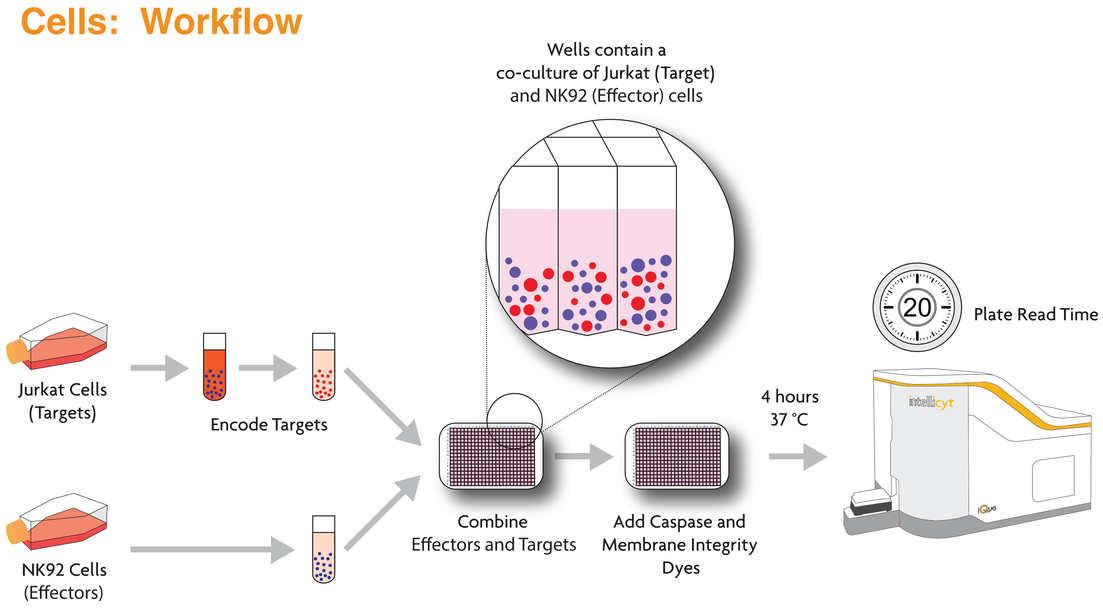

Figure 2. Screening Assay Workflow. Using a model of NK92 cells as effectors, and Jurkat cells as targets, a cell killing assay was demonstrated in a no wash workflow. First, target cells are encoded with Intellicyt's FL4 Proliferation and Encoding Dye, then combined in an assay plate with the target cells at various ratios. Caspase 3/7 activation and membrane integrity reagents are directly added to each well (ie. dyes are co-incubated with the cell treatments), and after a 4 hour incubation at 37°C, data are acquired on the iQue Screener. A 384-well plate can be read and fully analyzed in ~ 20 minutes, with approximately 500 target cells per sample.

Multiplexed Readouts Provide Context to Cell Killing

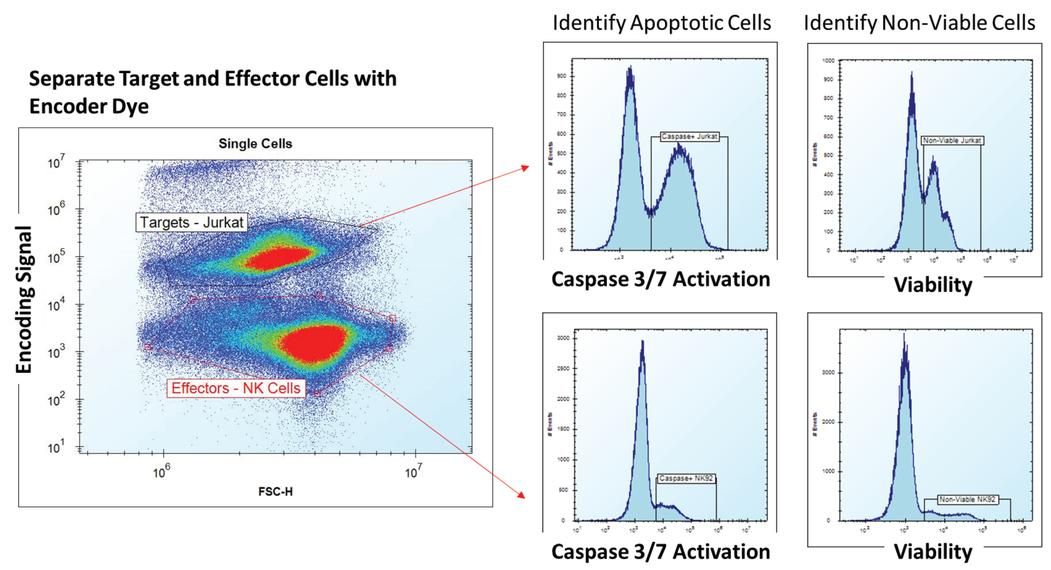

Figure 3. Multiplexing of the cell killing assay by encoding the target cells, and evaluating caspase 3/7 activation in addition to cell viability provides a much more robust, contextually relevant data set. The cell encoding reagent utilized for this study non-specifically binds to intracellular proteins, imparting a strong fluorescence signal and allowing the clear differentiation of target cells from effector cells mixed into a single sample. Once the cell populations have been defined, each cellular subset can be individually queried for downstream endpoints. The activation of caspase 3/7 and loss of membrane integrity of the target cells can be assessed, individually from the same signals being expressed by the effector cells.

