Quantify Antibody-Dependent Cell Phagocytosis (ADCP): Application of a Robust, Non-Radioactive KILR Cytotoxicity Platform

Surekha Bonasu, Vandana Kaul, Hanako Daino-Laizure, Debatri Chatterjee, Gaurav Agrawal, and Jane E. Lamerdin
Eurofins DiscoverX I Fremont, CA 94538

Abstract

Cell-based assays play an important role in determination of mechanism of action (MOA) for therapeutic antibodies, particularly when evaluating their potential for effector functions. Antibody-dependent cellular phagocytosis (ADCP) has gained prominence as an important MOA to evaluate, especially for IND-enabling studies of antibodies with modified Fc regions. ADCP is mediated by a variety of effector cells, namely monocytes, macrophages, dendritic cells, and neutrophils, through multiple FcγRs. While the Fab region of the antibody binds to a specific antigen on the surface of target cells, the Fc region of the antibody binds and activates various Fcγ receptors on effector cells. Activation of specific Fcγ receptors, like FcγRIIa, FcγRI, and FcγRIIIa, leads to activation of a complex pathway that results in phagocytosis and destruction of the target cells in the lysosome of the effector cells.

ADCP assays can be particularly difficult to standardize due to the variability of primary human Fcγ-expressing effector cells and the lengthy and difficult protocols required for immune cell differentiation. We have applied our industry-validated KILR® cytotoxicity assay platform, which specifically measures killing of target cells in co-culture with immune cells, to quantitation of ADCP in a homogeneous plate-based assay format, serving as an inexpensive alternative to laborious and expensive FACS or imaging approaches. Measurement of ADCP activity with KILR cells is a simple, reproducible and scalable method that directly measures the physiologically relevant endpoint: target cell destruction inside the lysosomes of effector macrophages. KILR ADCP assays are fast, robust, and reproducible, supporting screening and characterization of antibody drugs during early phase bio-comparability as well as in late stage characterization work. The broad application of KILR assays along the drug development pipeline, together with the ability of each stable KILR model to quantify CDC, ADCC, as well as ADCP allows this assay platform to support the development of almost any antibody therapeutic.

Specifically Measure Phagocytosis of Target Cancer Cells with KILR ADCP Assays

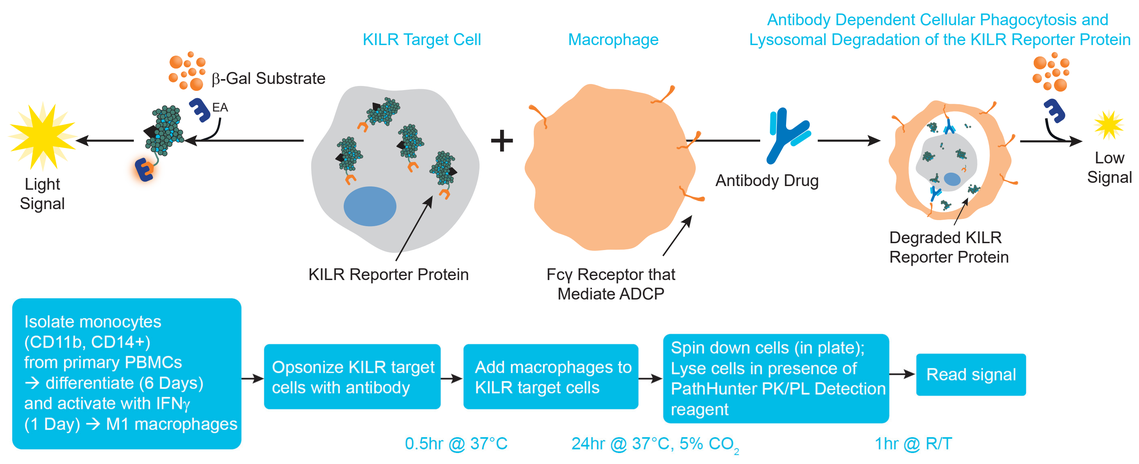
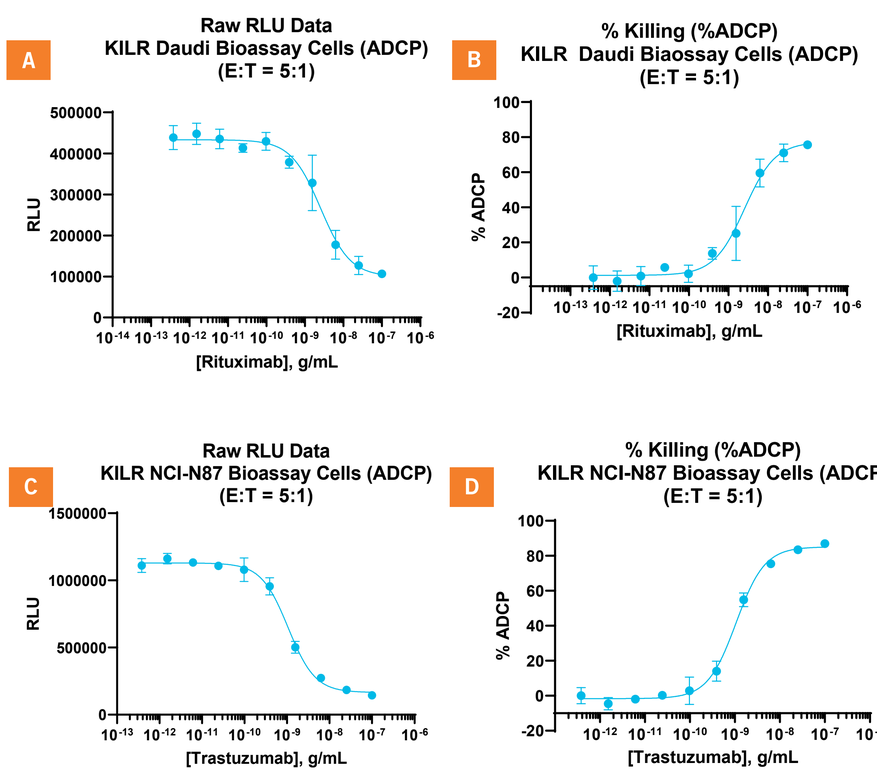


