Implementing MOA-Reflective ADCC Assays using Ready-to-Use KILR Target and Effector Cells from Screening to Lot Release

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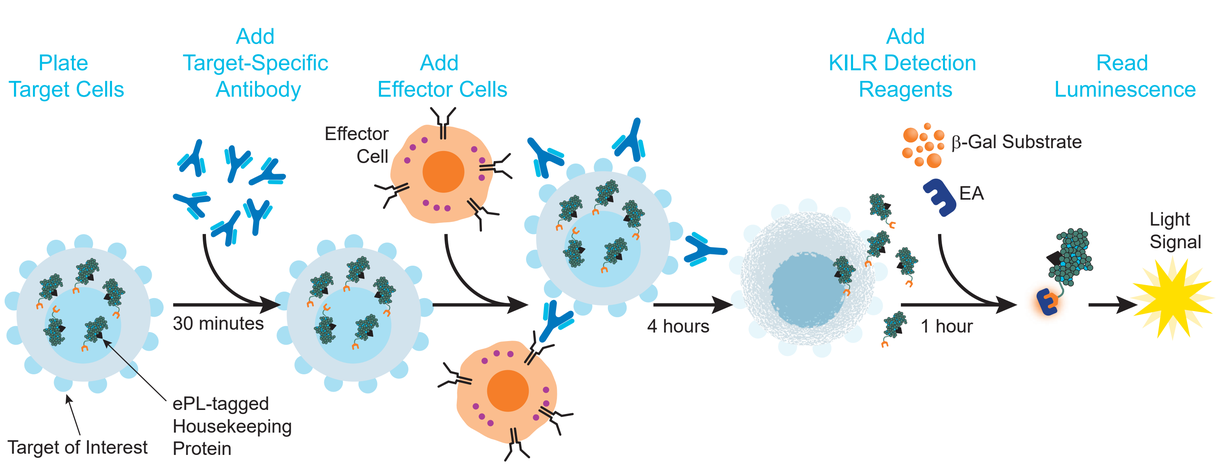
Abstract

The clinical success of an ever-increasing array of biologics has led to the development of a wide spectrum of immunomodulatory agents with distinct mechanisms-of-action (MOA) targeting novel antigens. During development of antibody-based biologics, evaluation of antibody (Fc) effector functions is required by regulators as is needed for antibody dependent cell-mediated cytotoxicity (ADCC). Increasingly regulators are requiring that ADCC assays, especially those used for lot release applications, measure immune cell-mediated killing rather than a surrogate endpoint for antibody engagement of antigen on target cells.

Eurofins DiscoverX has developed a novel cytotoxicity assay platform technology (KILR®) that specifically measures killing of antigen-expressing target cells in a co-culture with immune cells, in an easy-to-use, dye-free, and radioactivity-free assay format. Based on the industry-validated Enzyme Fragment Complementation (EFC) technology, the KILR platform can be applied to multiple immune cell-mediated killing events, including ADCC, CDC, ADCP, and T-cell redirection.

In this poster, we share examples of the universality of the KILR platform for development of ADCC assays for multiple antigens (e.g. HER2, CD20, CD19, CD38, CD33), and with a variety of effector cell types. Importantly, to ensure long-term assay reproducibility many KILR target cell models have now been developed in an assay-ready format. When used in combination with our engineered KILR CD16 Effector Cells, these KILR assay-ready target cells produce robust assay windows with excellent assay repeatability. We share phase-appropriate qualification data for the KILR Raji bioassay model that demonstrates that these assays are fit-for-purpose for screening and relative potency applications in lot-release testing.

