Driving Robust and Reproducible ADCC and T Cell Redirection with Single Donor KILR® CD16 Effector Cells

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Abstract

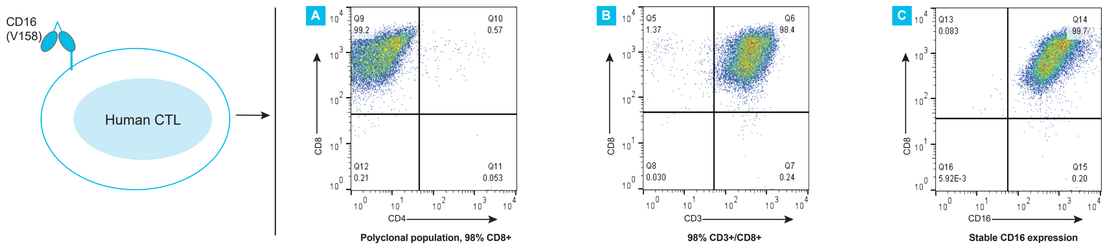
Success of ADCC assays is highly dependent on the quality of effector cells used. However, human primary cells (such as PBMCs or NK cells) suffer from inter-individual variability, while NK cell lines engineered to overexpress CD16, often show high background lysis in susceptible cell models, and high functional variability under different culture conditions.

In this poster, we present data on cytotoxicity assays using single donor-derived, engineered effector cells to stably express CD16, the KILR CD16 Effector Cells. These uniformly manufactured cells maintain their T cell phenotype (TCRαβ+, CD3+ and CD8+), and have the added ability to drive ADCC through the overexpression of CD16. Importantly, multiple batches of manufactured cells demonstrate consistent CD16 expression and killing capacity, further reducing long-term variability. These cells are optimized as frozen ready-to-use cells and generate robust assay windows with excellent repeatability and precision, enabling their use for lot release and characterization studies. The results of an evaluation study with MabThera (rituximab) using the Raji model demonstrate excellent accuracy, linearity, and intermediate precision of the ADCC assay using the KILR CD16 Effector Cells. Furthermore, we demonstrate that these effector cells are compatible with T cell redirection applications, using the clinical molecule blinatumomab.

Materials

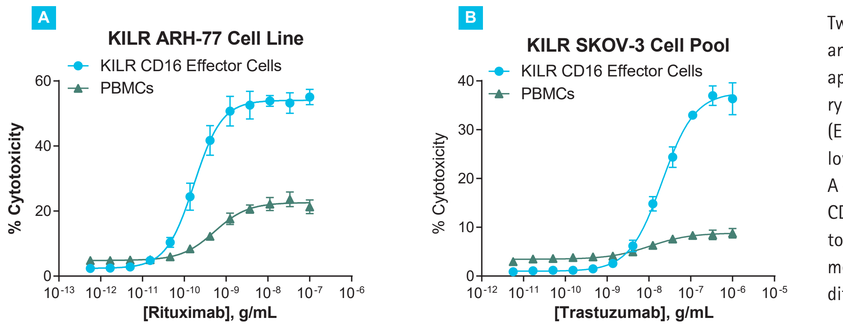
All KILR target cell lines and KILR CD16 Effector Cells were propagated and prepared for the cytotoxicity assays according to the protocols and recommendations provided in their respective datasheet and user manuals. ADCC assays were performed using conditions recommended in the relevant KILR ADCC User Manual. Pan T cells were isolated from frozen primary PBMCs using the EasySep™ Human T Cell Enrichment Kit (STEMCELL Technologies), as per the manufacturer's 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 A. that are also positive for CD3 B., with robust and stable expression of CD16. C. KILR CD16 Effector Cells have functional CD3, as demonstrated by ability of cells to stimulate T cell redirection in the presence of blinatumomab (see section on T cell redirection).