Figure 1. Overview of the KILR ADCP assay, which is based on the industry-validated Enzyme Fragment Complementation (EFC) technology. Target cells expressing the relevant antigen are engineered to stably express a housekeeping protein (KILR Reporter Protein) that is tagged with small enhanced ProLabel® (ePL), a β-galactosidase (β-gal) enzyme donor fragment. 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 a complementing β-gal enzyme acceptor (EA) 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.

Percent ADCP Measured in a Variety of KILR Bioassay Cells

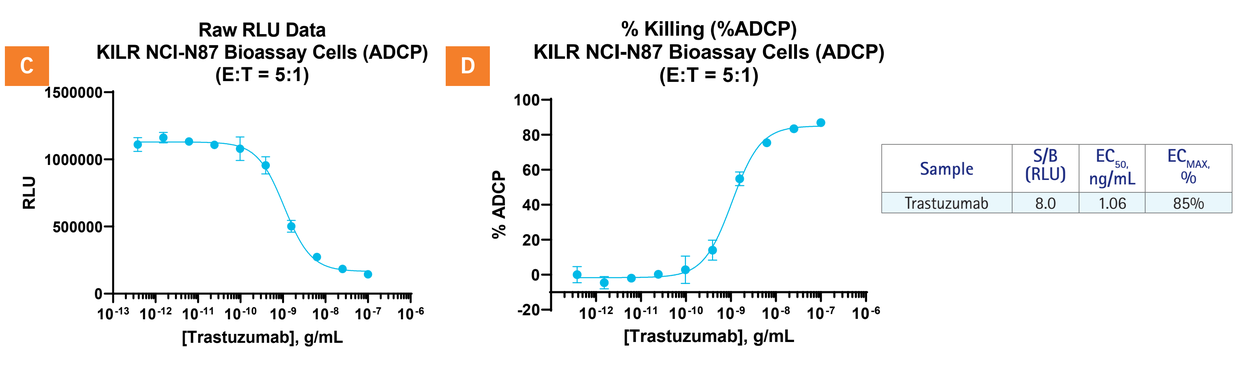


Sample	S/B (RLU)	EC_{50}, ng/mL	EC_{MAX}, %
Rituximab	4.1	2.56	77%

Sample	S/B (RLU)	EC_{50}, ng/mL	EC_{MAX}, %
Trastuzumab	8.0	1.06	85%

Figure 2. Representative ADCP data for Rituximab in KILR Daudi cells and Trastuzumab for KILR NCI-N87 bioassay cells, respectively, where bioassay cells are an assay-ready format of indicated KILR cell models, where bioassay cells are an assay-ready format of indicated KILR cell models. Following the protocol in previous panel, here we present 10-point dose-response curves, wherein macrophages were polarized in situ followed by addition of antibody serial dilutions and co-incubated with target cells for 24 hours. Effector to target ratio (macrophages to target cells (E:T)) was 5:1. A. for the KILR Daudi target cells, the raw RLU data demonstrates a robust dose dependent decrease in signal (S/B = 4.1) 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 77% was obtained in