Specifically Measure Target Cell Death with KILR ADCC Assays

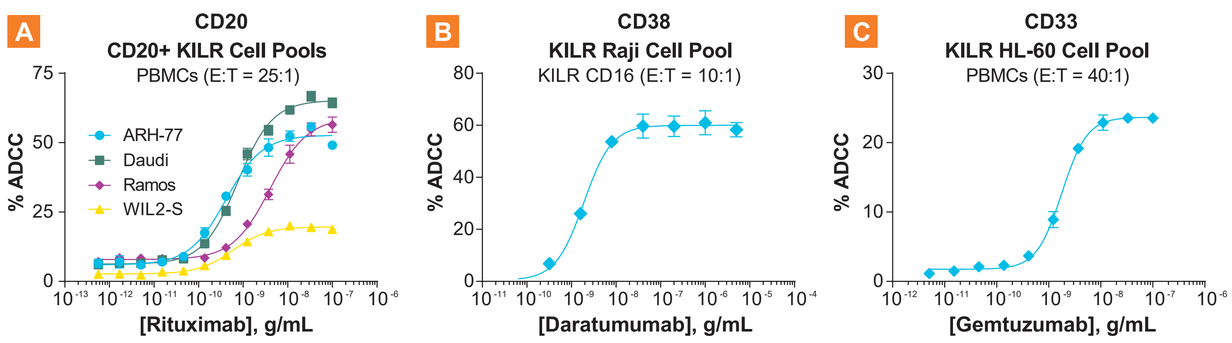


Target Cells
- KILR target cell models (>35)
- Target cells generated using KILR retroparticles

Effector Cells
- PBMCs, NKs
- NK-92 cell line
- KILR CD16 Effectors

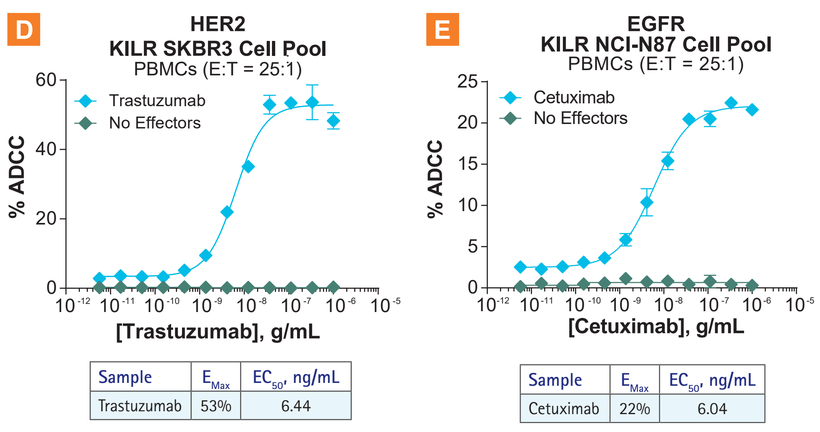
Figure 1. KILR ADCC cytotoxicity assay principle is based on the industry-validated EFC technology. Target cells expressing the relevant antigen are engineered to stably express a housekeeping protein that is tagged with a reporter fragment called enhanced ProLabel® (ePL). This reporter fragment is the small enzyme donor fragment of β-galactosidase (β-gal) that is inactive when not paired with its larger enzyme acceptor (EA) fragment. When the stable target cell line is incubated with appropriate effector cells and a test antibody, effector-mediated killing releases the tagged protein into the media. The ePL-tagged reporter protein (also called the KILR Reporter Protein) is detected in the media by the addition of detection reagents containing the EA fragment of β-gal. This leads to the formation of the active β-gal enzyme, which hydrolyzes the substrate to give a chemiluminescent output detected on any bench top luminometer. Overall, the KILR platform is extremely flexible, and can be used with many different effector cell types (PBMCs, purified NK or T cells, engineered effectors, macrophages, etc), allowing the assay to be used for quantitation of multiple MOAs, including ADCC, CDC, T-cell redirection, killing mediated by TILs or CAR-T's, and even ADCP.

