



# Accelerating Immuno-oncology Drug Discovery with MOA-reflective, Functional Cell-based Assays

Venkatesh Chari, Ph.D.

Scientific Market Development Manager

Eurofins DiscoverX Products LLC

Introduction to Eurofins DiscoverX and Immuno-oncology Therapeutics

Technology and Cell-based Assays Overview

Large Molecule Focused Assay Portfolio

- Immune Checkpoint Receptor Assays
- Internalization Assays for Antibody Drug Conjugates (ADCs)
- Assays for Screening and Characterizing Bi-Specific Antibodies (BsAbs)
- Cytokines, Chemokines, and Interleukin Assays
- KILR<sup>®</sup> Cytotoxicity Platform

End-to-end Support for QC Lot Release Testing

Conclusions

Visit us at US PEGS Booth #511

# Eurofins DiscoverX is a Global Leader of Cell-based Assays for Screening, Profiling, Potency, and Lot Release Programs



DiscoverX

From Discovery to Development to Clinic to Post-market

## 20+ Years Experience Building Cell-based Assays



Industry's Largest Cell-based Assay Portfolio

10+ Druggable Target Classes

Supporting 100+ Global Programs  
For Potency, Stability, and NAb testing

Global FAS Team | Assay Transfer Support

BIOASSAYS  
CERTIFIED CRO  
8 Certified CRO

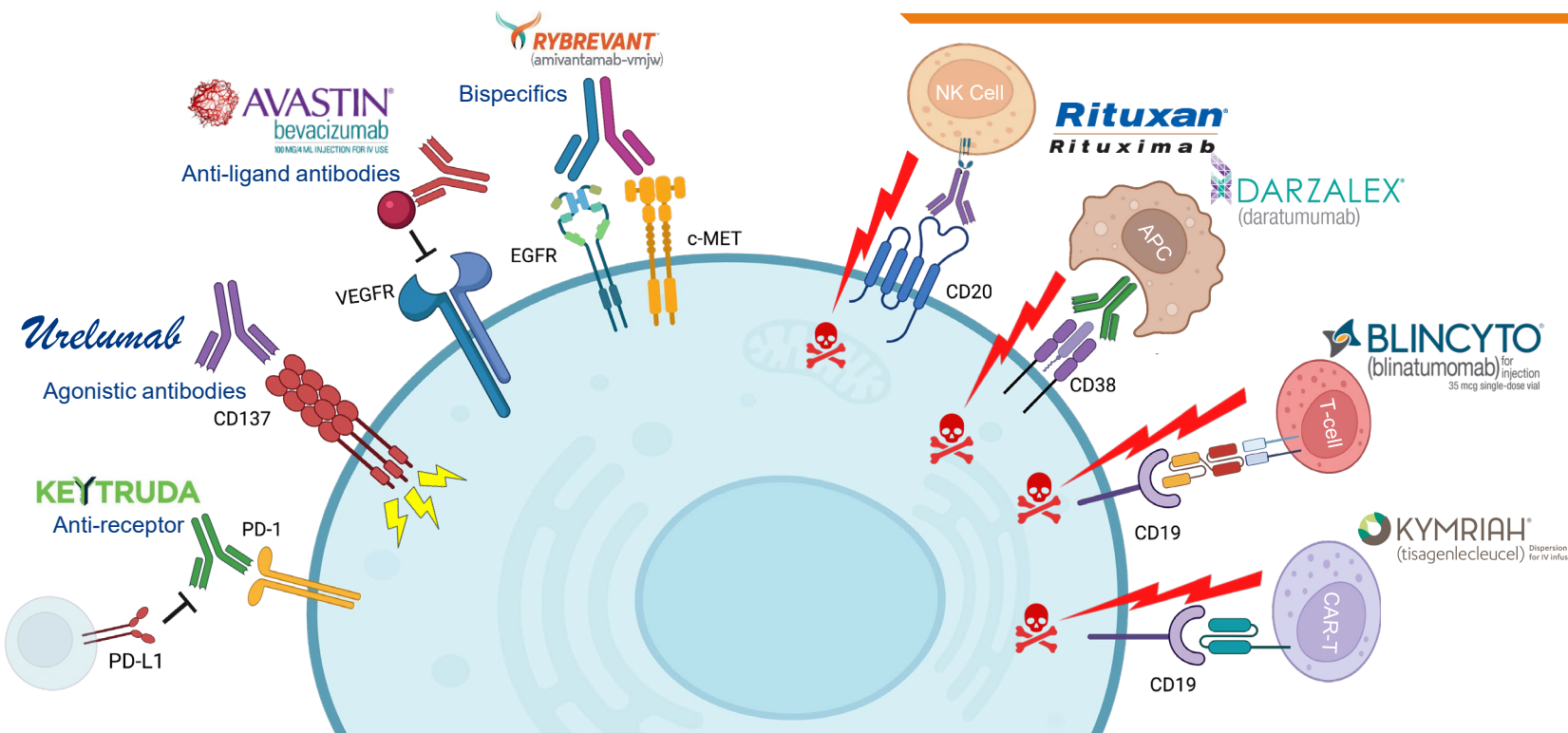
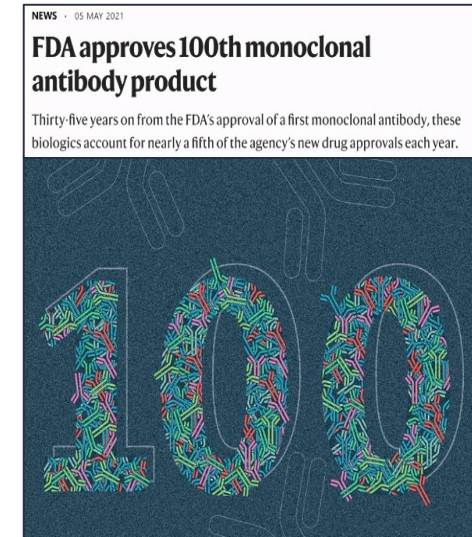
ICH-based Pre-qualified Bioassays

