Quantify Diverse Immune Cell-Mediated Killing Mechanisms: Applications of a Robust, Non-Radioactive KILR Cytotoxicity Platform

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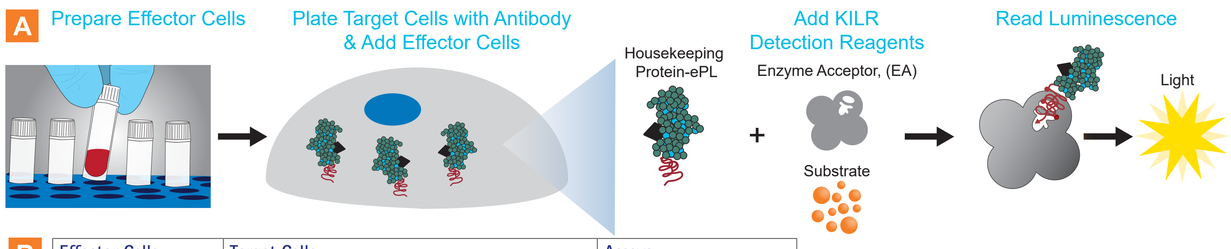
Abstract

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. These biologics include classical monoclonal antibodies, but also increasingly bi- and multi-specific antibodies designed to redirect T-cells to tumors and modulate anti-tumor T-cell responses. During the development of such therapeutics, classical antibody (Fc) effector functions are monitored, such as antibody dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). However, the measurement of another type of effector-mediated killing, known as antibody-dependent cellular phagocytosis (ADCP) has gained traction. Here, we present KILR®, a novel cytotoxicity assay platform technology that specifically measures killing of target cancer cells in a co-culture with immune cells, in an easy-to-use, dye-free, and radioactivity-free assay. Based on the industry-validated Enzyme Fragment Complementation (EFC) technology, the KILR platform is useful for multiple applications such as ADCC, CDC, ADCP, and T-cell redirection.

We discuss examples where the same engineered target cell line is used to determine ADCC, CDC, and ADCP capabilities of an antibody drug. Generating a very low background, these assays produce robust assay windows with excellent precision, and are applicable to various stages of drug development, ranging from screening to use in QC lot release of complex biologic drugs. KILR Cytotoxicity Assays have been tested using multiple primary effector cells (PBMCs, Macrophages, and NKs) or engineered cells lines (NK-92). Further, these assays can be used to evaluate other immunotherapy drugs such as T-cell redirecting bi-specific antibodies and chimeric antigen receptor T-cells (CAR-T), and tumor-infiltrating lymphocytes (TILs).