Evaluate Diverse Cancer Models with KILR ADCC Assays



Sample	E_{Max}	EC_{50}, ng/mL
Daratumumab	60%	1.89

Sample	E_{Max}	EC_{50}, ng/mL
Gemtuzumab	24%	1.78

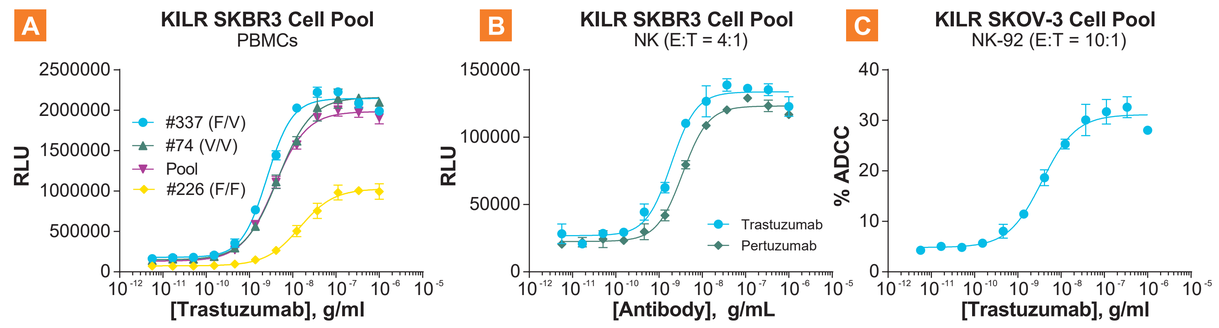


Sample	E_{Max}	EC_{50}, ng/mL
Trastuzumab	53%	6.44

Sample	E_{Max}	EC_{50}, ng/mL
Cetuximab	22%	6.04

Figure 2. The KILR platform is suitable for the detection of ADCC mediated by antibodies targeting multiple antigens in diverse cancer models (stable pools expressing the KILR reporter, to maintain heterogeneity of the native cell line), with different effector types. A. Rituximab-mediated ADCC in four different CD20+ B-lymphoblast KILR models (ARH-77, Daudi, Ramos and WIL2-S) using primary PBMCs at an effector-to-target ratio (E:T) of 25:1. B. ADCC mediated by the anti-CD38 therapeutic antibody, Daratumumab, in the KILR Raji cell model using engineered effector cells (KILR CD16 effector cells) at an E:T of 10:1. C. ADCC mediated by the anti-CD33 therapeutic antibody, Gemtuzumab (approved for treatment of AML), in the KILR HL-60 cell model using primary human PBMCs at an E:T of 40:1. D. ADCC mediated by the anti-HER2 therapeutic antibody, Trastuzumab (approved for treatment of metastatic breast cancer), in the KILR SKBR3 cell model using primary human PBMCs at an E:T of 25:1. E. ADCC mediated by the anti-EGFR therapeutic antibody, Cetuximab (approved for treatment of metastatic colorectal cancer), in the KILR NCI-N87 cell model using primary human PBMCs at an E:T of 25:1.

KILR ADCC Assays are Compatible with Multiple Effector Cell Types



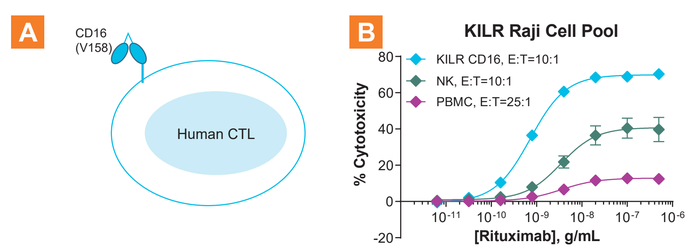
Donor	S/B	HillSlope	EC_{50}, ng/mL
#226 (F/F)	13.7	1.113	14.6
#337 (F/V)	13.9	1.486	2.53
#74 (V/V)	15.2	1.128	4.54
Pool	15.5	1.161	3.76

Sample	S/B	HillSlope	EC_{50}, ng/mL
Trastuzumab	5	1.501	1.92
Pertuzumab	5.5	1.463	3.48

Sample	S/B	HillSlope	EC_{50}, ng/mL
Trastuzumab	7.6	1.107	3.63

Figure 3. KILR ADCC Assays are compatible with multiple effector types. A. ADCC using primary PBMCs from 3 different donors characterized as a F158 homozygote (F/F; #226), a F158/V158 heterozygote (F/V; #337) or a V158 homozygote (V/V; #74). PBMCs with V/V or F/V genotypes should have higher affinity for the Fc portion of the IgG1-based antibody, and therefore are expected to produce a more potent ADCC response, whereas the F/F donor that is expected to have the least potent response, as observed here. B. ADCC with two HER2-targeting antibodies using primary NK cells in the KILR SKBR3 model. C. ADCC with Trastuzumab using an engineered NK-92 cell line as effector cells, with the NK-92-resistant cell line SKOV3.

