Single Donor KILR® CD16 Effector Cells to Drive Robust and Reproducible ADCC and T Cell Redirection

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Abstract

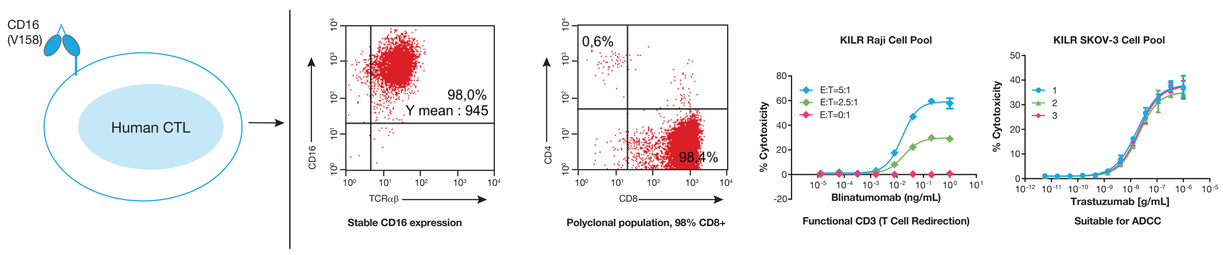
Class I therapeutic antibodies achieve their clinical efficacy not only by binding to their target antigen, but also through Fc domain-mediated recruitment of immune cell effectors to attack and kill the target cell. Therefore, developers of therapeutic antibodies must assess all possible mechanisms of action (MOA) of their molecules, including antibody-dependent cell-mediated cytotoxicity (ADCC). Success of ADCC assays is highly dependent on the quality of effectors used. However, primary human cells (such as PBMCs or NK cells) suffer from inter-individual or donor variability, while NK cell lines often show high background lysis in susceptible cell models and functional variability under different culture conditions.

In this poster, we present data on cytotoxicity assays using single donor-derived, engineered effector cells, the KILR CD16 T Effector Cells that stably express CD16. These uniformly manufactured cells maintain their T cell phenotype (TCRαβ-, CD3- and CD8-positive) after transfection. Importantly, CD16 expression and killing capacity are consistent across multiple batches of manufactured cells. When used in ADCC assays, these effector cells produce very low background, resulting in robust assay windows, with excellent repeatability and precision, making these cells well-suited for characterization and lot release assays. Further, we demonstrate that these effector cells are compatible with T cell redirection applications, using the clinical molecule blinatumomab.

Materials

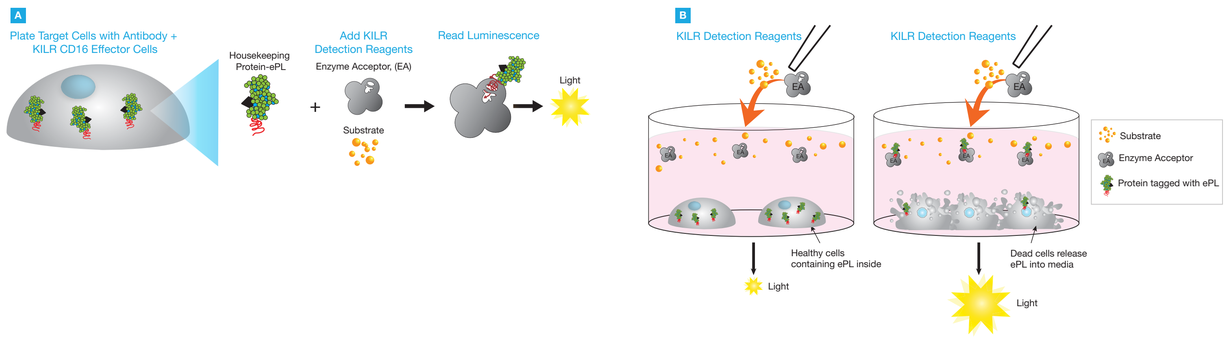
All KILR target cell lines and KILR CD16 Effector Cells were propagated and prepared for the cytotoxicity assays according to the protocols and recommendations provided in their respective datasheet and user manuals. ADCC assays were performed using conditions recommended in the relevant KILR ADCC User Manual. Pan T cells were isolated from frozen primary PBMCs using the EasySep™ Human T Cell Enrichment Kit (STEMCELL Technologies), as per the manufacturers' recommendations.