KILR Cytotoxicity Assays



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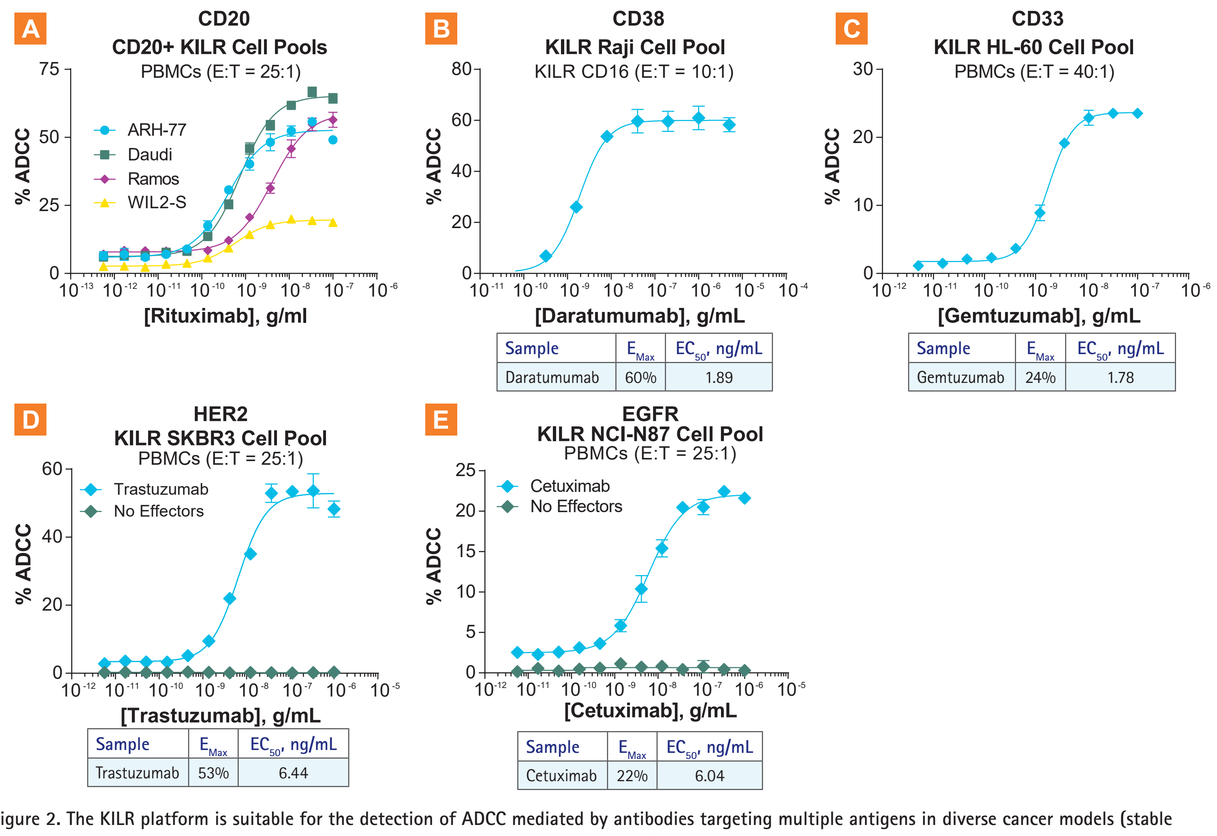
Effector Cells	Target Cells	Assays
PBMCs	KILR target cell models (>40)	ADCC
NKs	Target cells generated using KILR retroparticles	CDC
NK92 cell line		T-cell redirection
CAR-T		CAR-T
KILR CD16 effectors		ADCP

Figure 1. Overview of KILR Cytotoxicity Assays and their applications. A. Target cells expressing the relevant antigen are engineered to stably express a housekeeping protein that is tagged with small enhanced ProLabel® (ePL), a β-galactosidase (β-gal) enzyme donor fragment. When the stable target cell line is incubated with appropriate effector cells and the test antibody, effector-mediated killing releases the tagged KILR reporter (housekeeping) protein into the media. The reporter protein is then detected by the addition of detection reagents containing the enzyme acceptor (EA) fragment of β-gal. This leads to the formation of an active β-gal enzyme that hydrolyzes the substrate to give a chemiluminescent output, detected on any bench top luminometer. B. The KILR platform is versatile 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-Ts, and even ADCP.

