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Robust and Reproducible ADCC and T Cell Redirection with CD16-Engineered Effector Cells

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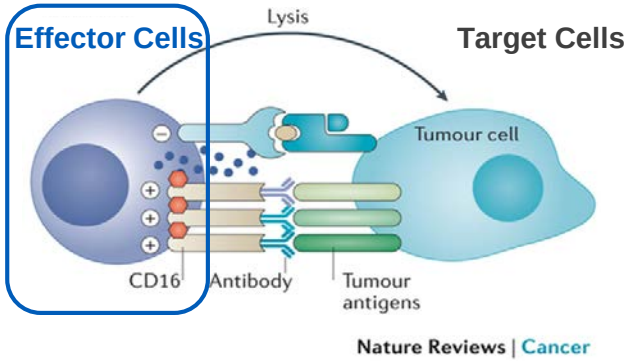
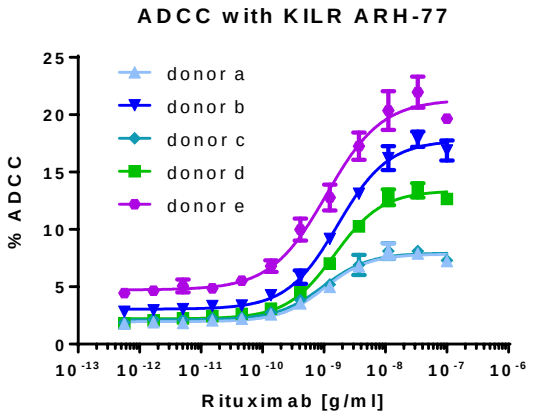
Limitations of Current Cytotoxicity Assays for Lot Release

Need for consistent performance from human effector cells for cytotoxicity assays

- PBMC-driven ADCC
  - High donor-to-donor variability
  - Logistical challenges with getting enough PBMCs or NKs
  - ADCC assays often have low signal to background ratios



- Engineered NK92-CD16 lines
  - Challenging to grow
  - Not compatible with many target cell lines (high background killing)
  - Requires expensive lysis fee



- Ideal ADCC assay for lot release
  - Reflective of drug MOA
  - Provides consistent performance from effector cells that eliminates donor variability and minimizes lot-to-lot variability

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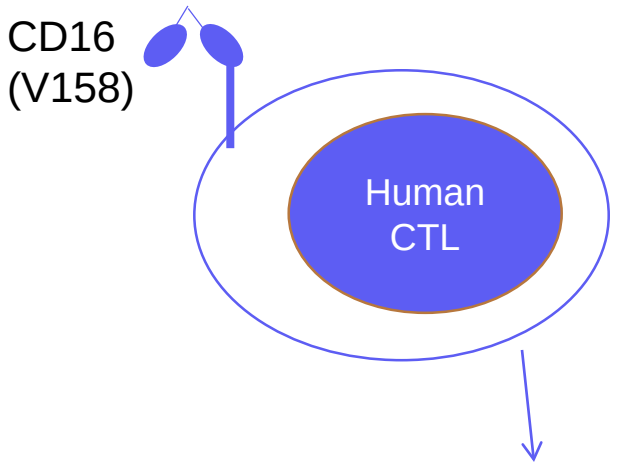
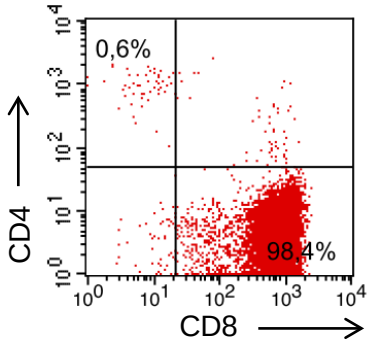
Single Donor-Derived Engineered Effector Cells

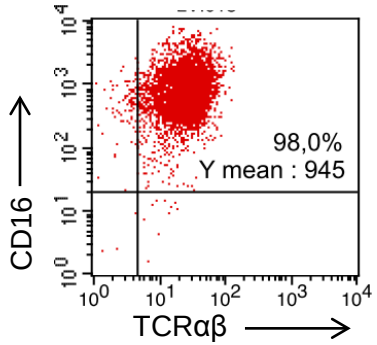
KILR CD16 (V158) Effector Cells



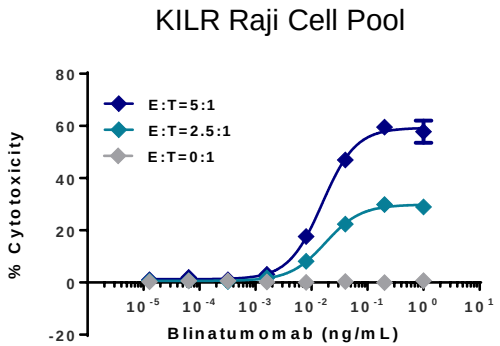
Primary human cytotoxic T lymphocytes (CTLs) engineered to express FcγRIIIa (CD16; V158) to enhance ADCC activity