CRO – Contract Research Organization, ICH – International Conference on Harmonization, NAb – Neutralizing Antibody, FAS – Field Application Scientist



# Cell-Mediated Cytotoxicity – A Rapidly Developing Area For Cancer Therapeutics

- Therapeutic mAbs are the fastest growing class of biologics
- Therapeutic mAbs targets various diseases and act through several different MOAs
- DiscoverX's cell-based assays supports the development of mAbs, bispecific antibodies, agonistic antibodies, and several cell and gene therapeutics



Need cytotoxicity assays for:

- Screening
- Characterization
- Lot release



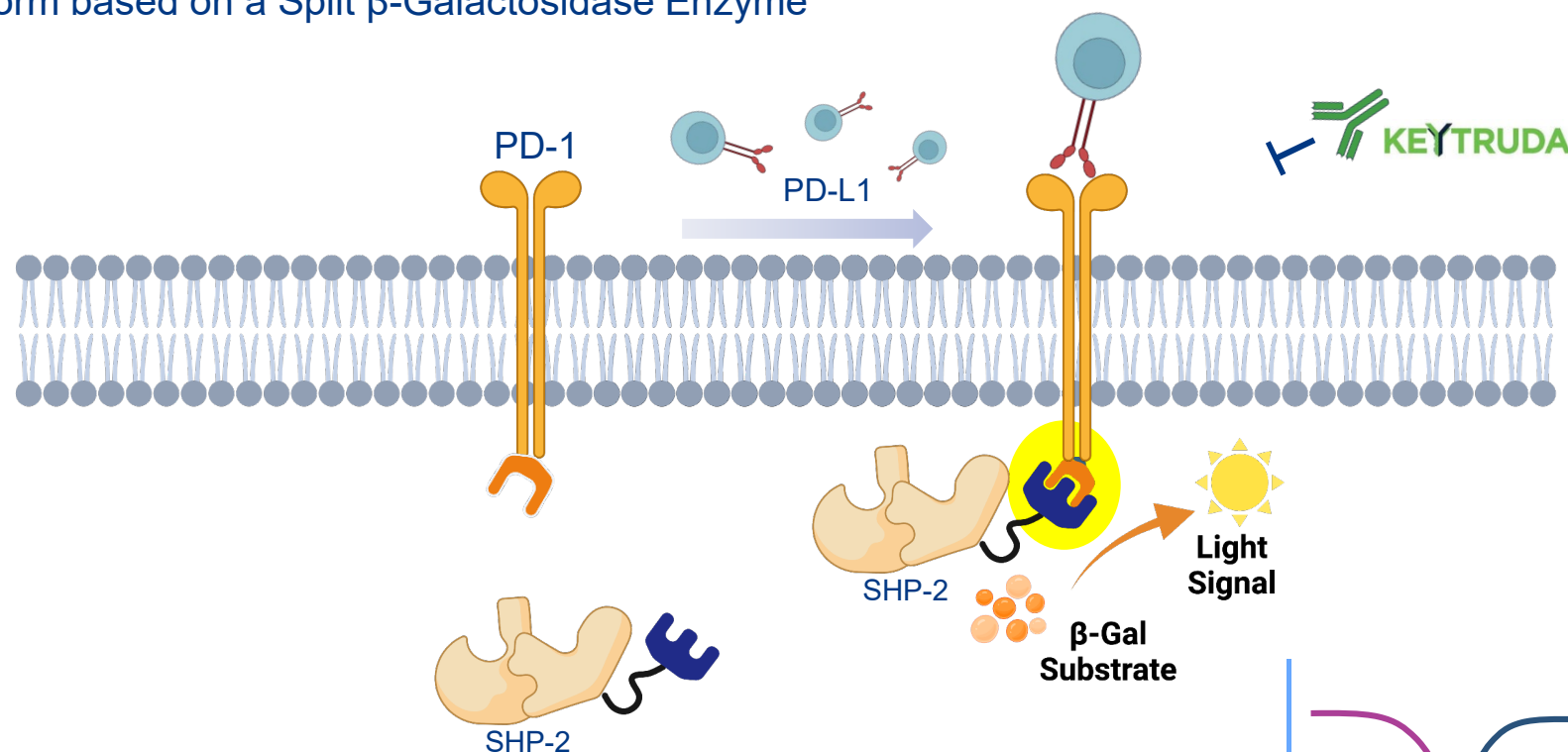
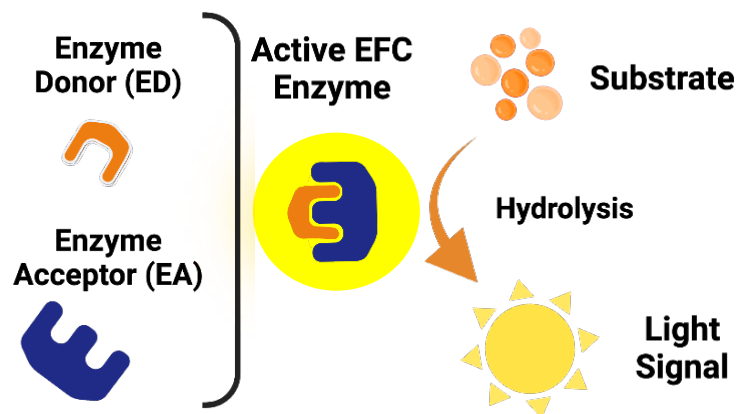
Technology and Cell-based Assays Overview

