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Accelerating Potency Bioassay Implementation from Development to QC Lot-Release & Ensuring Long-term Assay Reproducibility

Alpana Prasad, Ph.D.

Director, Innovations and Strategic Partnerships

Well Characterized Biologics & Biological Assays

November 1, 2022

Eurofins DiscoverX® Is a Global Leader in Cell-Based Assays

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For Screening, Profiling, Characterization, Potency, & NAb Applications

20+ Years of Enabling Drug Discovery and Development Programs

San Francisco Bay Area, California

R&D & Manufacturing
San Francisco Bay Area, California (HQ)
St. Charles, Missouri
Poitiers, France

10+
Druggable target classes

1500+
Stable cell line and membrane preps

20+
Core patents

2000+
Publications across multiple applications

55+
Qualified & MOA-based bioassays

Validated
>30 Billion Data Points
Screened in assay services with same assays

4 Certified CRO Partners
Scientific training to enable global CROs

ICH-based Bioassay Qualification
Facilitate downstream validation studies

Dedicated Scientific Support
Experienced team providing scientific support

20+ Successful Assay Transfers
At clients/affiliated CRO sites

70+ Global Programs
For potency, stability and NAb testing

MOA = Mechanism-of-Action; ICH = International Council for Harmonization; CRO = Contract Research Organizations; NAb = Neutralizing Antibody

Bioassay Implementation Workflow

A Holistic Approach Supporting Programs from Development to Post-Market

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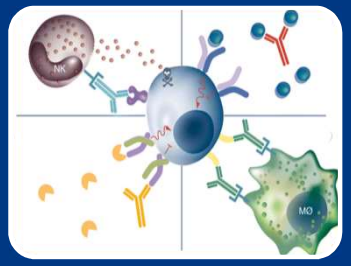
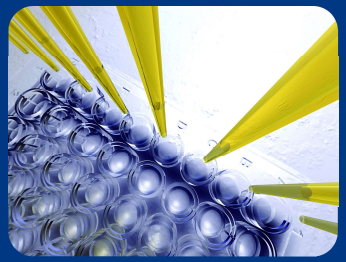


Assay Development

- Generate MOA-based cell lines
- Functional response established for cell pools
- Performance stability confirmed >50 passages
- Generate Ready-to-Use /Cryopreserved format
- Complex matrix (serum) tolerance
- Establish internal cell banks



Bioassay Qualification

- Qualified under ICH guidelines
  - Specificity, repeatability, precision, accuracy, dilutional linearity, parallelism
  - Reproducibility (intra- & inter-lot)
  - Relative potency range (50%-150% or client-defined)
- Stability-indicating
- Provided as a complete kit with optimized reagents & detailed protocols
- Multiple production lots

Transfer to Testing Sites (CDMO/CROs)

- Streamlined Method Transfer
  - Development dataset
  - On-site training (classroom & lab)
  - Validation study support
- Certified CDMOs/CROs
- Continued post-implementation support
- Global dedicated support teams

Long-term Critical Reagents Management

Bioassay Cells

- Dedicated well-characterized analytical cell banks for client's program
- Full traceability (audit-ready)

- Supply agreements
- Technology transfer

Enzyme Fragment Complementation (EFC)
Robust Platform for Interrogating Different MOAs

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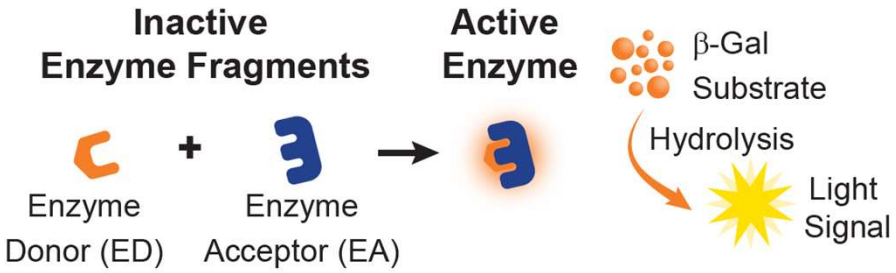
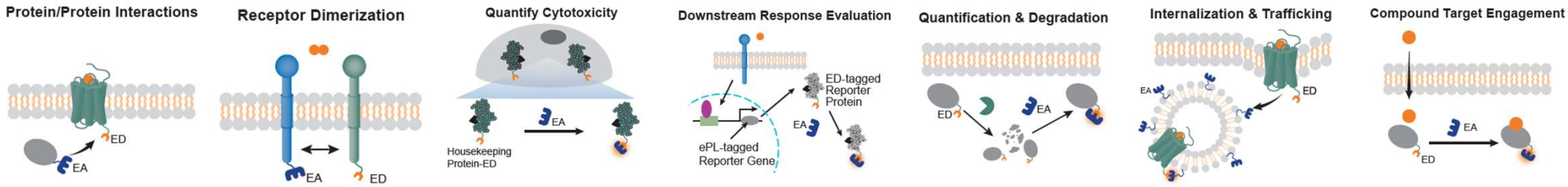


Homogenous
- Simple, add-and-read assay format
- Easily quantified luminescence read-out

Sensitive Detection
- Enzymatically-amplified assay
- High precision & accuracy

Robust
- Large dynamic range
- High assay reproducibility

Easy to Transfer
- Similar workflow across platform
- Compatible with most readers



Examples of Target Biology Translated into Assay Design

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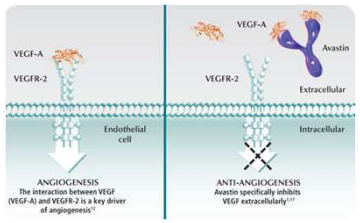
Molecular MOA

Anti-VEGFs



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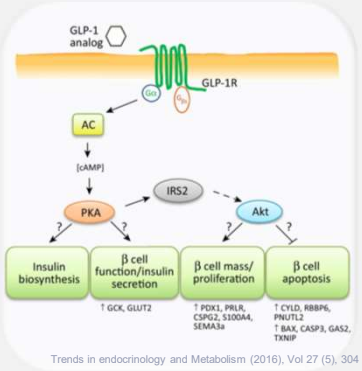
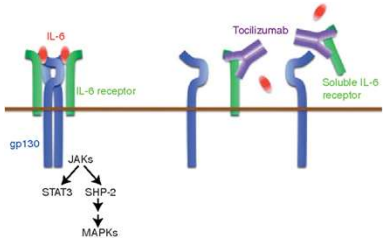
GLP-1R Agonists



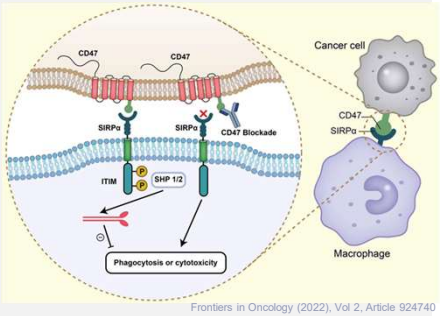
Trends in endocrinology and Metabolism (2016), Vol 27 (5), 304