ADCC Demonstrated with Antibodies to Multiple Antigens and Diverse Cancer Models



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

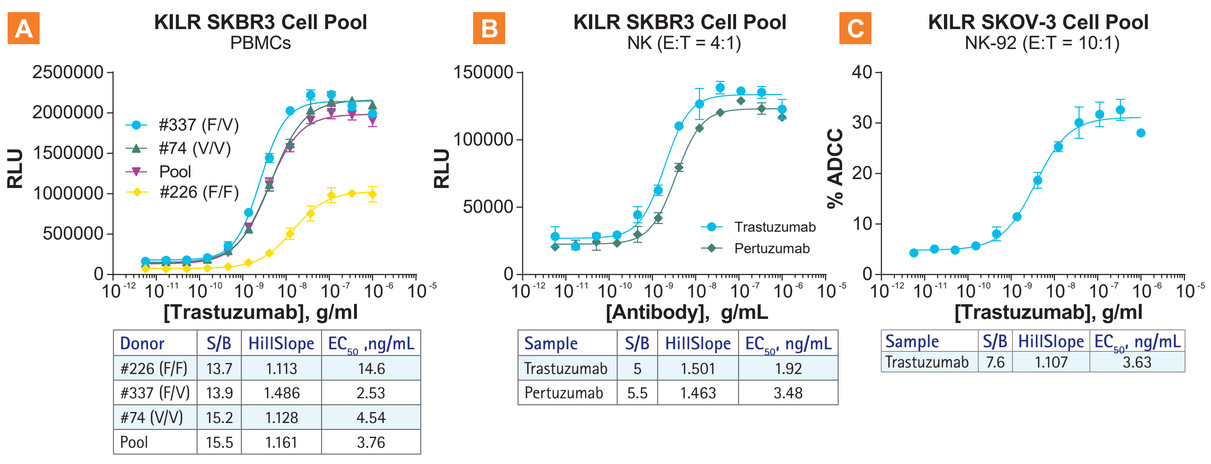
Sample	E_{Max}	EC_{50}, ng/mL
Gemtuzumab	24%	1.78

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.

The KILR ADCC Assay is Compatible with Multiple Effector Cell Types



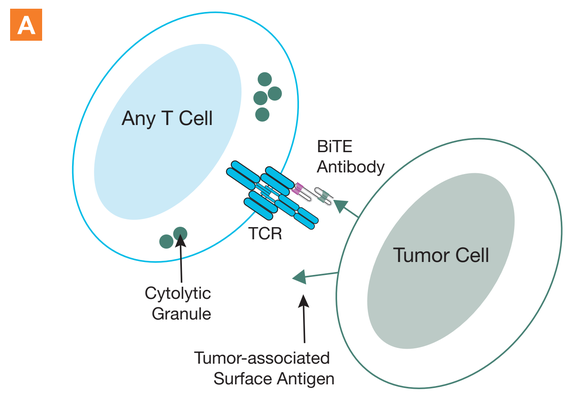
Donor	S/B	HillSlope	EC_{50} ,ng/mL
#226 (F/F)	13.7	1.113	14.6
#337 (F/V)	13.9	1.486	2.53
#74 (V/V)	15.2	1.128	4.54
Pool	15.5	1.161	3.76

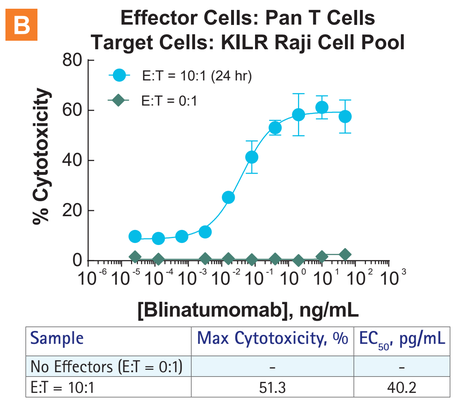
Sample	S/B	HillSlope	EC_{50}, ng/mL
Trastuzumab	5	1.501	1.92
Pertuzumab	5.5	1.463	3.48

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 and engineered NK-92 cell line as effector cells, with the NK-92-resistant cell line SKOV3.

T-Cell Redirection Assay with BiTEs





Sample	Max Cytotoxicity, %	EC_{50}, pg/mL
No Effectors (E:T = 0:1)	-	-
E:T = 10:1	51.3	40.2