this experiment with an EC_{50} of 2.56 ng/mL. C. for the KILR NCI-N87 target cells, the raw RLU data demonstrates a robust dose dependent decrease in signal (S/B = 8.0) with increasing concentrations of Trastuzumab. D. Data from panel C. plotted as % ADCP (each data point normalized to wells containing vehicle only). An excellent E_{MAX} value of 85% was obtained in this experiment with an EC_{50} of 1.06 ng/mL.

Efficacy of ADCP Correlates with Receptor Density



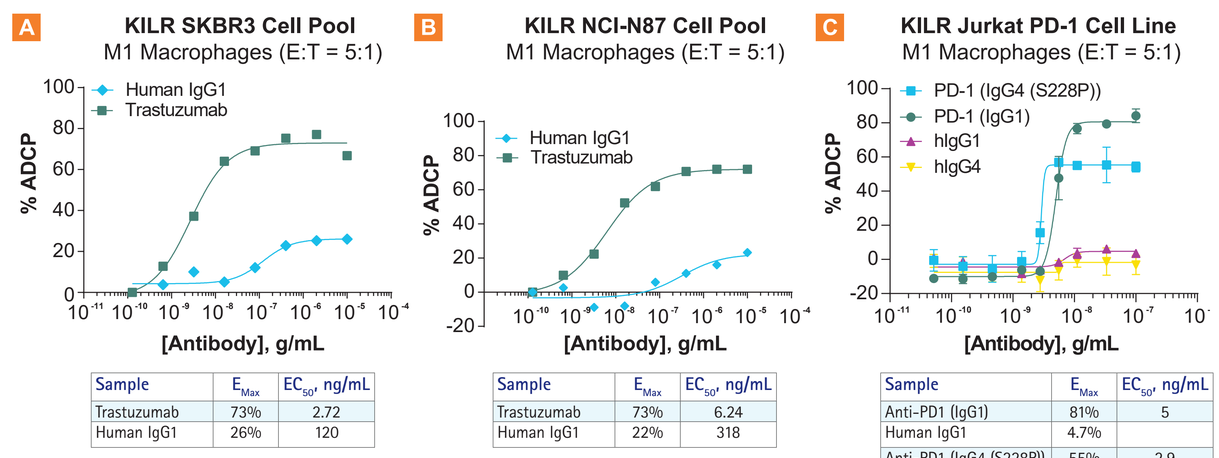
Cell Model	CD20 (Receptors/Cell)	CD38 (Receptors/Cell)
MOLT-4	n. d.	833
Raji	132K	1898
Daudi	184K	7300

Sample	E_{Max}	EC_{50}, ng/mL
Anti-CD20	67%	21.6
Anti-CD38	35%	7.45

Sample	E_{Max}	EC_{50}, ng/mL
Anti-CD20	91%	1.13
Anti-CD38	86%	2.64

Figure 3. Efficacy of ADCP correlates with receptor density in two B-cell cancer models. A. Receptor density for CD20 and CD38, two cell surface receptors highly expressed in B-cell malignancies as determined by flow cytometry in KILR MOLT-4, Raji, and Daudi cell models (n.d. = not determine). B. An anti-CD20 antibody produces a robust ADCP response in KILR Raji cells with an E_{Max} of 67%, while response to an anti-CD38 antibody is lower, consistent with lower expression of CD38 on these cells. C. Both anti-CD20 and anti-CD38 antibodies produced a robust ADCP response in KILR Daudi cells, which have high expression of both receptors. Note that the rank order of the two antibodies is the same in both cell models, but ADCP is more efficient in the KILR Daudi cell model.