IL-6 Antagonists



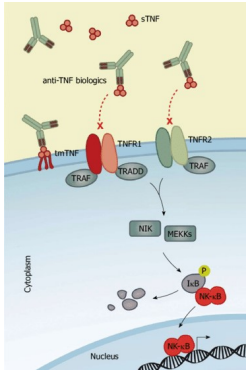
Cold Spring Harbor Perspectives in Biology (2014), 6 10: a016295

SIRPα | CD-47



Frontiers in Oncology (2022), Vol 2, Article 924740

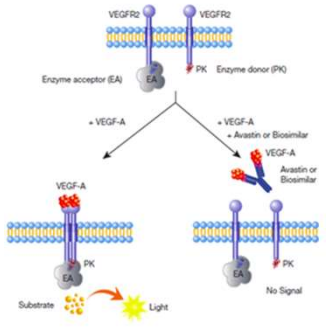
Anti-TNFs



World J Gastroenterology (2014), Vol 20(1), 64

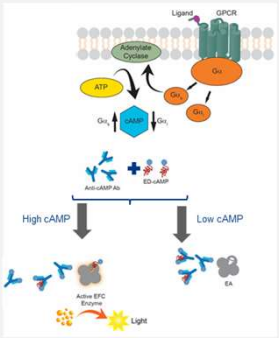
Assay Design

Receptor Dimerization



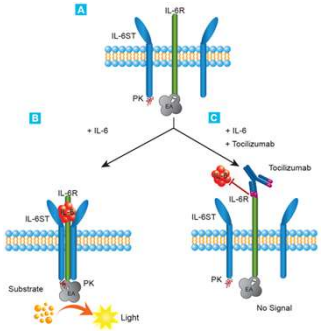
Bevacizumab, Ranibizumab & Aflibercept

cAMP Accumulation



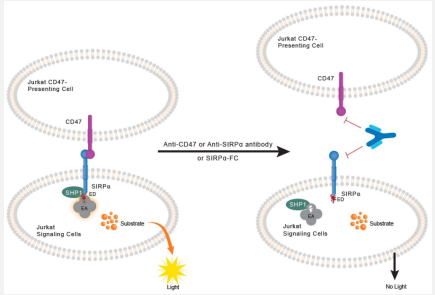
Semaglutide, Liraglutide & Exenatide

Receptor Dimerization



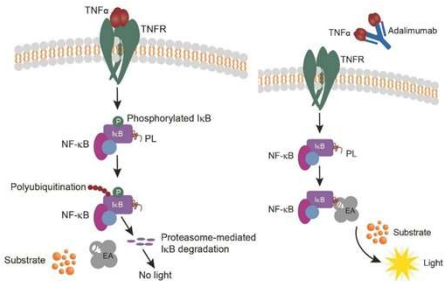
Tocilizumab

SH2 Recruitment



Anti-SIRPα antibody

Ikb Degradation



Adalimumab

Ready-to-Use Qualified Bioassay Method
From Assay Development to Transfer

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MOA-based Bioassays

Qualified Bioassays

Assay Selection & Cell Line Characterization

- **Choose assay format that best reflects MOA of drug**
- Select relevant MOA
- Establish stable cell line
  - Retrovirally engineered
  - Cell backgrounds
  - Functional response
  - Assay windows
- Passage stability
- Sterility

Assay Optimization

- **Optimize assay parameters (thaw-&-use cells only)**
- Culture & freeze conditions
- Cell density
- Assay reagents & buffers
- Ligand incubation time
- Cell plating time
- Assay plate type
- Incubation time with detection reagents
- Assess sensitivity to matrix (normal human serum)

Testing Innovator Drug

- **Refine conditions with originator drug or clinical molecule**
- Optimize with marketed drug or reference standard
- Dilution series
- Establish suitability of assay to determine relative potency
- Determine range and linearity of the assay over 50%-150% with single analyst (at least 3 independent runs)

Assay Qualification

- **Establish robustness, precision, accuracy & linearity of assay**
- Robustness: 11-point DRC
- Plate uniformity: EC_{80}/IC_{80} across entire plate
- Reproducibility and intermediate precision: 3 plates with 11-point DRC over 3 days
- Second analyst: Determine range and linearity over 50% -150% of assay

Confirm manufacturability
- Multiple lots
- Scale-up (~500/lot)

Establish QC release criteria for production lots

Bioassay kit & detailed protocol

Transfer method

Strong Relationships with Global CDMOs/CROs for Seamless Method Transfer & Implementation

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BIOASSAYS CERTIFIED Eurofins DiscoverX

Catalent

KBI BIOPHARMA

vela Laboratories
A Tentamus Company

eurofins | Advantar

eurofins | BioPharma Product Testing

eurofins | Lancaster Laboratories

PPD

QUALITY ASSISTANCE
Contract Research Organisation

eurofins | PROXY Laboratories

eurofins | DISCOVERY

charles river

intertek
Total Quality. Assured.

SARTORIUS

BioAgilytix

Lonza

QPS
CUSTOM-BUILT RESEARCH

ABZENA

Cytovance Biologics

WuXi Biologics
Global Solution Provider

SGS

LabCorp
COVANCE

Assays Implemented in Global Biologics Programs
(Characterization, Stability Studies, NAb Detection & Potency Testing in Lot Release)

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Anti-SIRPα Multiple programs in NA & EU for originator biologics Potency & NAb	**Anti-PD1** Multiple programs in NA & APAC for originator & biosimilars Potency	**BTLA** US-based program for originator biologic Not disclosed	**Anti-VEGF** Multiple programs in NA, EU & APAC for biosimilars Potency	**IGF1R** Multiple programs in NA and APAC for originator biologic Potency	**GLP1R** Multiple programs in NA, EU & APAC for originator & biosimilars Potency	**CNR1** US-based program for originator biologic Potency	**BRDKBR2** US-based CRO Potency	**INSRb** Multiple programs in NA, EU & APAC for biosimilars Potency
IL-17R EU biopharma for biosimilar Not disclosed	**IL-2R** Multiple programs in NA and EU for originator biologic Not disclosed	**IL-31R** US-based pharma for originator biologic Potency & NAb				**MCxR** Multiple programs in NA & EU for originator biologic Not disclosed	**FSHR** APCA-based program for biosimilar Potency	**PTHR1** Multiple programs in NA, EU & APAC for biosimilars Potency
IL-10R Multiple programs in NA for originator biologic Potency	**IL-23R** US-based biopharma for originator biologic Potency	**IL-7R** EU-based program for originator biologic Potency				**CALCRL/RAMP3** NA-based pharma for originator biologic Potency	**CXCR4** EU-based pharma for originator biologic NAb	**CXCR2** US-based program for originator biologic Not disclosed
TSLPR EU-based pharma for originator biologic Potency	**CSF2R** Multiple programs in NA & EU Potency	**CSF1R** Multiple programs in NA & EU Potency	**RANK** Multiple programs in NA & APAC for biosimilars Potency	**FGFR** US-based biopharma for originator biologic NAb	**ErbB2/ErbB3** Multiple programs in NA, EU & APAC for biosimilars Potency	**GM-CSF** Multiple programs in NA & EU for originator biologic Potency	**AXL** US-based program for originator biologic Not disclosed	**CD16 Effectors** Multiple programs in NA & EU for ADCC Potency