Figure 4. Application of KILR in T-Cell Redirection Assays. A. Mechanism of Action of BiTEs (Bi-Specific T-cell Engager). BiTEs are bispecific molecules wherein one end engages a specific antigen on the surface of the tumor cell and the other end engages CD3 expressed on the surface of T-cells. BiTE molecules activate endogenous T-cells by connecting CD3 in the T-cell receptor (TCR) complex with the antigen on the tumor cells. This causes the formation of a synapse between the T-cell and the tumor cell that results in production of cytolytic proteins, release of inflammatory cytokines, and proliferation of T-cells, ultimately leading to the redirected lysis of the tumor cells. B. Representative T-cell Redirection data with the CD19 BiTE, Blinatumomab. Pan T-cells (unactivated) were co-incubated with KILR Raji cells and the therapeutic agent, Blinatumomab for 24 hours at 37°C at an E:T of 10:1 (no effectors are also shown as a negative control). EMax (max killing) of >50% was observed with an EC_{50} of 40.2 pg/mL, well within the range of EC_{50} values reported for killing assays in the BLA for Blinatumomab (10-100 pg/mL).

Plate-Based Antibody-Dependent Cellular Phagocytosis (ADCP) Assay

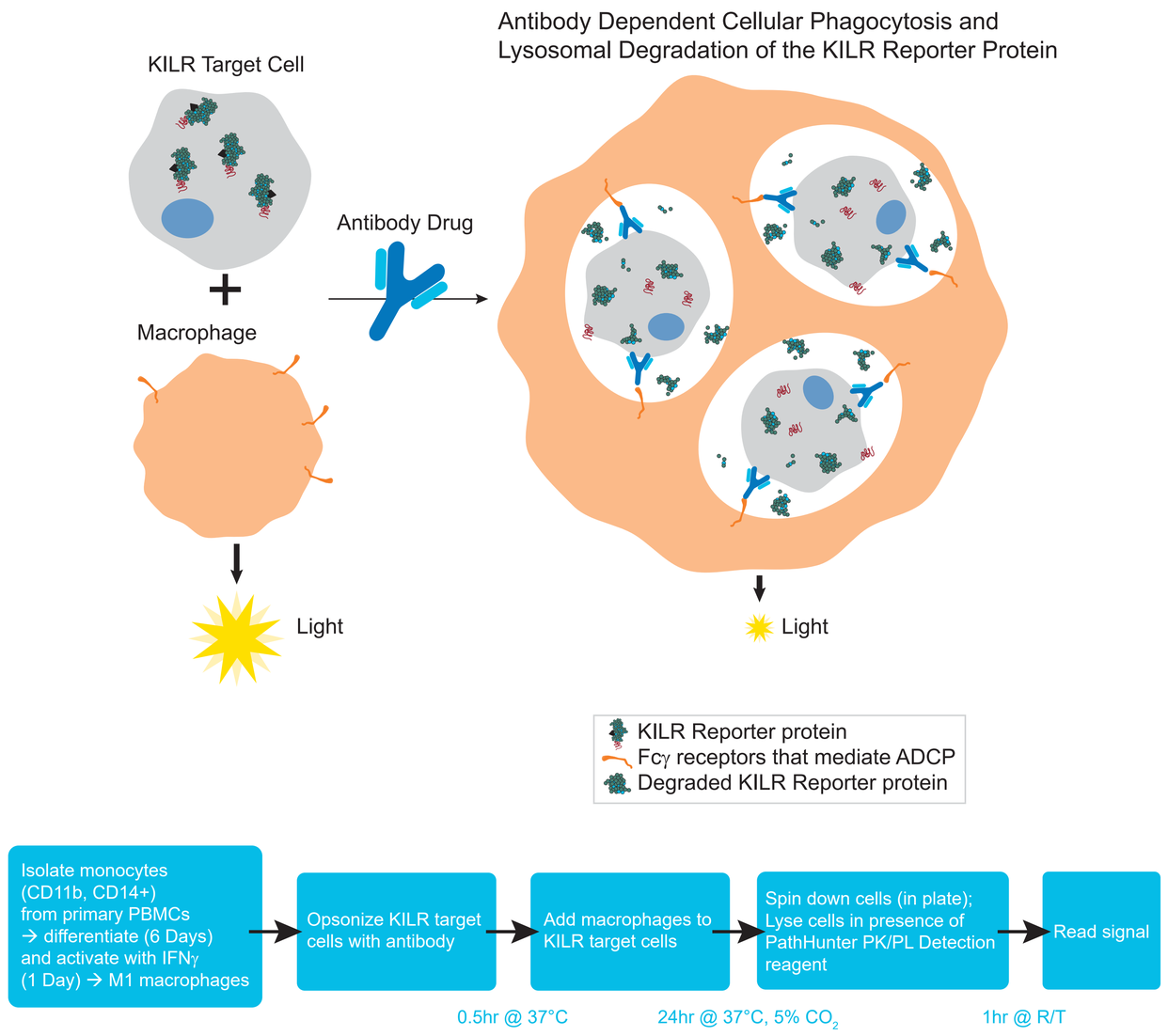
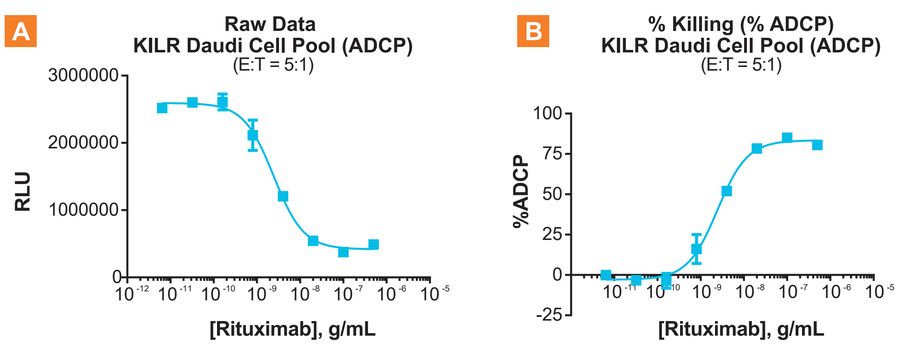