Single Donor-Derived KILR CD16 Effector Cells



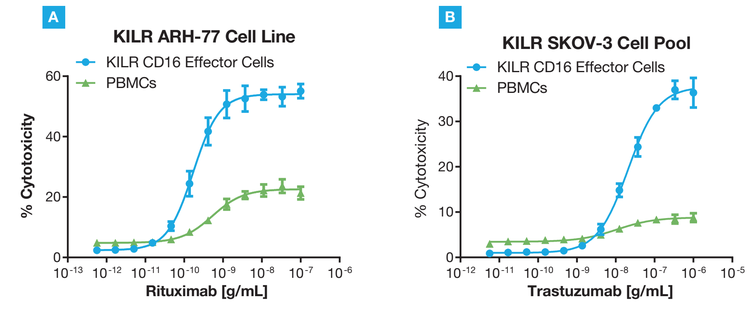
Single donor-derived cytotoxic T lymphocytes (CTLs) transfected with CD16 (FcγRIIIa- V158). KILR CD16 Effector Cells are a polyclonal population of predominantly (98%) CD8+ cells with robust and stable expression of CD16. KILR CD16 Effector Cells have functional CD3, as evidenced by ability of cells to stimulate T cell redirection in the presence of blinatumomab. An example of robust trastuzumab-mediated ADCC of three experimental replicates (each prepared in duplicate) is shown in the HER2+ KILR model, SKOV-3.

Technology Principle for KILR Cytotoxicity Assays



A.The KILR CD16 Effector Cells are added to the plated target cells expressing the receptor antigen, which have been engineered to stably express a housekeeping protein that is tagged with enhanced ProLabel® (ePL), a β-gal enyme fragment using the KILR Retroparticles. When the stable target cell line is used in a cytotoxicity assay, and its membrane is compromised due to cell death, it will release the tagged reporter protein into the media. We can detect this reporter protein by the addition of detection reagents containing the enzyme acceptor (EA) fragment of the β-gal enzyme. 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 luminescence reader. B. In the figure, the well on the left contains healthy, intact target cells that are alive in the presence of immune effector cells. When detection reagents are added to the well, we cannot detect chemiluminescence as the reporter protein does not leak out through an intact cell membrane into the media. Alternatively, in the well on the right, the target cancer cells are killed by the KILR CD16 Effector Cells, releasing the reporter protein into the media. Addition of the detection reagents leads to the recognition of the reporter protein and generation of a chemiluminescent signal that is proportional to the number of dead cells. Death of any other cell type, including the KILR CD16 Effector Cells present within the co-culture will not affect the assay output, giving the KILR cytotoxicity assay an unparalleled specificity to detect target cell death within a co-culture system.

Significantly Larger Assay Window to Better Analyze Antibody Activity

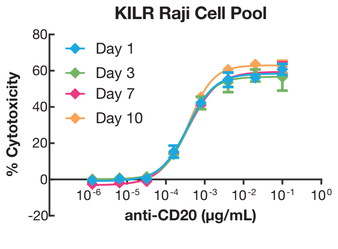


Effector Cell Type	EC_{50} (ng/mL)	S/B	Max. Cytotoxicity (%)
KILR CD16 Effector Cells (10:1)	0.17	23	54%
PBMCs (25:1)	0.53	5.5	22.6%

Effector Cell Type	EC_{50} (ng/mL)	S/B	Max. Cytotoxicity (%)
KILR CD16 Effector Cells (12.5:1)	20.6	41.6	37%
PBMCs (25:1)	10.6	2.6	8.8%

Two KILR cytotoxicity models, A. ARH77 (CD20+) and B. SKOV-3 (HER2+) were opsonized with the appropriate antibody, then incubated with primary PBMCs (E:T=25:1) or KILR CD16 Effector Cells (E:T=10:1 or 12.5:1, respectively) for 3 hours, followed by the addition of KILR Detection Reagent. A 4-fold larger assay window was observed in the CD20 model with KILR CD16 Effector Cells relative to PBMCs, while an even larger (16-fold) improvement in assay window was observed with the more difficult to kill SKOV-3 cell model.