NA = North America; EU = Europe; APAC = Asia Pacific

Case Study:

KILR® Raji Bioassay for Therapeutics Driving ADCC Mechanism

Challenges with Existing Cytotoxicity Assays Addressed by KILR® Platform

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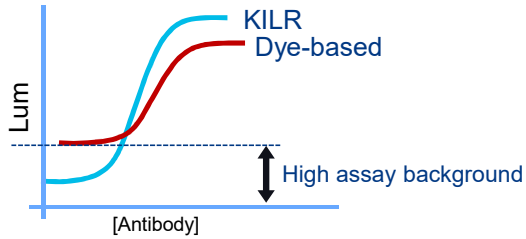
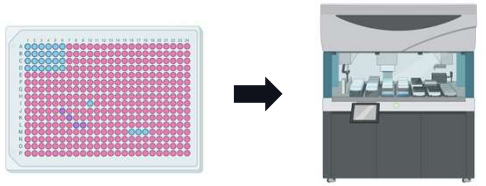
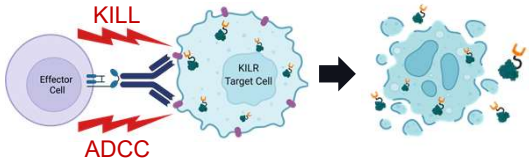
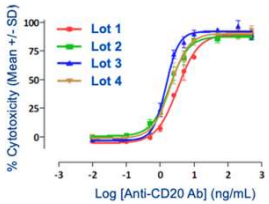
What assay development scientists face….

Radioactivity or fluorescent dye-based assays are inherently leaky, less robust & produce high background	*KILR assays are non-radioactive and not leaky*	*Little to no background*
Low assay throughput restricts screening and characterization efficiency	*KILR assays are high-throughput compatible (384-well)*	*Ease of implementation from screening to lot release*
Surrogate assays fail to reflect the MOA. These are *"Predictive" and not "Reflective"*	*KILR assays are end-point assays and specifically measure target cell killing*	*Physiologically-reflective of TRUE MOA*
High donor-to-donor variability associated with effector cells	*KILR CD16 Effector Cells are single donor-derived*	*Eliminate donor variability*









KILR® Cytotoxicity Assay for ADCC Overview

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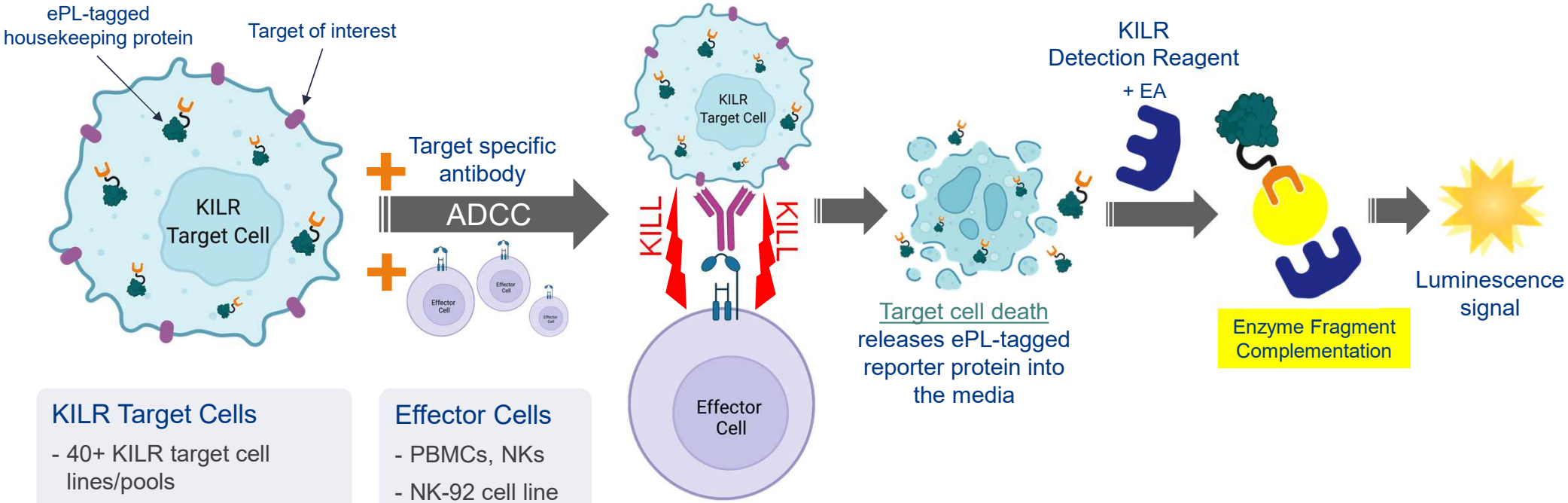
An easy-to-use assay to specifically measure target cell death



KILR Target Cells

- 40+ KILR target cell lines/pools
- 3 KILR RTU models
- Target cells generated using KILR retroparticles

Effector Cells

- PBMCs, NKs
- NK-92 cell line
- CAR-T
- KILR CD16 Effector Cells

KILR® Assay ADCC Demonstrated Using a Variety of Antibodies, Antigens, and Target Cell Types

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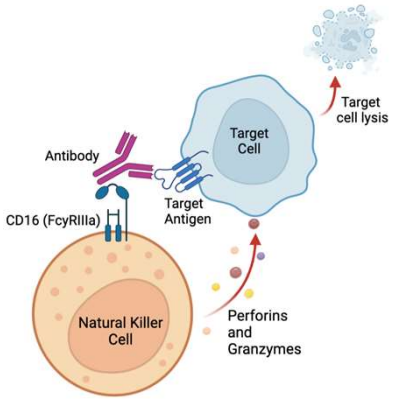
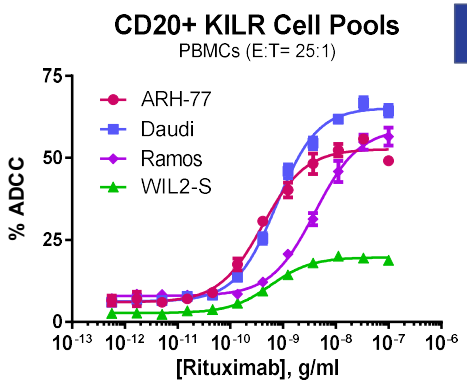
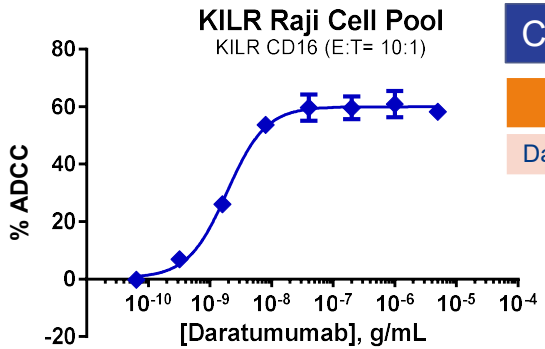