# Enzyme Fragment Complementation

# Enzyme Fragment Complementation (EFC) Technology

Enabling Technologies with a Flexible Platform based on a Split  $\beta$ -Galactosidase Enzyme

## EFC Technology Principle



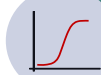
### Homogenous

- Simple add-and-read assay format
- No media change, shaking or filtration



### Sensitive Detection

- Enzymatically-amplified Assay
- High precision & accuracy



### Robust

- Large dynamic range
- High assay reproducibility



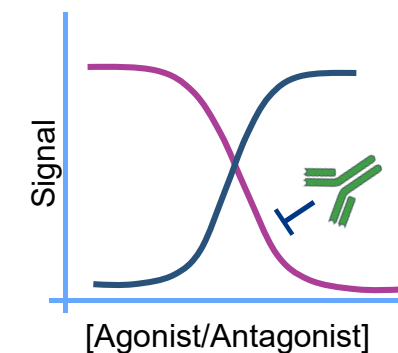
### Luminescence Readout

- Easily quantified luminescence readout
- Compatible with most readers



### Easy to Transfer

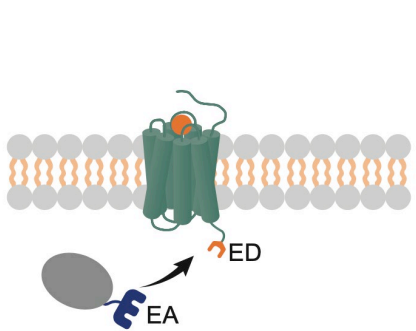
- Assay protocol is similar across the platform
- Detailed user manuals