Significantly Larger Assay Window to Better Analyze Antibody Activity

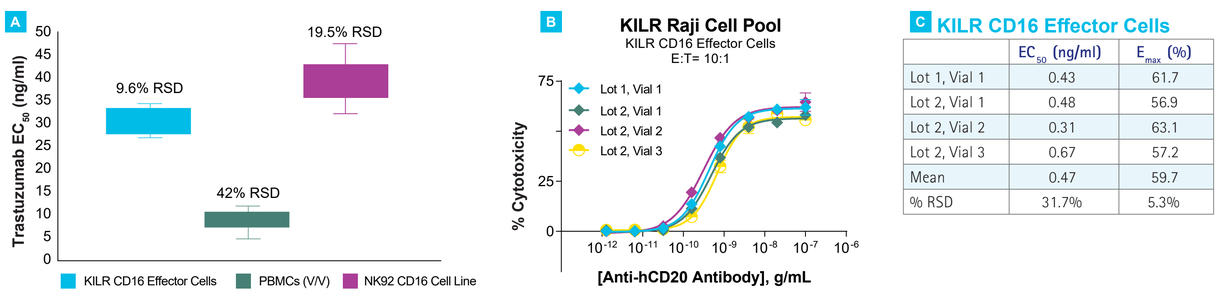


Two KILR cytotoxicity models, A. ARH77 (CD20+) and B. SKOV-3 (HER2+) were opsonized with the appropriate antibody, then incubated with primary PBMCs (E:T=25:1) or KILR CD16 Effector Cells (E:T=10:1 or 12.5:1, respectively) for 3 hours, followed by the addition of KILR Detection Reagent. A 4-fold larger 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.

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%

Excellent Intermediate Precision of ADCC and High Lot-to-Lot Reproducibility



C KILR CD16 Effector Cells

	EC_{50} (ng/ml)	E_{max} (%)
Lot 1, Vial 1	0.43	61.7
Lot 2, Vial 1	0.48	56.9
Lot 2, Vial 2	0.31	63.1
Lot 2, Vial 3	0.67	57.2
Mean	0.47	59.7
% RSD	31.7%	5.3%

A. Comparison of intermediate precision of ADCC in SKOV-3 cells with KILR CD16 Effector Cells, PBMCs isolated from a V158/V158 donor and NK92 CD16 cell line. B., C. Evaluation of intra- and inter-lot performance of KILR CD16 Effector Cells in a Rituximab ADCC model.

Rituximab Qualification Study: Implementation in Comparability Studies and as a Lot Release Assay

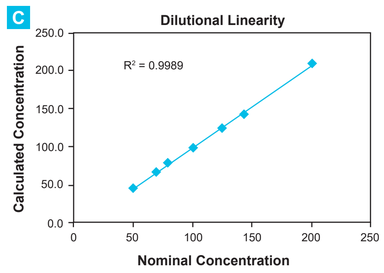
A

Assessment #	Day	Operator	Vial #	Samples (%)		
1	1	A	1	50	100	200
2		B		100		
3	2	A	2	80		125
4		B		50	100	200
5	3	A	3	70	143	200
6		B		50	200	Specificity

B

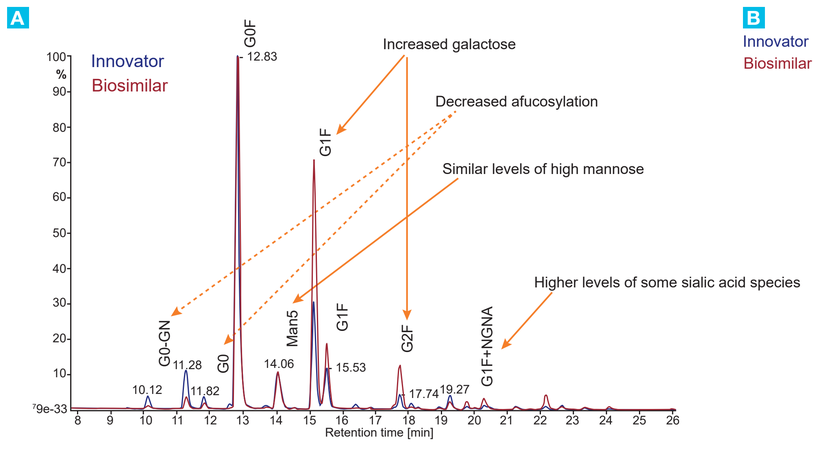
Nominal Concentration	Result	% Accuracy	% Inaccuracy
50	47.8	95.6	-4.4
50	39.6	79.2	-20.8
50	52.7	105.4	5.4
70	67.7	96.7	-3.3
80	81.1	101.4	1.4
100	89.8	89.8	-10.2
100	112.1	112.1	12.1
100	98.0	98.0	-2.0
125	125.5	100.4	0.4
143	143.3	100.2	0.2
200	227.0	113.5	13.5
200	168.5	84.3	-15.8
200	216.0	108.0	8.0
200	225.7	112.9	12.9