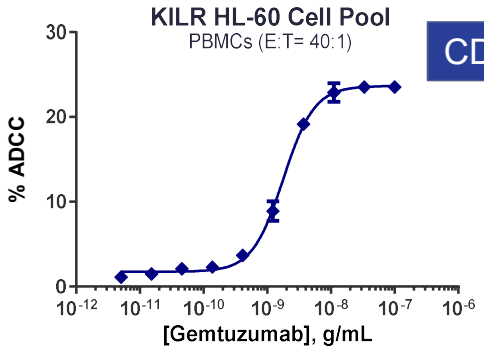


CD20



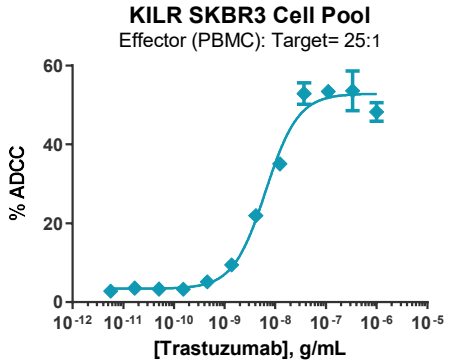
CD38

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



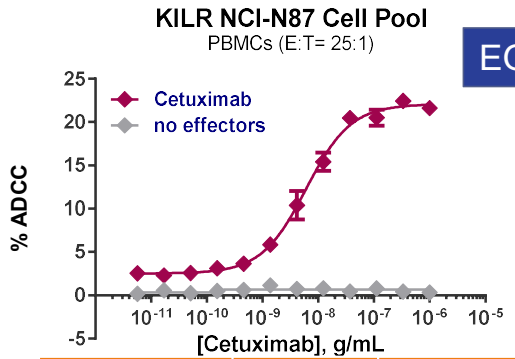
CD33

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



HER2

Sample	E_{Max}	EC_{50}, ng/mL
Trastuzumab (5K, 10K)	53.6%	6.44



EGFR

Sample	E_{Max}	EC_{50}, ng/mL
Cetuximab	22%	6.04

Eliminating Donor Variability in ADCC Assays
Using KILR® CD16 Effector Cells

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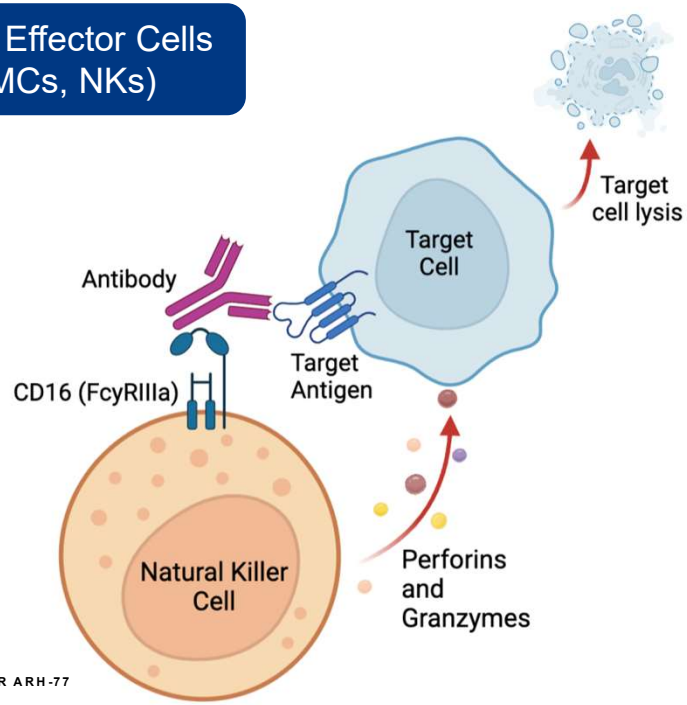
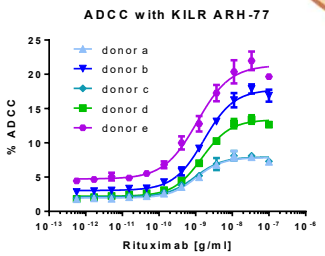
Isolated Effector Cells (PBMCs, NKs)

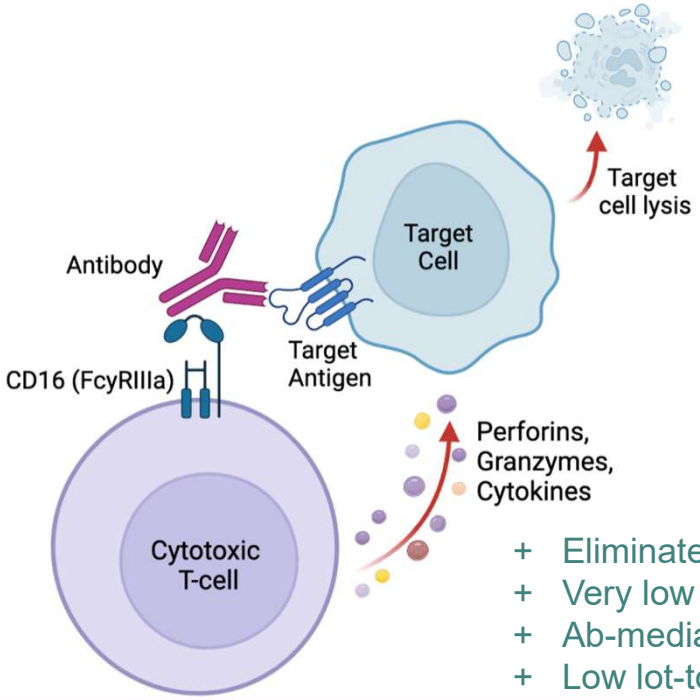


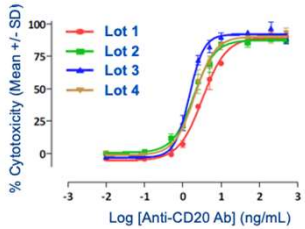


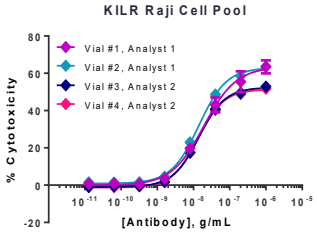
- High donor variability
- Heterogenous population (PBMCs)
- High lot-to-lot variability
- High background killing (NK cells)

KILR CD16 Effector Cells

Single donor-derived Cytotoxic T-cells engineered to express CD16 receptor







- \+ Eliminates donor variability
- \+ Very low background killing
- \+ Ab-mediated rapid killing kinetics
- \+ Low lot-to-lot variability