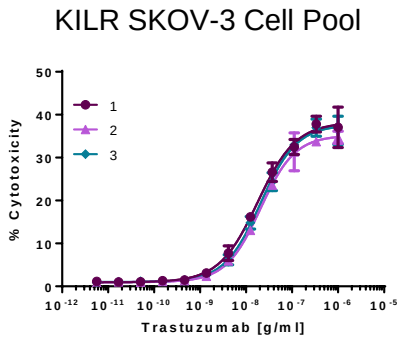
Polyclonal population, 98% CD8+



Stable CD16 expression



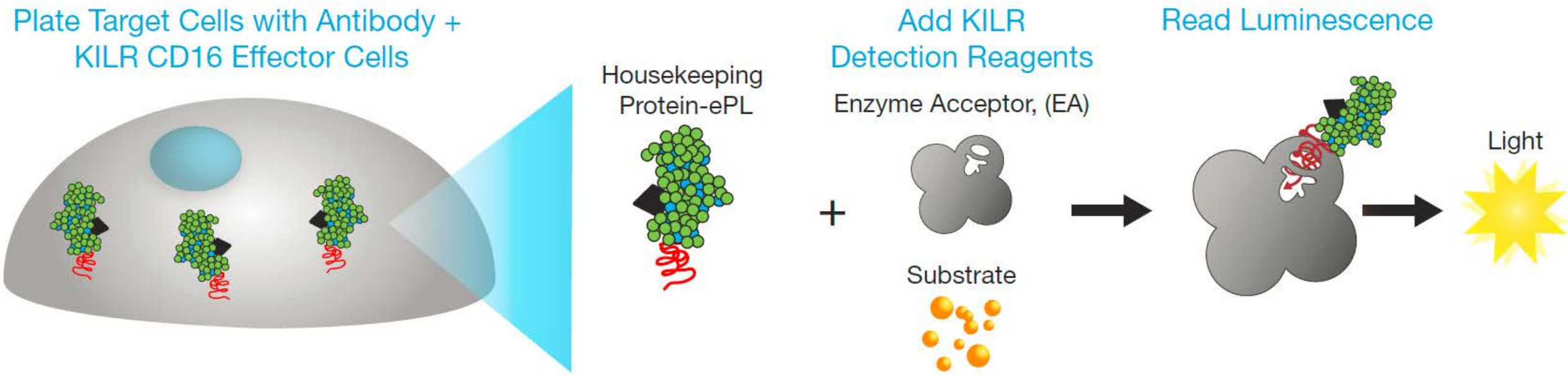
Functional CD3
(T Cell Redirection)



Suitable for ADCC

KILR® CD16 Effector Cells for ADCC Assay

Example: KILR ADCC assay



Target Cells:
- KILR target cell lines/pool
- Target cells generated using KILR retroparticles
- Customer's in-house target cells/cell line

Assays:
- KILR ADCC
- Chromium 51 release
- Calcein release

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KILR® CD16 Effector Cells Highlights

Compatible with Different Cytotoxicity Assays (KILR, Chromium Release, Calcein Release, etc.)

- Eliminate Donor Variability - Primary effector cells from a single donor
- Easily Implement in Any Lab - Frozen ready-to-use cell
- Fit for Long-Term QC Testing - High lot-to-lot reproducibility
- Measure Target Cell Death - Relevant measure of ADCC and TCR