Consistent Performance Over Several Days with No Loss in Killing Capacity



Days Post-Thaw	EC_{50} (µg/mL)	Max. Cytotoxicity (%)
Day 1	361	58.5
Day 3	371	56.7
Day 7	371	59.5
Day 10	400	62.9

KILR CD16 Effector Cells can be maintained in culture for 10 days with no reduction in killing capacity. KILR CD16 Effector Cells were thawed, then cultured in AssayComplete® Culture Kit 117 for the indicated number of days prior to use in ADCC assay with KILR Raji cells. The table shows the EC_{50} of anti-CD20 antibody varied by 4.5% RSD over the 4 days, while maximum cytotoxicity values varied by 4.4% RSD.

KILR CD16 Effector Cells Deliver Excellent Intermediate Precision of ADCC and High Lot-to-Lot Reproducibility

A KILR SKOV-3 Target Cells with KILR CD16 Effector Cells

E:T = 12.5:1	EC_{50} (ng/mL)	Max. Cytotoxicity (%)	% RSD
Day 1, R1	33	14.8%	
Day 1, R2	31.6	14.3%	
Day 1, R3	27.7	13.0%	
Mean, Day 1	30.8		8.84%
Day 2, R1	30.1	28.5%	
Day 2, R2	26.9	40.3%	
Day 2, R3	34.4	37.6%	
Mean, Day 2	30.5		12.35%
Mean, Inter-day	30.6		9.6%

Range in EC_{50} (over 2 days) = 1.28-fold

B KILR SKOV-3 Target cells with PBMCs (V/V Donor)

E:T = 25:1	EC_{50} (ng/mL)	Max. Cytotoxicity (%)	% RSD
Day 1	4.76	21%	
Day 2	11.9	9.8%	
Day 3	8.98	14.6%	
Mean	8.54		42%

Range in EC_{50} (over 3 days) = 2.5-fold

C KILR CD16 Effector Cells

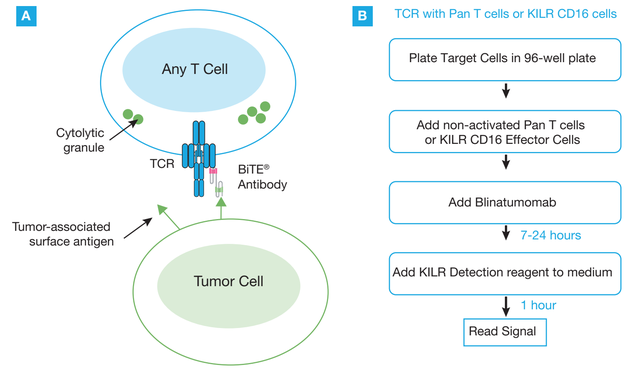
	EC_{50} (ng/mL)	% RSD
Lot 1	3.26	
Lot 2	1.93	
Lot 3	1.49	
Lot 4	2.05	
Mean	2.18	34.7%

D PBMCs

	EC_{50} (ng/mL)	% RSD
Donor 1	0.68	
Donor 2	0.49	
Donor 3	0.301	
Donor 4	1.21	
Mean	0.67	58.5%

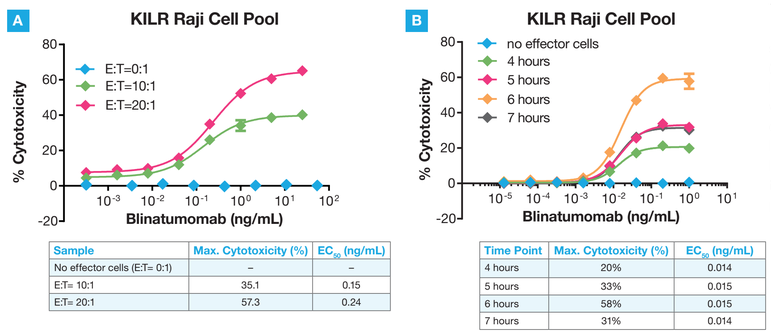
Comparison of intermediate precision of ADCC in SKOV-3 cells with A. KILR CD16 Effector Cells vs. B. PBMCs isolated from a V158/V158 donor. Evaluation of lot-to-lot performance of KILR CD16 Effector Cells (C) in a Rituximab ADCC model, compared to PBMCs from different donors (D).