Continuous Culture vs Ready-to-Assay Cell Formats

KILR® Raji ADCC Bioassay Kit Performance

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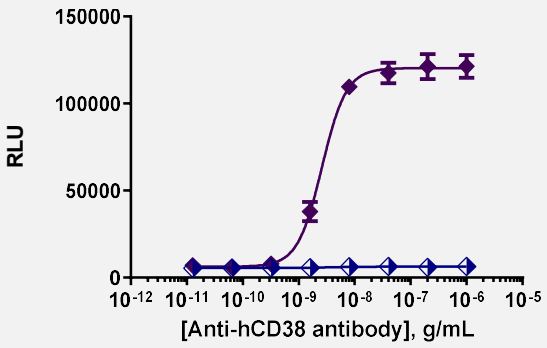
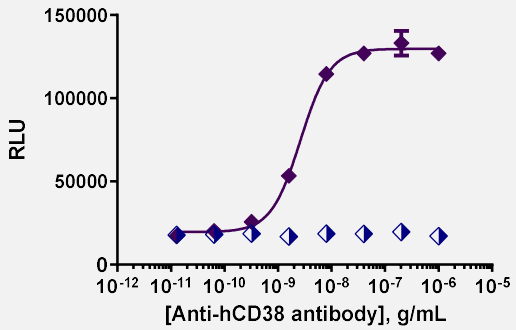
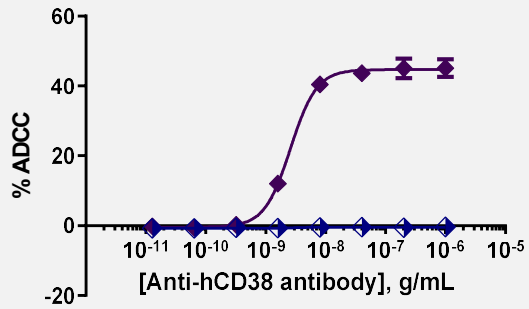
Continuous Culture **Assay Ready**

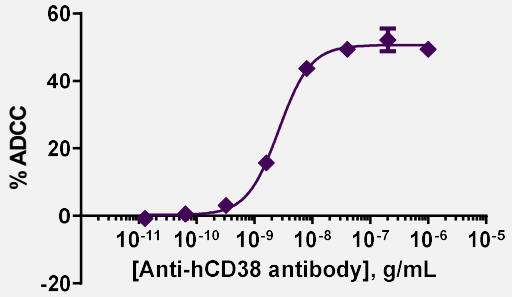
Raw Luminescence (RLU)





%Killing (%ADCC activity)





Condition	S/B	EC_{50}, ng/mL
Continuous Culture	19	2.6
Assay Ready	6.7	2.6

Excellent Concordance between EC_{50} of the two formats

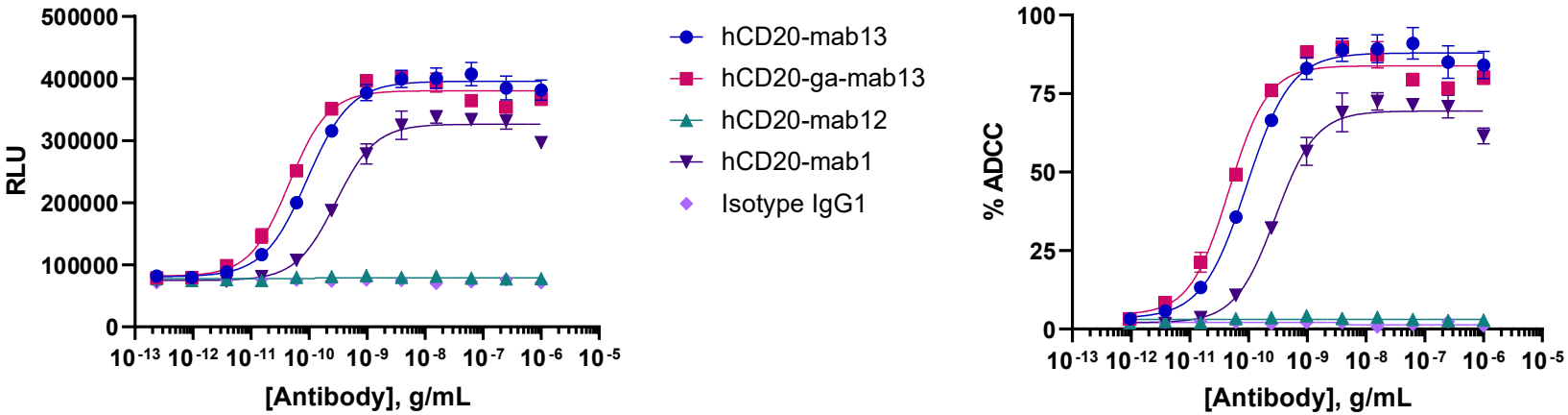
Condition	E_{Max}	EC_{50}, ng/mL
Continuous Culture	45%	2.6
Assay Ready	51%	2.6

Comparable % Killing (% ADCC) with KILR Raji Bioassay Cells Relative to Continuous Culture

Effectors used: KILR CD16 Effector Cells

Suitable for Screening and Rank Ordering of Antibodies

KILR® Raji ADCC Bioassay Kit



Sample	Description	S/B	HillSlope	E_{Max}	EC_{50}, pg/mL
hCD20-mab13	Non-fucosylated anti-CD20 (rituximab)	5.4	1.152	88%	92.8
hCD20-ga-mab13	Non-fucosylated anti-CD20 (obinutuzumab)	5.2	1.307	84%	46.5
hCD20-mab12	Fc Null variant of rituximab	--	--	--	--
hCD20-mab1	Wild-type rituximab	4.5	1.270	69%	288
Isotype (IgG1)	Human IgG1 isotype control	--	--	--	--

KILR Raji Bioassay allows discrimination of differences in Fab and effector regions of related antibodies

Repeatability (over 3 Days); Single Analyst
KILR® Raji ADCC Bioassay Kit

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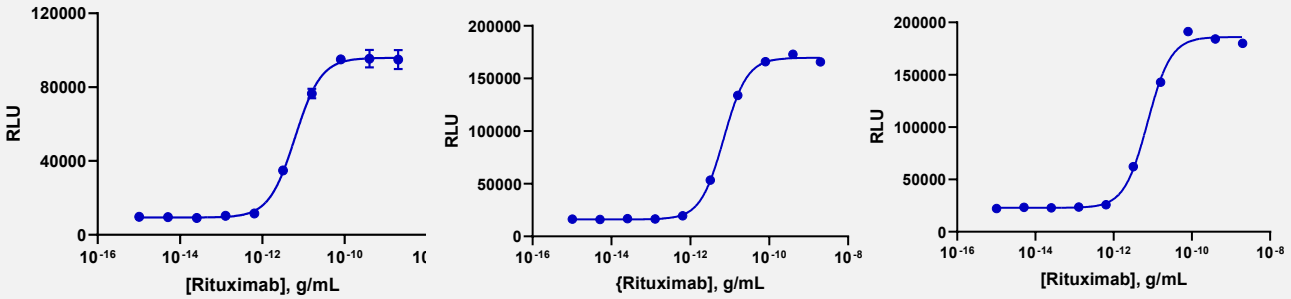
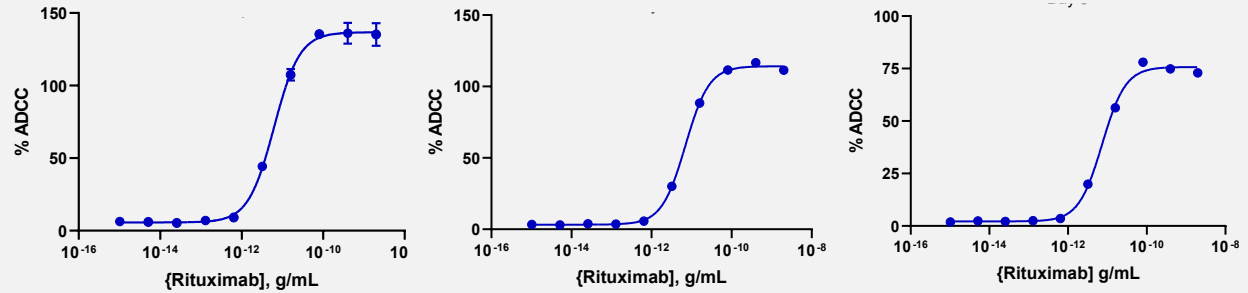
Day 1 **Day 2** **Day 3**