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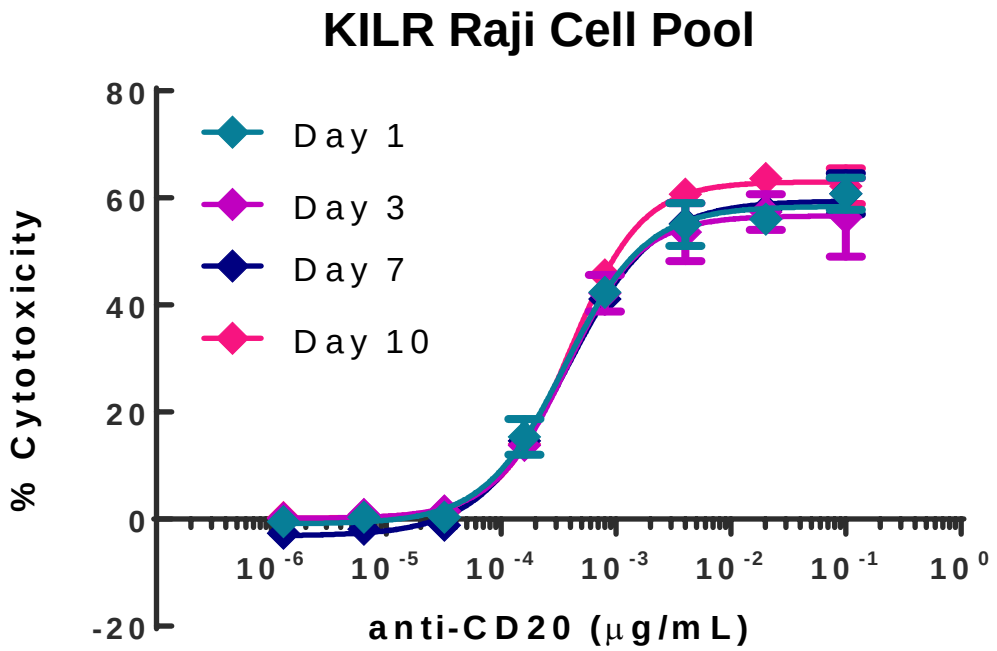
Consistent Performance of KILR® CD16 Cells Post-Thaw

No reduction of killing capacity over 10 days in culture



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

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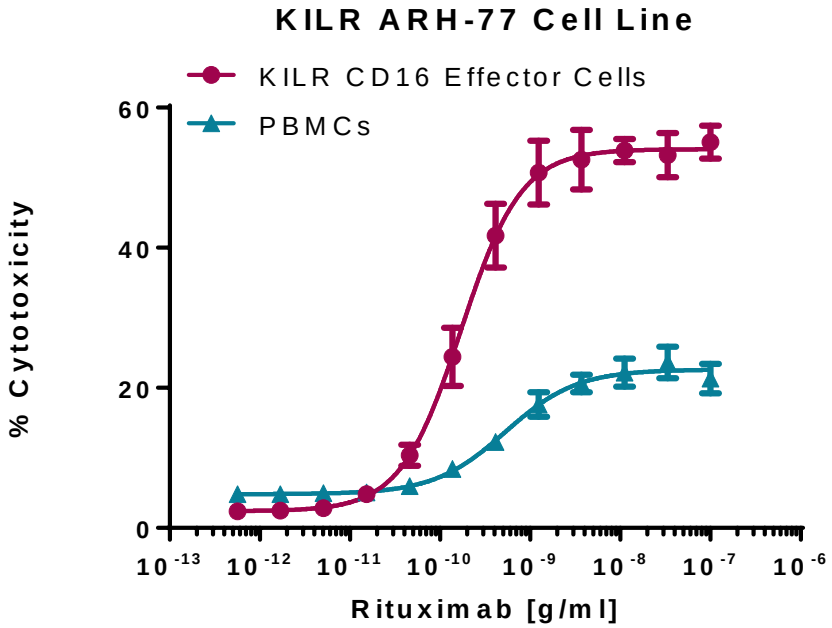
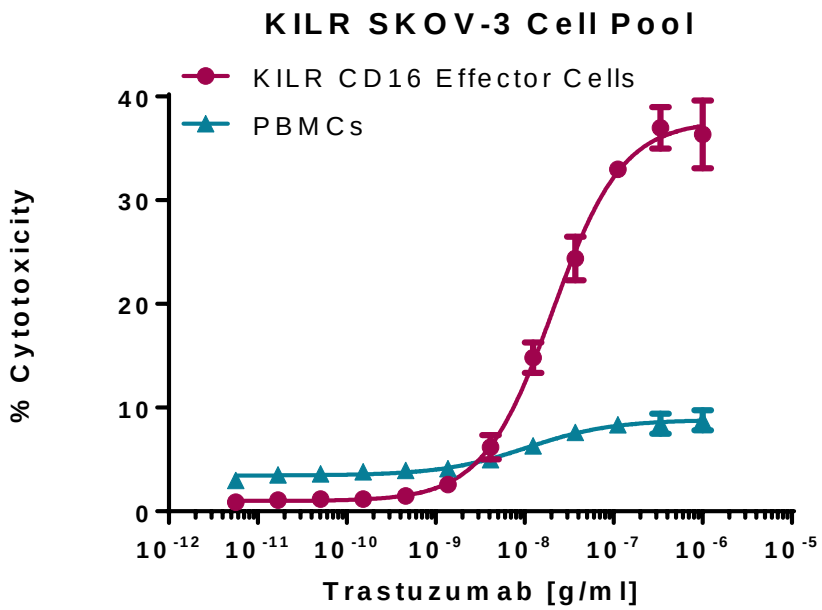
Significantly Larger Assay Window to Better Analyze Antibody Activity

