Using the Intellicyt® iQue Screener PLUS to Profile Antibody Dependent Cell Cytotoxicity

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Introduction:

During the antibody discovery workflow, functional studies are needed to screen for desired antibody characteristics. Antibody mutational screening and other processes to increase antibody potency or functionality require multiple rounds of monitoring.

Therapeutic antibodies often mediate direct killing of cancer cells through antibody dependent cell-mediated cytotoxicity (ADCC). Traditional ADCC assays use a homogenous live/dead readout, which greatly limits the contextual and correlative value of the screening data. To overcome this limitation, a high throughput, multiplex screening assay profiling ADCC was developed and analyzed using Intellicyt's iQue Screener PLUS and integrated ForeCyt® software. This screening assay can discriminate between effector and different target cell populations in the same sample, and performed in 96 or 384 well plates. The Panorama feature in ForeCyt allows for easy data visualization, even in large, multi-plate screening campaigns.

Methods:

Cells and Reagents: The CD20 positive human B-cell lines Ramos and Raji (ATCC, Manassas VA) were used for the studies. The CD20 negative HL-60 cell line was used in multiplex assays. Cryopreserved PBMCs were purchased from Astarte Biologics (Bothell WA) and cultured for 20-24 hours prior to the assay. Truxima was a generous gift from BioOutsource (Glasgow Scotland). Anti-hCD20 IgG1, IgG2 and IgG mutants were purchased from InvivoGen (San Diego CA).

ADCC Assays: Assays were done in either 96- or 384-well plates. Briefly, target cells were stained with different concentrations of VL1 encoding dye (Intellicyt, Albuquerque NM) in order to discriminate the different cell populations. A dilution series of the various antibodies (25,000 – 0.01 ng/ml) were added to the target cells. PBMCs were added at various E:T ratios (5-40). After overnight incubation, the cells were stained with FL1/BL1 viability dye (Intellicyt, Cat# 90342) prior to data acquisition on iQue Screener PLUS.

Data Acquisition and Analysis: Data was acquired on iQue Screener PLUS using 7 second sips (~12 µl) per well. Plate level gating is shown in Figure 1. Four parameter curve fits, EC_{50} values, and heat maps were generated using the integrated ForeCyt software (v6.2). Heat maps, profile maps and hit ranking from the multi-plate antibody mutation screen was produced using the Panorama feature in ForeCyt.

Plate Design and Gating Strategy:

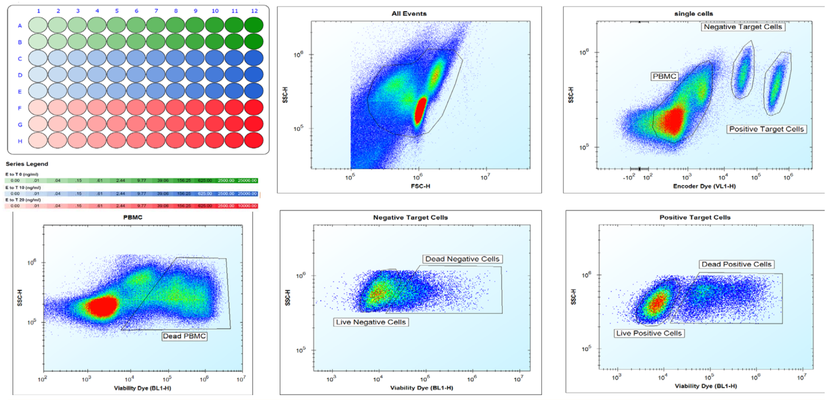


Figure 1: Plate Level Gating. ADCC assays were set up in 96-well plates. CD20 antibodies were diluted across the plate using different E:T ratios. Positive and negative target cells and PBMC were gating on VL1 (ex 405 nm; detect 445 nm) encoding dye intensity. Cell viability was assessed for each population using FL1/BL1 membrane integrity dye (ex 488 nm; detect 530nm).

ADCC Characterization and EC_{50} Assays:

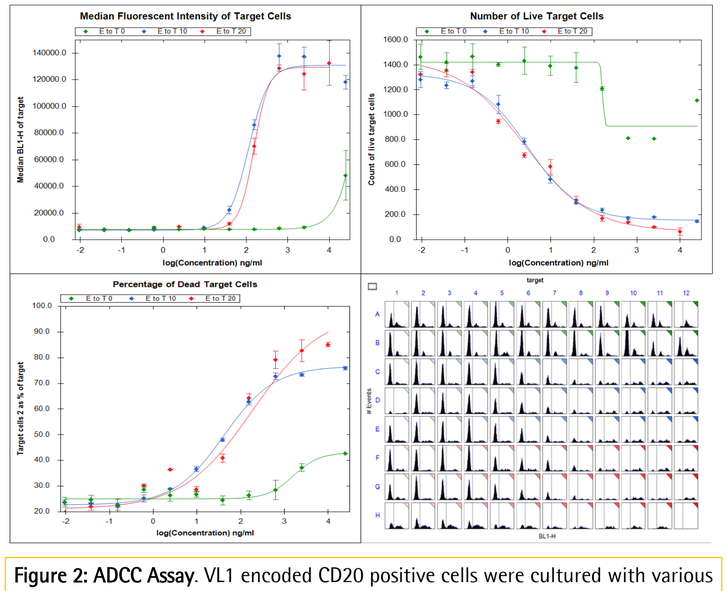


Figure 2: ADCC Assay. VL1 encoded CD20 positive cells were cultured with various dilutions of Truxima and with PBMCs (E:T of 0, 10 or 20). Target cell viability was measured by BL1 median fluorescence intensity, live cell counts and dead (BL1 positive) target cells as a percentage of total target cells. In all parameters, cell death rose with increasing antibody concentration in the presence of effector cells. Cell death occurred at the high antibody concentration in the absence of effector cells, consistent with published *in vitro* studies using CD20 antibodies.