C



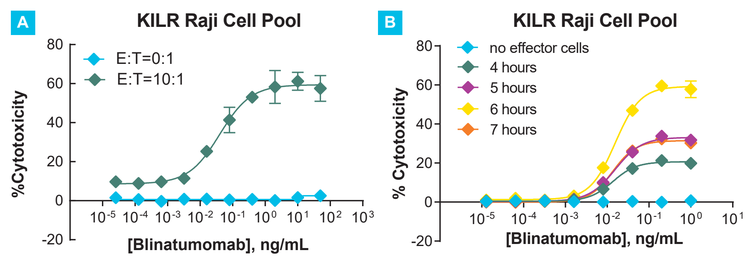
ADCC activity of Rituximab (Rituxan®) was assessed using Raji cells and KILR CD16 Effector cells. A. The qualification study was designed to assess the major requirements of ICHQ2, including Accuracy, Intermediate Precision, Linearity, Range, and Specificity. The study used two different operators, three different vials of KILR CD16 Effector Cells, and the assays performed over three separate occasions, to assess the Intermediate precision over a range of 50-200%. Specificity controls are of the same isotype as the molecule undergoing assessment, but are not expected to elicit a positive response. B. Across the range of concentrations tested, from 50 to 200%, there is no pattern in the level of inaccuracy observed for the multiple samples assessed, indicating that there is no inherent bias in the assay. This, coupled with a maximum inaccuracy of 20.8% suggests that these effector cells are suitable for assessing potency across this range in an ADCC assay. C. The data generated for the accuracy assessment was used to assess the linearity of the assay, resulting in an R^2 value of 0.9989, which indicates that an accurate result would be obtained over the range of the assay. Taken together, these data indicate that the KILR cells are a good alternative to isolated PBMC preparations from blood and could be used from clone selection and process development to comparability studies as well as lot release assays. This data is part of a qualification study conducted by BioOutsource Sartorius Stedim. For details, please email information@sartorius-stedim.com.

Infliximab Comparability Study: KILR CD16 Effector Cells Are Able to Detect Differences in Glycan Profiles When Used in an ADCC Assay



A. Glycosylation profile of the innovator and biosimilar infliximab. The glycan profile of both molecules are consistent with a monoclonal antibody that has been expressed in mammalian cell lines. Most peaks in the biosimilar chromatogram are also observed in the innovator chromatograms although there are some variations. There are higher levels of some sialic acid species and an increase in galactosylation of G1F and G2F. There is decreased afucosylation in the biosimilar profile, which can have an impact on ADCC activity. B. ADCC activity of infliximab was subsequently assessed using purified Natural killer (NK) cells from V/V and F/F donors, and compared with KILR CD16 Effector Cells. The following potencies were returned for the biosimilar molecule during the assay: V/V donor - 29.7%, F/F donor - 38.7%, KILR CD16 cells - 41.0%. This data is part of a comparability study conducted by BioOutsource Sartorius Stedim. For details, please email information@sartorius-stedim.com.

Rapid Killing Kinetics with T Cell Redirecting Antibody, Blinatumomab, Compared to Isolated Pan T Cells



Sample	Max. Cytotoxicity (%)	EC_{50} (pg/mL)
No effector cells (E:T= 0:1)	–	–
E:T= 10:1	51.3	40.2

Time Point	Max. Cytotoxicity (%)	EC_{50} (pg/mL)
4 hours	20%	13.7
5 hours	33%	15.5
6 hours	58%	15.5
7 hours	31%	13.6

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 0:1 or 10:1 effector: target ratios 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 >50%) was observed at an E:T of 10:1 for pan T cells (24 hours) and at 6 hour incubation with KILR CD16 effectors. Note that various EC_{50} observed with blinatumomab with pan T cells (40.2 pg/mL) and KILR CD16 Effector Cells (13-15 pg/mL) are comparable to the EC_{50} observed for blinatumomab in the original BLA (10-100 pg/mL).

Summary

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 – Can be maintained in culture for up to 14 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