Raw Luminescence (RLU)



% Killing (% ADCC activity)



Parameter	Day 1	Day 2	Day 3	% RSD
EC_{50} (pg/mL)	6.2	7.03	7.4	8.9%
S/B	9.8	10.6	8.3	12.2%
E_{Max} (%)	135%	114%	75.8%	27.7%

Excellent inter-day repeatability

Effectors used: KILR CD16 Effector Cells

Consistent Performance Between Analysts (Relative Potency)

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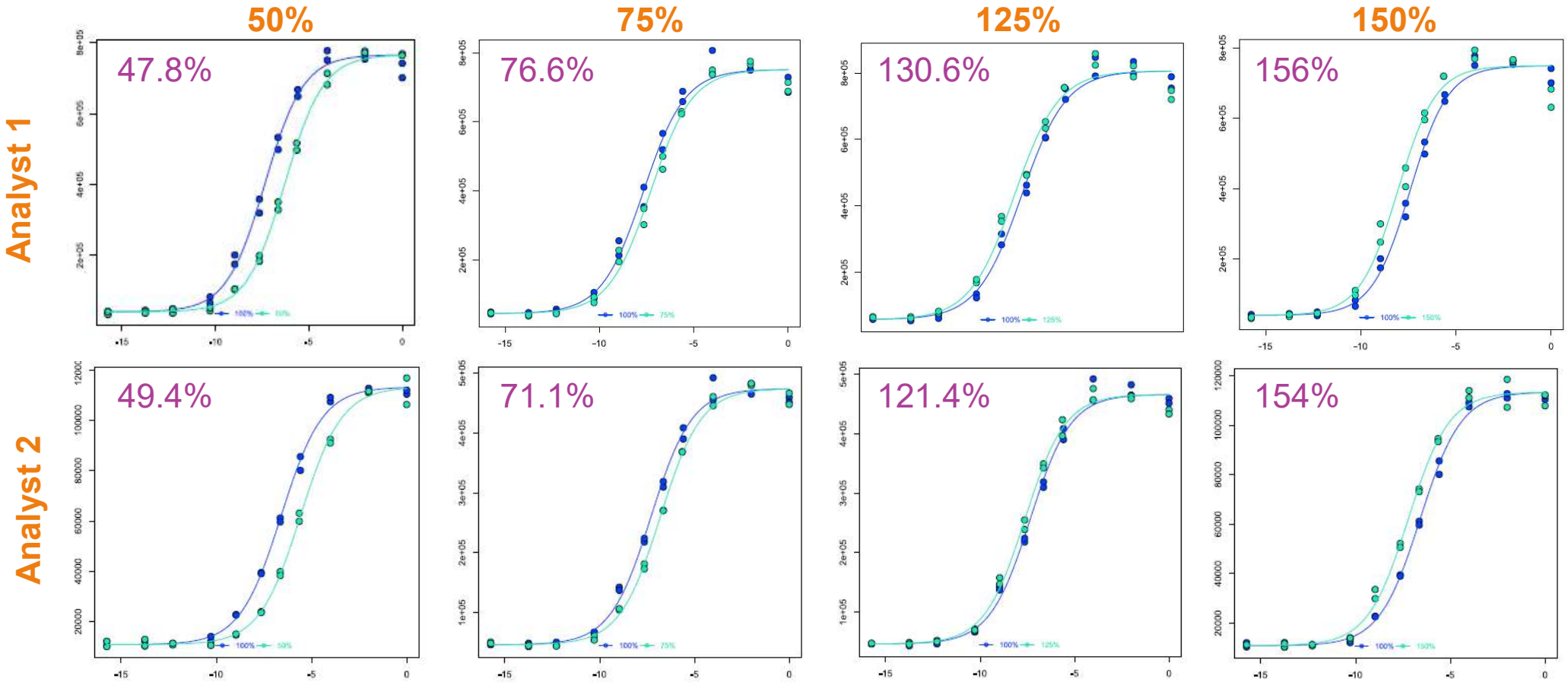

Effectors used: KILR CD16 Effector Cells

Phase-Appropriate Method Qualification

Suitability of KILR® Raji Bioassay Cells for Relative Potency Assays

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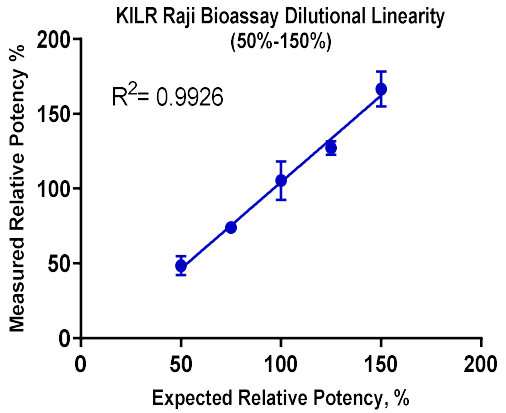
Nominal RP, %	Analyst	Observed RP, %	Average RP, %	% RSD	Average % Recovery
150	1	156	166.6	7.0	111.1%
	1	179.4			
	1	177.4			
	2	166			
	2	154.3			
125	1	130.6	127.3	3.6	101.8%
	1	131.2			
	1	126			
	2	121.4			
100	1	82	106.5	13.0	106.5%
	1	116			
	1	112.7			
	1	98.4			
	1	117.8			
	2	112.2			
75	1	70.4	73.9	4.9	98.5%
	1	76.6			
	1	77.3			
	2	71.1			
50	1	47.8	48.5	13.2	96.9%
	1	45.8			
	1	40.9			
	2	58.4			
	2	49.4			



Parameter	Value	Specification
Accuracy (Average % Recovery)	103%	100% ± 20%
Repeatability	14.2%	≤20%
Intermediate Precision	≤13.2%	≤20%
Linearity (R^2)	0.9926	≥0.95