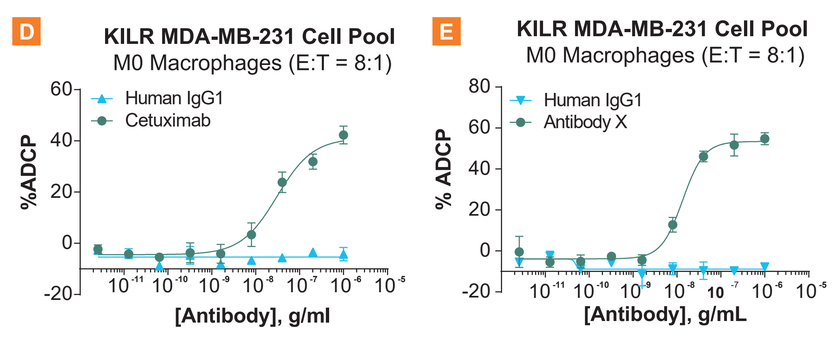
Evaluate Diverse Cancer Models with Robust KILR ADCP Assays



Sample	E_{Max}	EC_{50}, ng/mL
Trastuzumab	73%	2.72
Human IgG1	26%	120

Sample	E_{Max}	EC_{50}, ng/mL
Trastuzumab	73%	6.24
Human IgG1	22%	318

Sample	E_{Max}	EC_{50}, ng/mL
Anti-PD1 (IgG1)	81%	5
Human IgG1	4.7%	
Anti-PD1 (IgG4 (S228P))	55%	2.9
Human IgG4	-	-



Sample	E_{Max}	EC_{50}, ng/mL
Cetuximab	44.7%	30.6
Human IgG1	-	-

Sample	E_{Max}	EC_{50}, ng/mL
Antibody X	53.5%	13.5
Human IgG1	-	-

Figure 4. Robust ADCP observed for diverse antigens in multiple solid and liquid tumor models. ADCP mediated by Trastuzumab relative to an IgG1 control in HER2+ A. KILR SKBR3 cells, a breast cancer model, and B. KILR NCI-N87 cells, a gastric cancer model. Note the human IgG1 control of this particular macrophage donor shows some activity in both cell models, but it is roughly 50-fold less potent than Trastuzumab. Both cell models used M1 macrophages (E:T= 5:1) as effectors. C. ADCP in the KILR Jurkat PD-1 cell model with two commercial anti-PD-1 antibodies (IgG1 and IGG4 (S228P) formats) relative to the two isotype controls (human IgG1 and human IgG4) using M0 macrophages (E:T= 5:1). Note that as reported in the literature, the IgG4 (S228P) isotype is able to mediate ADCP nearly as effectively as the IgG1 formatted antibody, suggesting this assay may be useful for characterizing Pembrolizumab biosimilars. ADCP in KILR MDA-MB-231 breast cancer cells using D. Cetuximab or E. Antibody X, which recognizes a novel antigen expressed on MDA-MB-231 cells, using M0 macrophages (E:T= 8:1) as effectors.