Consistent pharmacology with higher signal





Sample	EC_{50}, ng/mL	S/B	Max Cytotoxicity, %
KILR® CD16 cells (10:1)	0.17	23	54%
PBMCs (25:1)	0.53	5.5	22.6%

Sample	EC_{50}, ng/mL	S/B	Max Cytotoxicity, %
KILR CD16 cells (12.5:1)	20.6	41.6	37%
PBMCs (25:1)	10.6	2.6	8.8%

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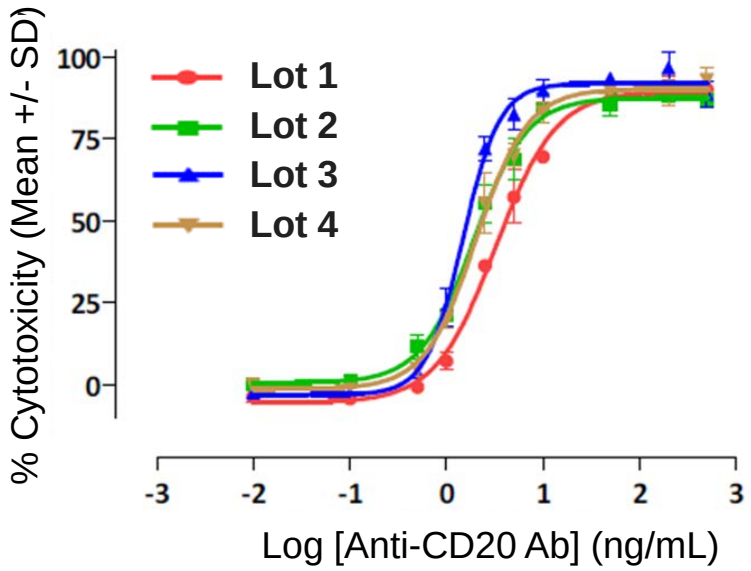
Excellent Inter-Lot Reproducibility

Lower lot-to-lot variability than typical inter-donor variability



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%**

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%**

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Intermediate Precision in KILR® ADCC Assay

Lower inter-assay variability than for PBMCs (V158 variant)