Profiling of Compound Effect on Target and Effector Cells Yields Insight into Specificity

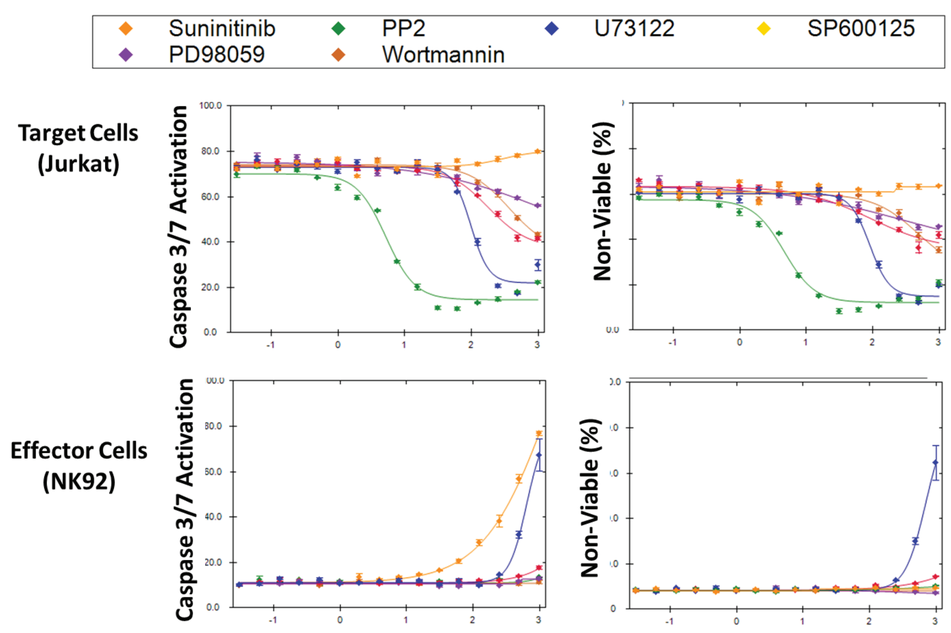

Figure 4. The ability to individually analyze the target and effector cell populations enables the assessment of compound specificity. Six compounds that were reported to affect NK cell function according to different mechanisms were profiled, and several distinct profiles were observed. In a highlighted example, Suninitinib (orange diamond) did not significantly activate caspase 3/7 in the target cells, but was shown to be cytotoxic to the NK92 cells. PP2 (green diamond) showed specificity towards killing of the target cell, this time with no effect to the effector cell on either caspase activation or cell viability.

Screening for Chimeric Antigen Receptors (CAR T-Cells)

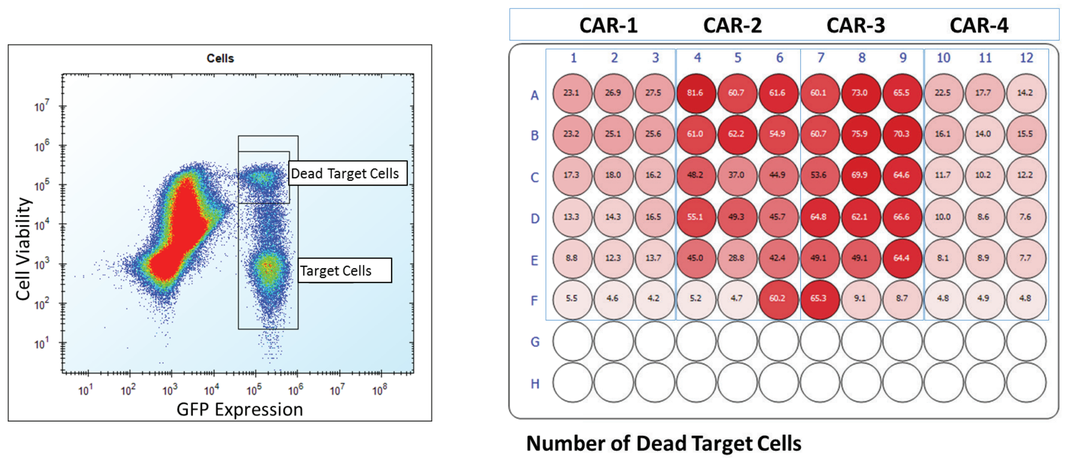

Figure 5. In an assay model screening for efficacy of CAR T-cells, various CARs were constructed and tested against a NALM-6 cell line. The target cells were added to the assay at a fixed number (5000/sample) and were GFP-tagged. Effector cells in varying numbers (E:T ratios ranging from 20:1 down to 1:1) were tested. Based on the heatmap visualization in ForeCyt, the CARs with higher killing activity (CAR-2 and CAR-3) are easily identified.

Summary

The iQue Screener, with IntelliCyt's MultiCyt cell based reagents provides a robust multiplexed assay platform for immunotherapy studies. In examples of cell-mediated killing assays, the system features:

- Simultaneous detection of target and effector cell biology
- High throughput: Less than 20 minutes to run 384 well plate
- Straightforward data analysis of plate level data using ForeCyt analysis software

Because of the speed of the iQue Screeer and the high content information of this assay, it can easily be incorporated into profiling screens aimed at defining functional activity for cell-mediated killing assays.

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