ADCC Antibody Specificity:

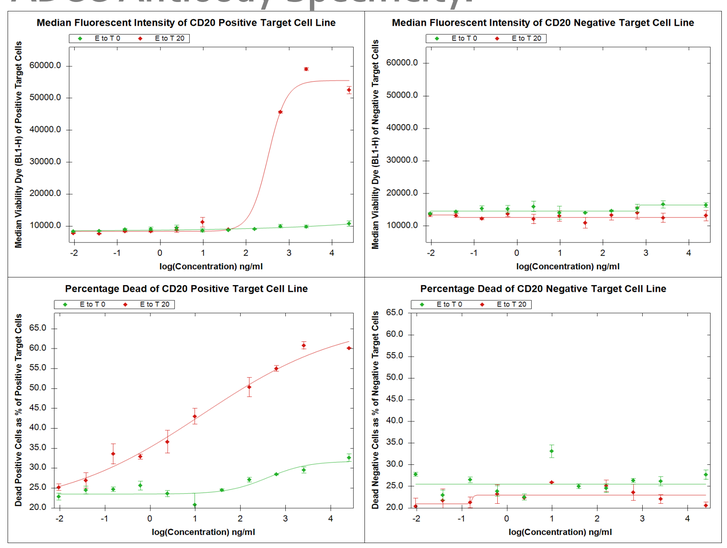
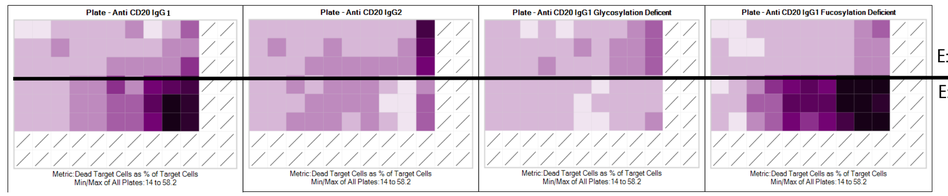


Figure 3: ADCC Antibody Specificity. VL1 encoded CD20 positive and CD20 negative cells were cultured in each well. The ADCC assay was performed and data was analyzed as in Figure 2. Concentration dependent cell death was observed in the CD20 positive cells, but no loss in cell viability was seen in the CD20 negative cell lines

Multi-Plate Screening of Antibody Mutants:

A) Heat Map: Percentage of Target Cell Death



B) Profile Map: Criteria >2-fold Target Cell Death and < 50% Monocyte Death

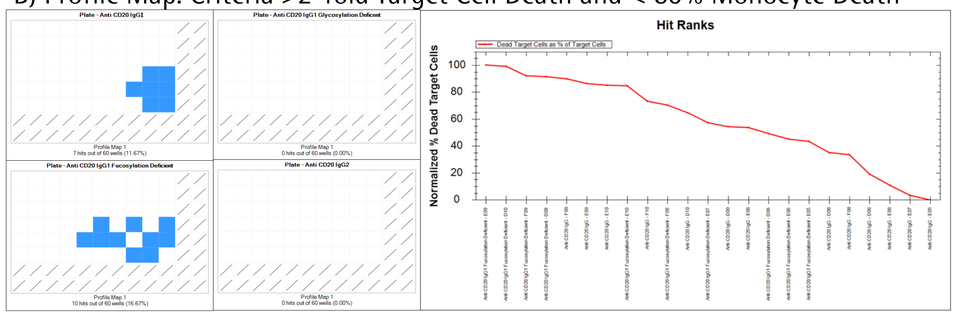


Figure 4: Screening of CD20 Antibody Mutants. VL1 encoded CD20 positive cells were cultured with increasing amounts of CD20 IgG1, CD20 IgG2 or IgG1 mutants that inhibit either Fc glycosylation or fucosylation. Published studies show the glycosylation mutants inhibit ADCC activity while fucosylation mutants enhances ADCC. A) Heat maps show target cell death across the entire screening campaign. The top half of each plate contained no effector cells while PBMCs (E:T 40) were added to the wells of the bottom half of the plates. B) Effector cell death can occur during ADCC and we observed an anti-CD20, dose response loss of monocytes. Profile maps were created showing wells that had high ADCC activity with low monocyte death. Line graph of hits from the profile map rank the wells containing the highest ADCC activity.

Conclusion:

- The iQue Screener PLUS can be used to perform multiplexed ADCC assays for functional analysis during antibody discovery and in antibody engineering/mutation/humanization screens
- Cell viability can be measured from positive and negative target cell lines and effector cells from the same sample well.
- Q-bead-based assays to measure levels of secreted cytokines and other protein can be multiplexed with the ADCC assay
- Antibody specificity and EC_{50} measurements are quickly and easily analyzed using ForeCyt software
- Antibodies with the desired functional criteria can be easily identified and ranked from large multi-plate screening campaigns using the Panorama feature of ForeCyt