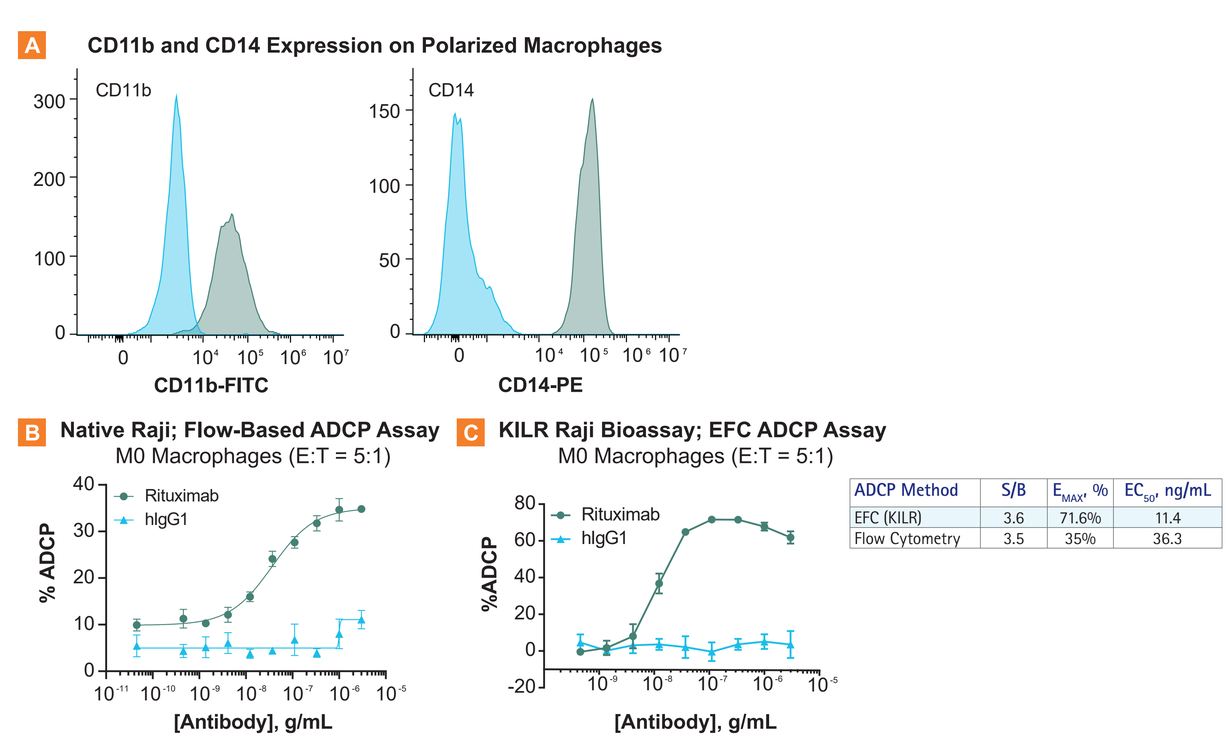
KILR ADCP Assay Delivers High Reproducibility

Inter-Plate Variability – Same Day (EC_{50}, ng/mL of Anti-HER2)				
Plate 1	Plate 2	Plate 3	Mean	%CV
7.99	6.12	8.95	7.69	18.76

Inter-Day Variability (EC_{50}, ng/mL of Anti-HER2)				
Day 1	Day 2	Day 3	Mean	%CV
*7.69	5.3	6.3	5.8	12.19

Figure 5. ADCP assay with KILR SKBR3 cell pools shows high reproducibility between experiments. The same antibody, anti-HER2, was run on three different plates on the same day (inter-plate experiment) and over the course of two days. EC_{50}'s (ng/mL for Anti-HER2) between plates varied by less than 20% CV, while inter-day %CV was only 12.2%. * Average of plate 1, 2, and 3 data.

Higher Percentage Phagocytosis Seen with Plate-Based, EFC Assay Compared to Flow-Based Assay Formats



ADCP Method	S/B	E_{MAX}, %	EC_{50}, ng/mL
EFC (KILR)	3.6	71.6%	11.4
Flow Cytometry	3.5	35%	36.3

Figure 6. Comparison of Rituximab-mediated ADCP in two different ADCP assay formats (flow vs EFC). Monocytes from the same healthy donor were isolated and differentiated to M0 macrophages prior to use in each assay. A. Expression of CD11b and CD14 was assessed on the polarized macrophages by flow cytometry at time of harvest to confirm differentiation state. B. For flow-based assay, polarized macrophages were incubated with CFSE labeled Raji target cells (E:T= 5:1) and a dose response of Rituximab for 24h before quantifying co-localization of CD14+ effectors (with APC-labeled anti-CD14 antibody) and CFSE-stained target cells by flow cytometry. C. For EFC-based assay, monocytes polarized to M0 macrophages in situ in a 96-well assay plate for 7 days were co-incubated with KILR Raji Bioassay target cells (E:T= 5:1) and a dose response of Rituximab for 24h before addition of PathHunter PK/PL Detection Reagent and detection of assay signal on a plate-based luminometer. Data from each assay were normalized to vehicle treated cells to calculate % ADCP.

Summary

The KILR platform provides a sensitive, target-specific assay format for quantifying ADCP in a homogeneous assay format. Easily measure the physiologically relevant endpoint for ADCP: target cell destruction inside the lysosomes of effector macrophages.

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 are available off-the-shelf that express the KILR reporter. Each cell model can be used for multiple immune cell mediated killing assays, including CDC, ADCC, ADCP, T-Cell Redirection, etc.

Take advantage of our custom assay development program to generate custom target cell models, as well as provide assay optimization and data generation with client antibodies.

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