KILR CD16 Effectors			
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

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

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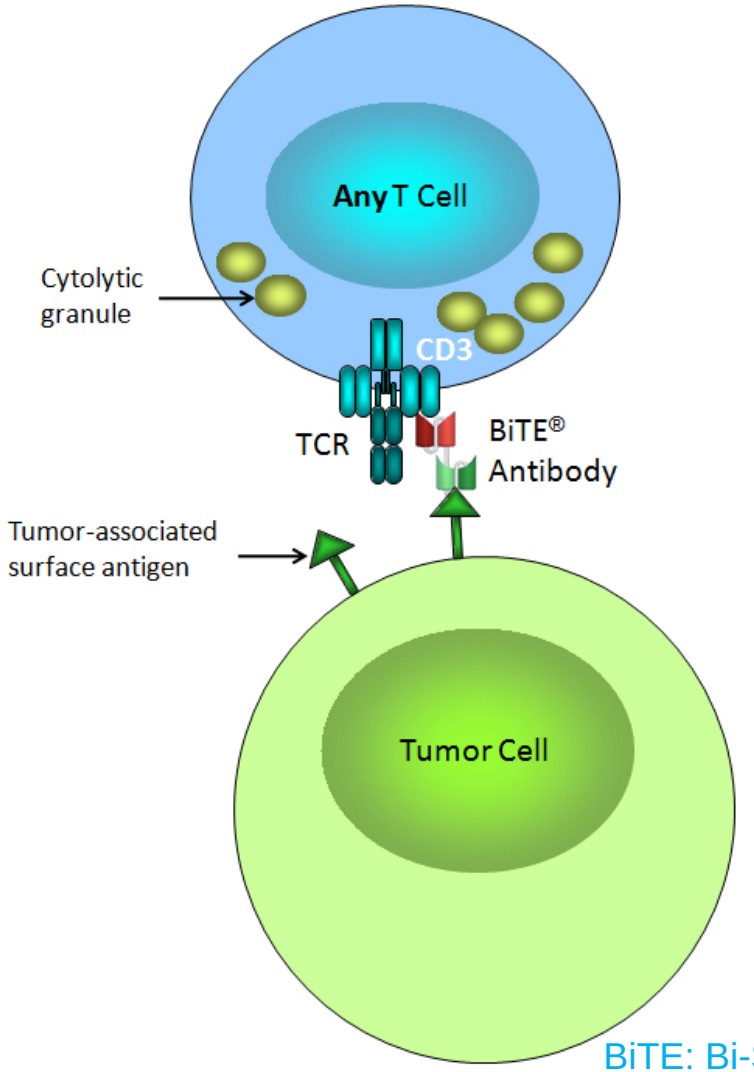
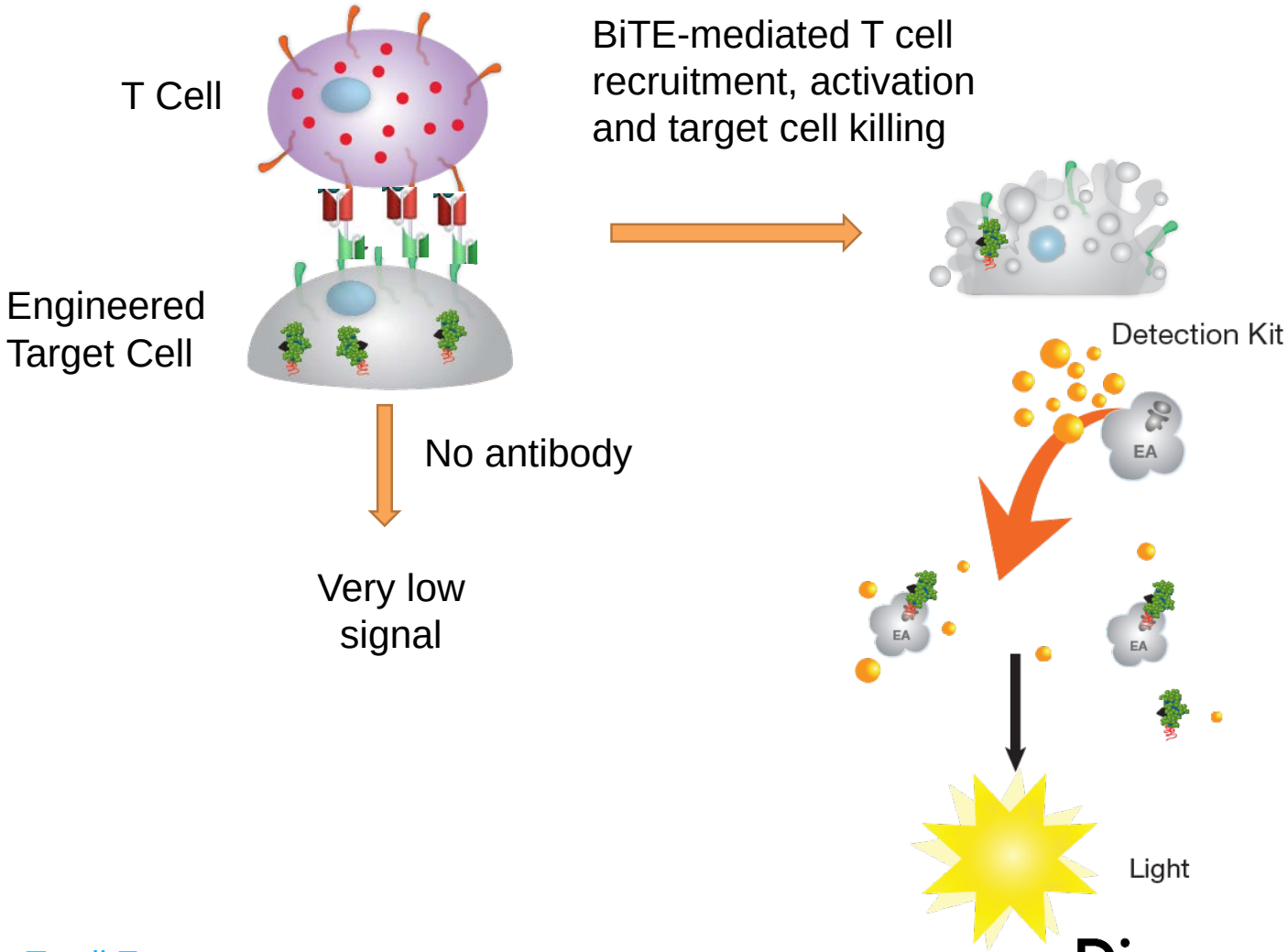
T Cell Redirection with BiTEs