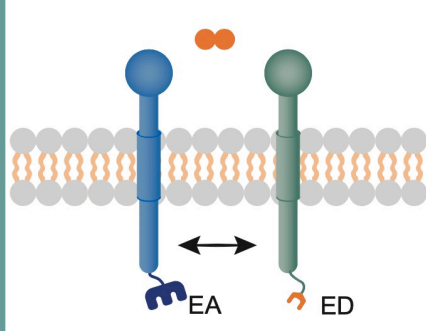
# Enzyme Fragment Complementation

## Versatile and Robust Platform for Cell-based Assays

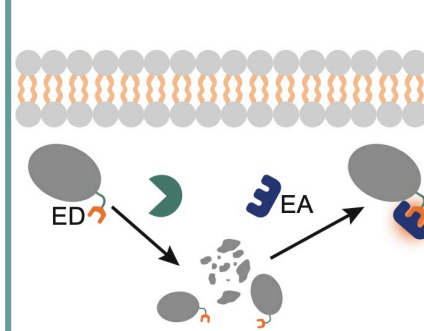
Protein-protein Interaction



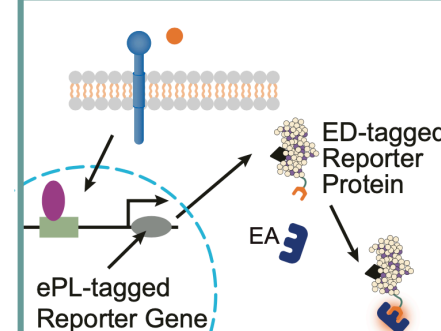
Receptor Dimerization



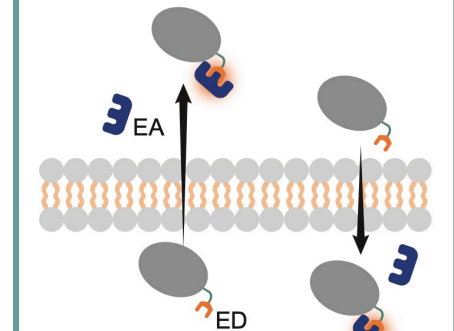
Quantitation and Degradation



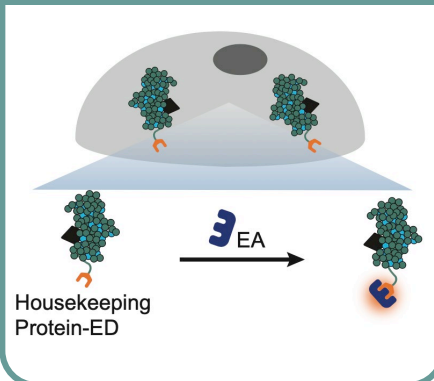
Downstream Signaling



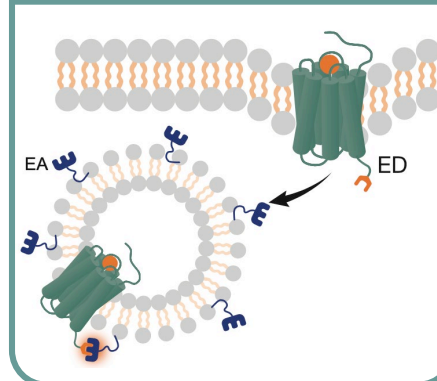
Secretion and Translocation



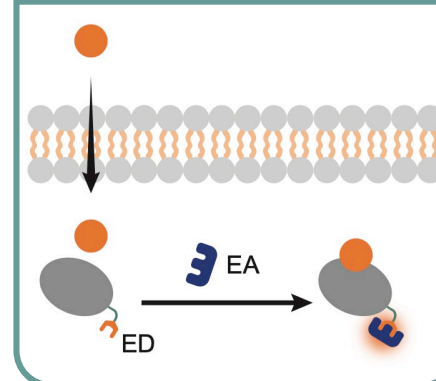
Quantify Cytotoxicity



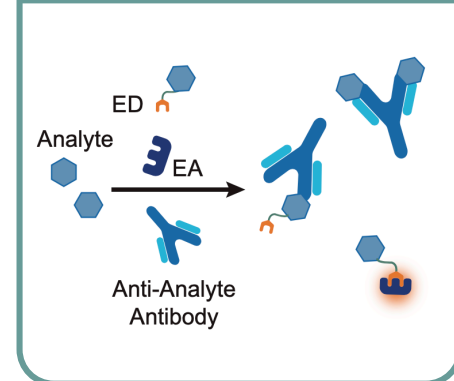
Internalization and Trafficking



Compound Target Engagement



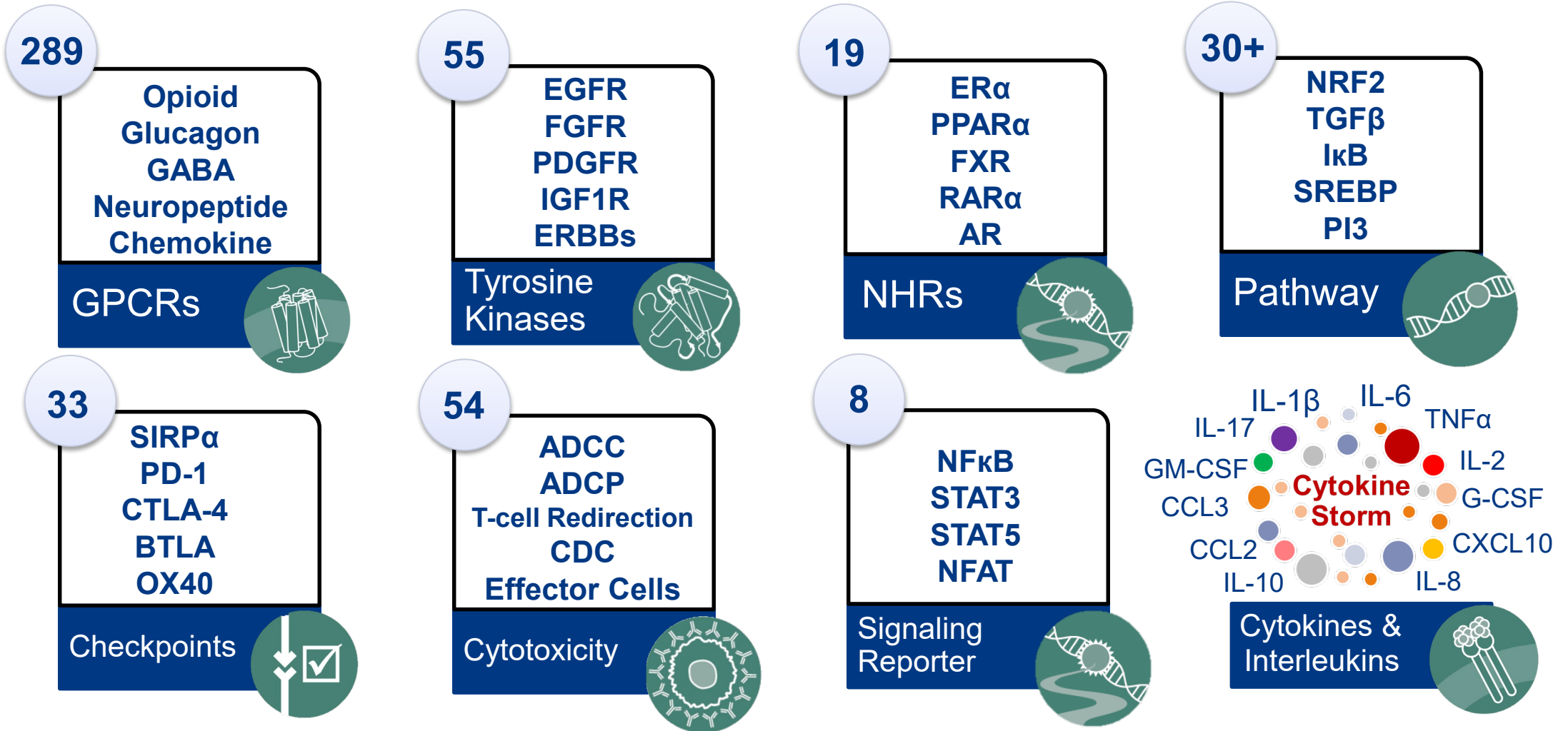
Biochemical Analyte Detection





# Largest Menu of Cell-based Assays for Discovery, Potency, and NAb Assay Development

>800 Cell lines to support bioassay development for major drug target classes





# Eurofins DiscoverX Assays are Widely Accepted for Lot Release and NAb Testing

CENTER FOR DRUG EVALUATION AND RESEARCH



APPLICATION NUMBER:  
**761061Orig1s000**

MULTI-DISCIPLINE REVIEW

Summary Review  
Office Director  
Cross Discipline Team Leader Review  
Clinical Review  
Non-Clinical Review  
Statistical Review  
Clinical Pharmacology Review


CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:  
**213793Orig1s000**

OTHER REVIEW(S)  




CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:  
**211939Orig1s000**

OTHER REVIEW(S)  
  




World Health Organization

WHO/BS/2020.2391  
ENGLISH ONLY

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION  
Geneva, 19 to 23 October 2020

WHO International Collaborative Study of the Proposed 1<sup>st</sup> International Standard for Bevacizumab

Haiyan Jia<sup>1</sup>, Parvathy Harikumar, Eleanor Atkinson, Peter Rigsby and Meenu Wadhwa

National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, United Kingdom  
<sup>1</sup>Email address: [haiyan.jia@nibsc.org](mailto:haiyan.jia@nibsc.org)

NOTE:  
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **5 October 2020** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: [knezevici@who.int](mailto:knezevici@who.int).