T Cell Redirection with Blinatumomab



T cell redirection with blinatumomab. A. Blinatumomab (Blincyto®) is a therapeutic antibody known as a BiTE or bi-specific T cell engager. One arm of blinatumomab targets CD19 expressed on malignant B cells, while the anti-CD3 arm recruits and activates T cells to kill the bound CD19-positive cells. B. Schematic of protocol for T Cell redirection with KILR Raji cells is shown on the right.

Larger Assay Windows and Rapid Killing Kinetics Compared to Isolated Pan T Cells



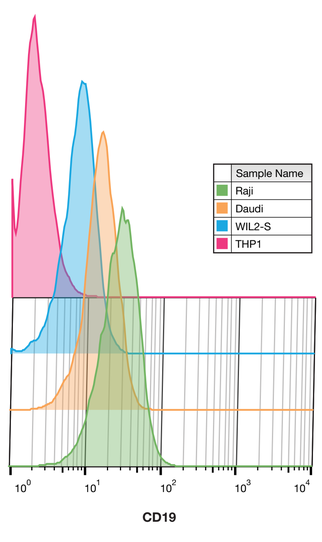
Sample	Max. Cytotoxicity (%)	EC_{50} (ng/mL)
No effector cells (E:T= 0:1)	–	–
E:T= 10:1	35.1	0.15
E:T= 20:1	57.3	0.24

Time Point	Max. Cytotoxicity (%)	EC_{50} (ng/mL)
4 hours	20%	0.014
5 hours	33%	0.015
6 hours	58%	0.015
7 hours	31%	0.014

T cell redirection with isolated (pan) T cells vs. KILR CD16 Effector Cells. A. Pan T cells isolated from PBMCs by negative selection were co-incubated at multiple effector: target ratios (0:1, 10:1, 20:1) with KILR Raji cells and the therapeutic antibody, blinatumomab for 24 hours at 37°C. B. KILR CD16 Effector Cells were incubated at an E:T of 5:1 with KILR Raji cells and blinatumomab for 4, 5, 6, or 7 hours at 37°C. Optimal assay window (max killing of >55%) was observed at an E:T of 20:1 for pan T cells (24 hours) and at 6 hour incubation with KILR CD16 effectors. Note that EC_{50} with KILR CD16 Effector Cells was almost 10-fold more potent than observed with pan T cells.

Blinatumomab-Mediated Killing Correlates with CD19 Receptor Density

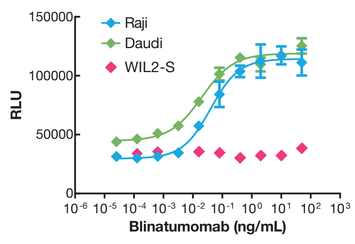
A Surface Expression of CD19 (FC)



B Receptor Density Data (CD19)

Cell Model	CD19 Density (x1000/Cell)
THP-1	0.7
WIL2-S	2.5
Daudi	4.5
Raji	7.4

C TCR with Blinatumomab in KILR Pools



Sample	% Max. Cytotoxicity	EC_{50} (ng/mL)
Raji	59.4	0.04
Daudi	44.7	0.02
WIL2-S	–	–

Blinatumomab-mediated killing correlates with CD19 receptor density. A. Receptor density of CD19 in 5 KILR cell lines was quantified using PE-labeled CD19 antibodies, and standard curve produced with BD Quantibrite PE beads. B. GraphPad Prism was used to interpolate geometric means of each sample to determine receptor density as shown in table. C. TCR response to blinatumomab in 3 KILR cell pools. No killing was observed in cell line with low CD19 receptor density (WIL2-S).

Summary

KILR CD16 Effector Cells are single donor-derived human Cytotoxic T-lymphocytes that have been stably transfected with human CD16 (V158) that maintain their T cell phenotype (TCR α/β+, and functional CD3) and are predominantly CD8+ cells.

Benefits of Using KILR CD16 Effector Cells for Cytotoxicity Assays

- Eliminate donor variability – Primary effector cells from a single donor. Higher S/B ratios than observed with PBMCs
- Fit for long-term QC testing – Excellent intermediate precision and lot-to-lot reproducibility
- Easily implement in any lab – Frozen ready-to-use cells (after 24 hours recovery) and can be maintained in culture for >7 days with no reduction in killing capacity
- Measure target cell death – Relevant measure of ADCC and TCR. Produce robust and reproducible data, with excellent intermediate precision. Rapid kinetics for T Cell Redirection relative to unstimulated Pan T cells