Robust ADCC Assays with Single-Donor Derived KILR CD16 Effector Cells



Effector Cells	EC_{50} ng/mL	S/B	E_{Max}
KILR CD16 (10:1)	0.73	28.2	70%
Primary NK's (10:1)	3.46	10.9	41%
PBMCs (25:1)	3.51	4.9	13%

Figure 4. Improved ADCC performance with KILR CD16 Effector Cells. A. Single Donor Derived KILR CD16 Effector Cells are engineered cytotoxic T lymphocytes (CTLs) that are transfected with CD16 (FcγRIIIa- V158). KILR CD16 Effector Cells are a polyclonal population of predominantly (>98%) CD8+ cells that are also positive for CD3 with robust and stable expression of CD16 that are also positive for CD3 with robust and stable expression of CD16. KILR CD16 Effector Cells have functional CD3 B. More robust ADCC observed with KILR CD16 Effector Cells than with primary cells. In KILR Raji cells treated with Rituximab, KILR CD16 Effector Cells mediate a 2.5-fold higher assay window and E_{max} than primary NK cells when used at the same effector-to-target (E:T) ratio (E:T= 10:1). The difference in assay window and E_{max} is even more pronounced when comparing primary PBMCs (used at an E:T= 25:1).

ADCC Workflow Using Assay-Ready KILR Target Cells

Thaw & Wash Cryopreserved KILR Target Cells → Count viable cells; add target cells to assay plate → Opsonize target cells with antibody → Add effector cells to assay plate → Incubate 4h @ 37°C → Add KILR Detection Reagent (1h @ R/T). Read plate

Qualification Data with Rituximab Demonstrates Suitability of KILR Raji Bioassay for Relative Potency Assays

A Qualification Study Design

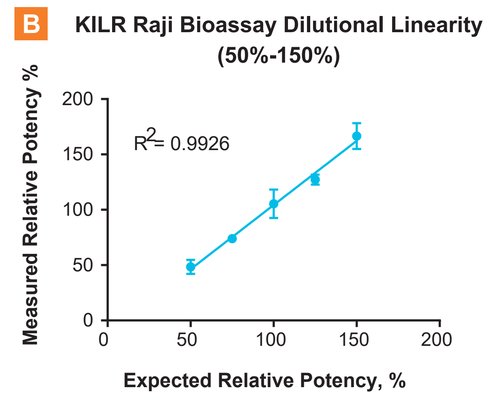
Details of Study Design

Evaluated 5 nominal concentrations (NC) over a range of 50%-150% (n ≥ 4 for each NC)

Repeatability: 4 Runs of 100% NC by single analyst

Intermediate precision incorporates:
- 2 Analysts
- Multiple days
- 2 Lots of Bioassay Target Cells
- 3 Lots of KILR CD16 Effectors

Day	Nominal Concentrations	Analyst
1	100% x 4 (Repeatability)	1
2	150%, 125%, 75%, 50%	1
2/3	150%, 100%, 50%	2
3	150%, 125%, 75%, 50%	1
4	125%, 75%	2
4	150%, 125%, 75%, 50%	1



Parameter	Value	Specification
Accuracy (Average % Recovery)	103%	100% +/- 20%
Repeatability	14.2%	≤20%
Intermediate Precision	≤13.2%	≤20%
Linearity (R2)	0.9926	≥0.95

Figure 5. Qualification data for KILR Raji Bioassay using Rituximab. A. Study design for qualification of KILR Raji ADCC Bioassay. Five nominal concentrations of rituximab were evaluated over a range of 50-150%. Repeatability (4 runs) was assessed at the 100% nominal concentration by a single analyst. Intermediate precision incorporated several sources of variability, including multiple analysts, multiple days, 2 lots of KILR Raji Bioassay target cells, and 3 different lot of KILR CD16 Effectors. B. Results obtained from qualification study indicate very good accuracy, high repeatability, intermediate precision, and dilutional linearity for KILR Raji Bioassay.

Summary

- MOA-reflective KILR cytotoxicity assays specifically measure direct killing of antigen-expressing target cells in co-culture with effector cells mediated via ADCC
- KILR target cell models are amenable to use in an assay-ready format and produce data with excellent repeatability and reproducibility
- KILR Raji and Daudi Bioassays are fit-for-purpose for screening and relative potency applications in lot-release testing
- Over 40 target cell models available for development of bioassay format and additional target cell models can be generated using KILR retroviral particles
- Single donor-derived KILR CD16 Effector Cells eliminate donor variability in ADCC assays and ensure assay reproducibility with high signal to background ratios
- IND-enabling characterization and assay qualification studies with the clinical molecules can be performed through Eurofins DiscoverX custom assay development program

Learn more about the Eurofins DiscoverX cytotoxicity KILR assays at discoverx.com/kilr