Assay demonstrates very good accuracy, repeatability, intermediate precision, and dilutional linearity

Effectors used: KILR CD16 Effector Cells

Ready-to-Use Format
Simple and Fast KILR® Raji ADCC Bioassay Kit Protocol

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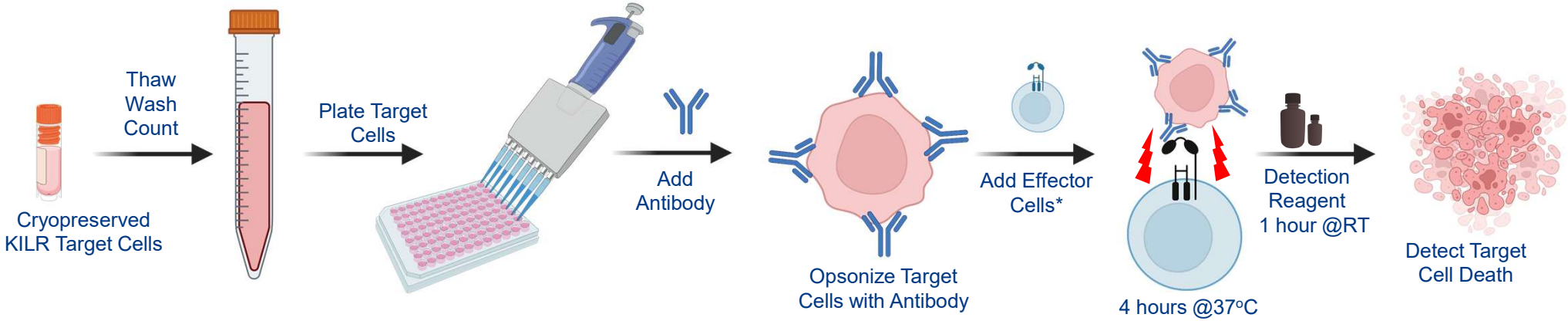
<8 hours



Bioassay Kit Includes Required Reagents for the Assay

*Effector cells sold separately

Same KILR® Target Cells Enable Assessment of Multiple MOAs

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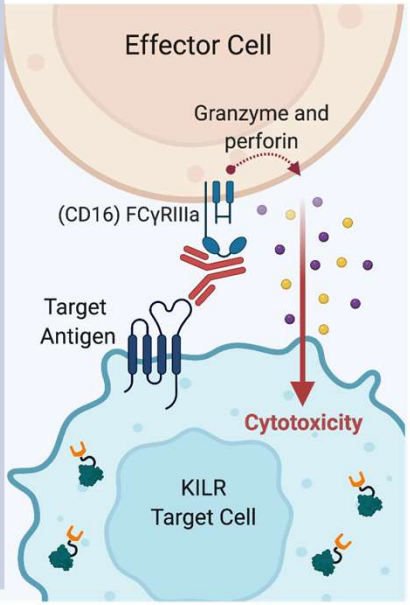
ADCC

Antibody-Dependent Cellular Cytotoxicity

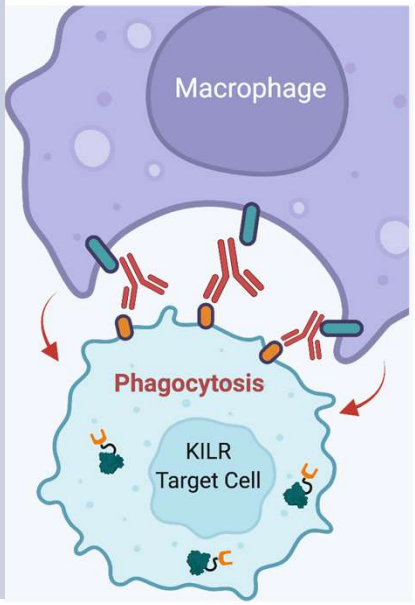
Effector cells engage with target cells through antibody and kill



ADCP

Antibody-Dependent Cellular Phagocytosis

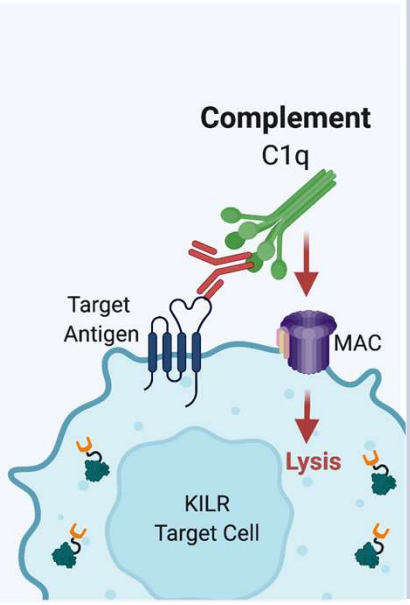
Fc-dependent phagocytosis and lysosomal degradation



CDC

Complement-Dependent Cytotoxicity

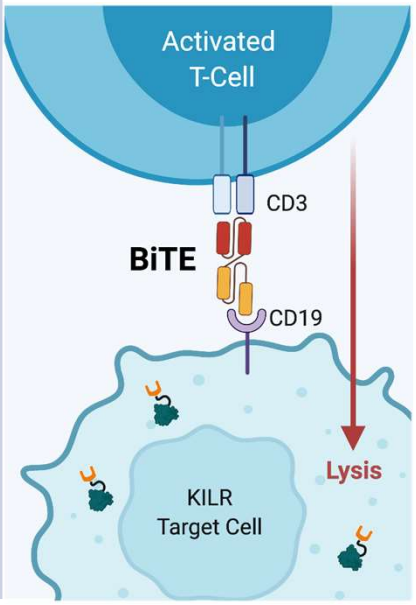
Activated complement system leads to formation of membrane attack complex (MAC)



TCR

T-cell Redirection (with T-cell Engagers)

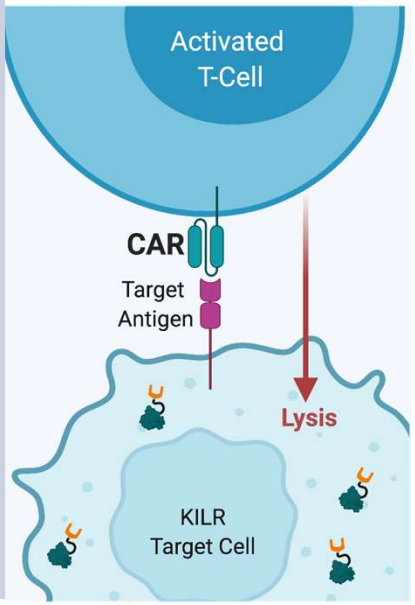
Bi-specific antibody engages T-cell with cancer cells for killing