Concept for KILR® TCR assay with Blinatumomab





BiTE: Bi-Specific T cell Engager

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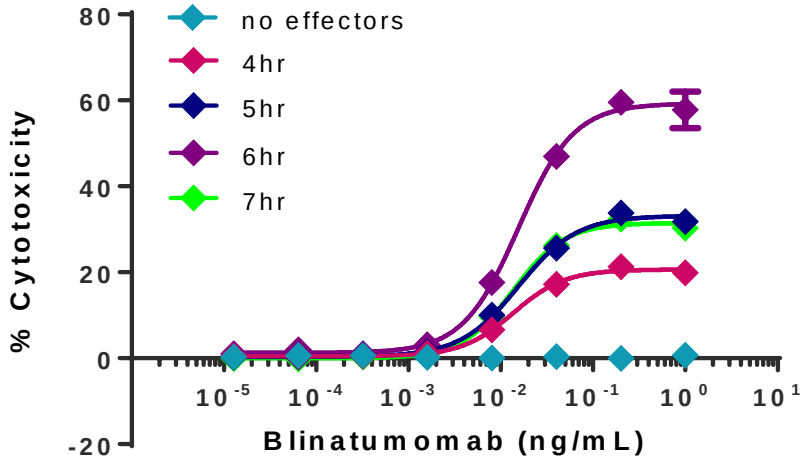
Potent and Rapid Killing Kinetics for TCR with KILR® CD16 Effectors

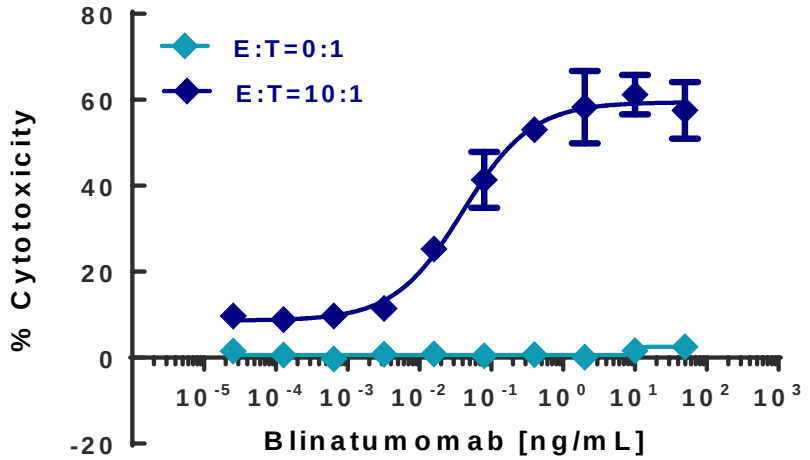
Faster results relative to pan T Cells

Effector Cells: KILR CD16 cells
Target Cells: KILR Raji Cell Pool
(E:T= 5:1); Incubation times: Varies



Time Point	Max Cytotoxicity, %	EC_{50}, pg/mL
4h	20%	13.7
5h	33%	15.5
6h	58%	15.5
7h	31%	13.6

Effector Cells: Pan T Cells
Target Cells: KILR Raji Cell Pool
(E:T= 10:1); 24hr incubation



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

Reported EC_{50} of Blinatumomab= 10-100 pg/mL

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KILR® CD16 Effector Cells

Driving robust and reproducible effector cells performance for cytotoxicity assays

Feature	Benefit
Single-donor derived primary human effector cells	Eliminate donor variability from your ADCC assays and get results with high reproducibility
Effector cells engineered to stably express CD16	Biologically-relevant true measure of ADCC
High lot-to-lot reproducibility	Enable long-term support of QC lot release bioassays
Drive and measure cell death of target cells	Reflective of true mechanism of action with target cell death driven by human effectors
Cryopreserved ready-to-use (24 hours post-thaw) primary cells	QC-friendly protocol for rapid adoption in any lab
Higher signal to background ratios	Improved performance compared to PBMCs enabling use in lot release assays

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Questions?

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