Figure 5. Overview of the KILR ADCP Assay. ADCP measures antibody dependent phagocytosis of target cells, typically mediated by primary human macrophages. M1 macrophages are obtained by isolating monocytes from primary human PBMCs from a single healthy donor, then differentiating with M-CSF for 7 days, followed by activation with IFNγ for 1 day. On the day of the assay, the KILR target cells are opsonized with the antibody of interest, then co-cultured with the isolated M1 macrophages for 24 hours at 37°C. Since macrophages digest the target cells, all cells are lysed and the detection reagent containing the complementing EA enzyme fragment is added to the lysed cells. Complementation of ePL and EA facilitates detection of the remaining amount of KILR protein present. A high chemiluminescent signal indicates minimum killing, while a low signal correlates with more killing.

ADCP in KILR Daudi Cells with Rituximab



Sample	S/B	E_{Max}	EC_{50}, ng/mL
Rituximab	6.2	83%	2.44

Figure 6. Representative ADCP data for Rituximab in KILR Daudi cells. A. Following the protocol in previous panel, KILR Raji cells were opsonized with an 8-point dose-response curve of Rituximab and co-incubated with M1 macrophages at an E:T of 5:1 for 24 hours. Shown are raw data, plotted as RLU, which demonstrate a robust dose-dependent decrease in signal in the assay (S/B = 6.2) with increasing concentrations of Rituximab. B. Data from panel A. plotted as % ADCP (each data point normalized to wells containing vehicle only). An excellent E_{Max} value of 83% was obtained in this experiment with an EC_{50} of 2.4 ng/mL.

Summary

- KILR is a sensitive, target-specific assay format for detecting antibody-mediated immune cell killing of target cells through a variety of different mechanisms
- Over 40 target cell models are available off-the-shelf that express the KILR reporter. Each cell model can be used for multiple immune cell-mediated killing assays such as CDC, ADCC, ADCP, T-Cell Redirection, etc.
- Additional target cell models can be generated under our custom assay development (CAD) program
- Assay optimization (ADCC and/or ADCP) and IND-enabling characterization studies with the client molecule can be performed under our CAD program

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