CAR-T

Chimeric antigen receptor T-cells

Engineered T-cells recognize and kill cancer cells



KILR Reporter Protein

Largest Menu of Cell-based Assays for Potency & NAb Assay Development

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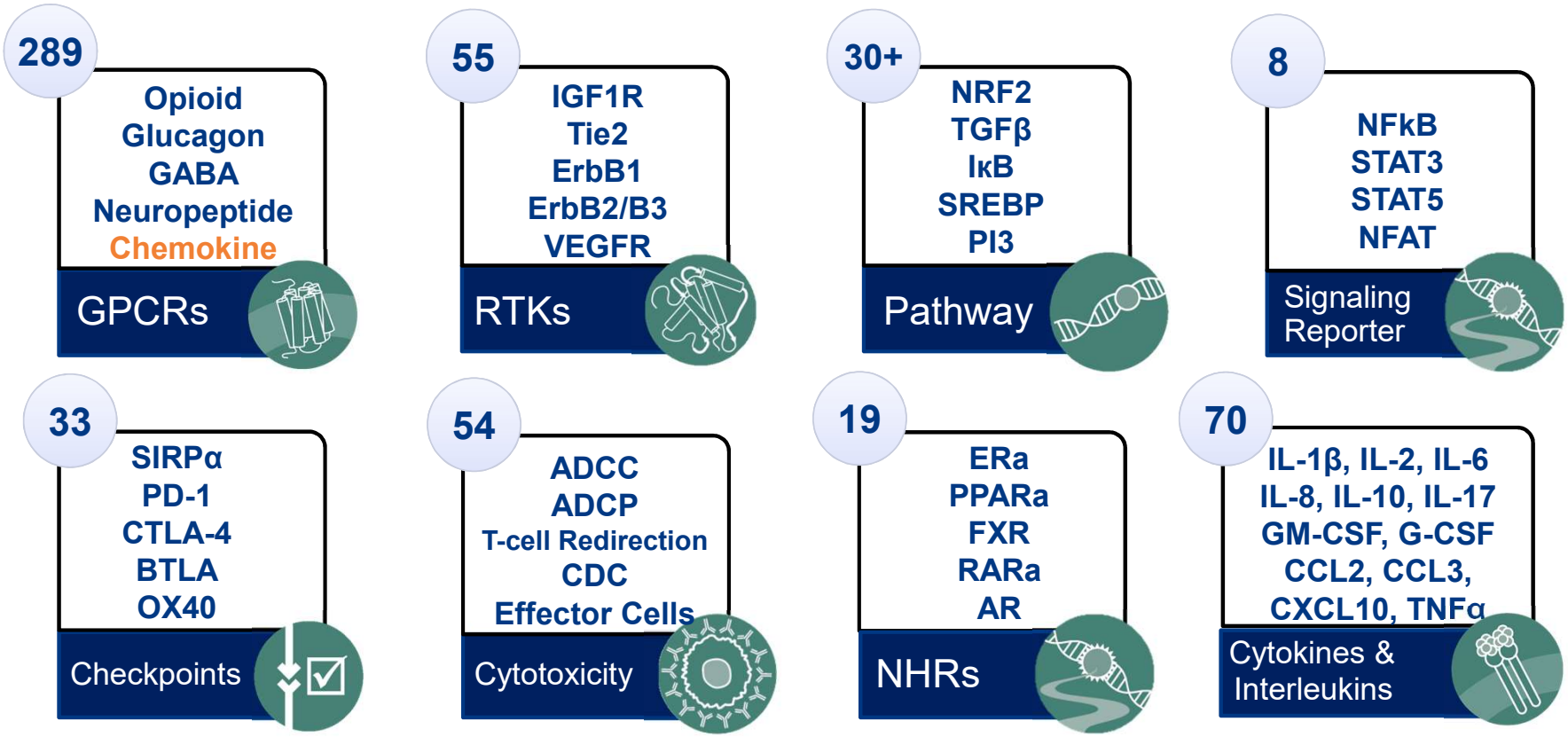
>1500+ Cell Lines and Membrane Preps to support bioassay development for major drug target classes



Options to Accelerate Your Bioassay Development Plan

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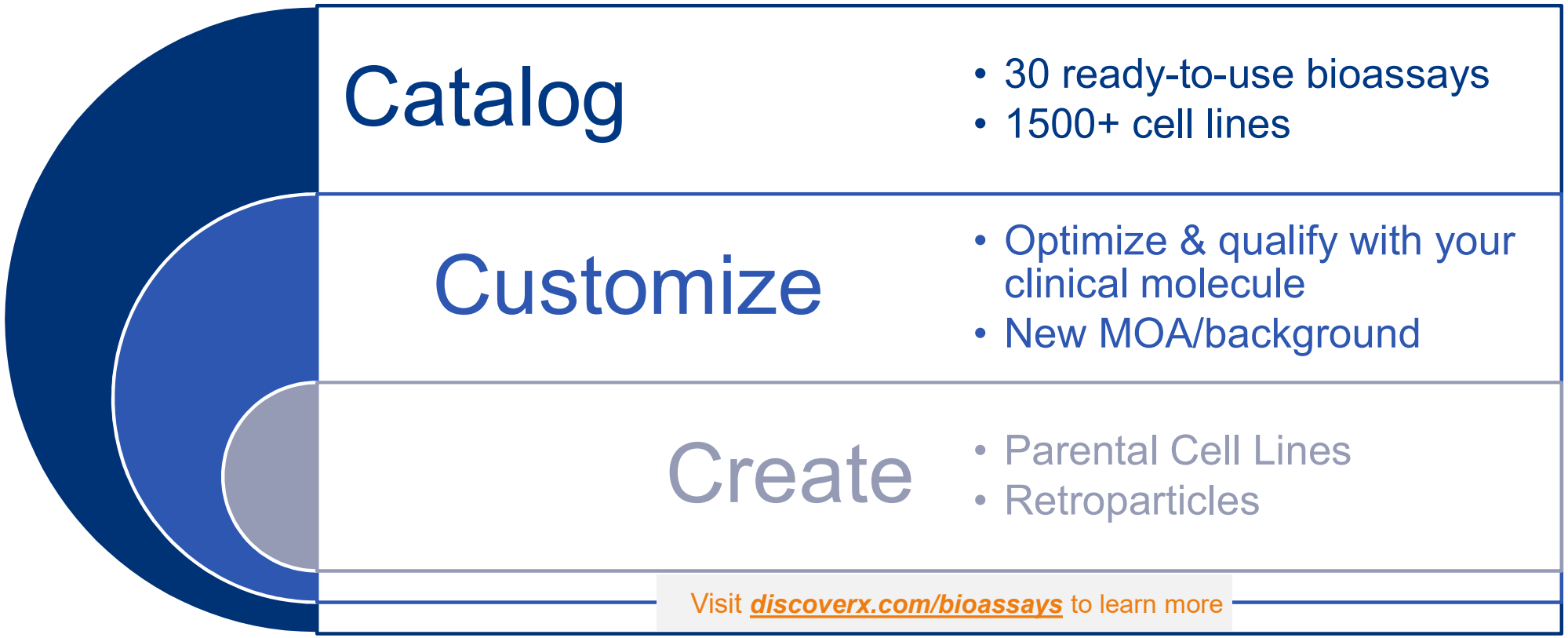

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Come visit us at our booth! We look forward to discussing your program requirements.