Development of a High-Throughput Imaging Screen for the Functional Assessment of Cell-Linking Moieties Using Effector And Target Cells In A Cell Kill Assay

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Abstract

Cell linking moieties (CLMs) are biologically inspired linkers designed to recruit CD3+ T cells to specific tumor cell targets via defined signaling events. In this study, we report the development of a high-throughput screen for CLM activity, which was designed to assess CLM-specific effector functions. Specifically, killing ability, and then a secondary screen is employed to provide dose-activity curve ranking of best performing CLMs. Data generated from the primary and secondary screen were analyzed to identify CLMs capable of testing a large number of candidate CLMs for specific tumor cell killing activity.

Experimental Objectives

1. To develop and implement an efficient high-throughput screen that can evaluate large numbers of candidate CLMs.
2. To evaluate CLM-directed killing ability against specific targets.
3. To provide dose-activity curve ranking of best performing CLMs.

Cell Linking Moieties (CLMs) for Directing Effector cells to Tumor Cell Lysis

High Throughput Image-Based Screening Overview

Image-Based Analysis Visualizes Kinetic PBMC Migration to and Killing of Cancer Cells, Corroborated by CytoTox-Glo Protease Release Assay

Consistent PCR Product Transfection allows for Reproducible Target Cell Killing in 384-Well Plates

Optimized Effector:Target Ratio selected with Reduced Background Killing Activity and Increased Assay Dynamic Range

Directed Killing of Cells by CLM Requires Specific Binding to Target Surface Markers

Hit Screening Depicts Similar Ranking of Highly Effective CLMs, Amongst Multiple PBMC Donors Evaluated

Secondary Screening of Top Candidate from Hit Screen Shows Dose Response and Ec50 in Multiple Cancer Cell Types

Conclusions

1. Successfully developed a high content fluorescent image-based screening assay capable of testing a large number of candidate CLMs for specific tumor cell killing activity.
2. This image-based screening platform allows for well- and cell-level killing information to be obtained in complex cell mixtures without the presence of radiative compounds.
3. Kinetic analysis achievable from analysis of the same well at multiple time-points to understand the dynamics of killing activity without having to sacrifice assay wells.

Through this POC study, a platform assay using non-terminal high content fluorescent imaging was primed for screening new target candidate CLMs and can be used to rapidly select optimal constructs, resulting in a shortening of the developmental timeline.