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UNITED STATES  
SECURITIES AND EXCHANGE COMMISSION  
WASHINGTON, D.C. 20549

Amendment No. 3 to

Form F-1  
REGISTRATION STATEMENT  
UNDER  
THE SECURITIES ACT OF 1933

OKYO Pharma Limited  
(Exact name of Registrant as specified in its charter)

IMMUNOBIOLOGY AND IMMUNOTHERAPY



The SUMOylation inhibitor subasumstat potentiates rituximab activity by IFN1-dependent macrophage and NK cell stimulation



625.LYMPHOMA: PRE-CLINICAL-CHEMOTHERAPY AND BIOLOGIC AGENTS | NOVEMBER 5, 2020

KPMW135, a Biosuperior CD3 Bispecific Version of Rituximab Created By a Novel Chemical Conjugation Technology Demonstrates Increased Anti-Tumor Activity By Adding T Cell-Mediated Cytotoxicity Activity to the Existing Mechanisms of Rituximab

Ublituximab Is a Novel, Next-Generation Glycoengineered Anti-CD20 mAb



Xtandi<sup>®</sup>  
(enzalutamide)



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Trusted Products to pedite Your Discovery & Development – Advance Confidently

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## Immuno-oncology Cell-based Assays

- Immune Checkpoint Receptor Assays
- Internalization Assays for ADCs
- Assays for Bi-specific Antibody Engagement
- Cytokines, Chemokines, and Interleukin Assay
- KILR® Cytotoxicity Platform

# Immuno-oncology Cell-based Assays

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## Functional Cell-based Assays

Immunoglobulin Superfamily (IgSF)

ICOS

CD28

CD33

Co-culture Model

SIRPα	CD47
CD200R	CD200
PD-1 (SHP1)	PD-L1
PD-1 (SHP2)	PD-L2
BTLA	HVEM
CTLA4	CD86

Signaling Cell Lines

Ligand Cell Lines

TNFR Superfamily (TNFRSF)

CD137

CD40

OX40

Pathway Signaling Reporter Assay

PD-1 NFAT PD-L1 Reporter

NF-κB CD40L Reporter

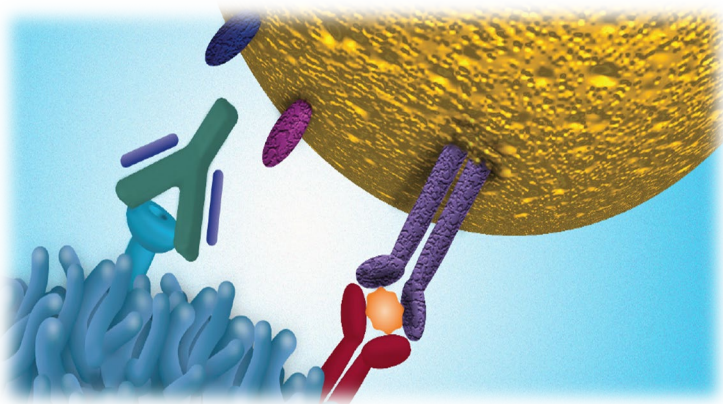
NF-κB CD27L Reporter

Clustering Cell Lines

FcγRIa

FcγRIIa

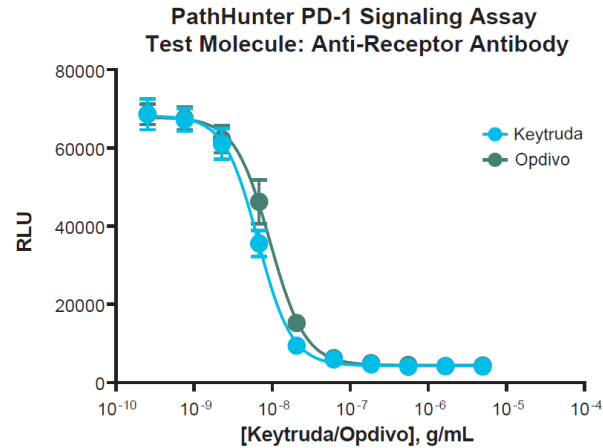
FcγRIIb



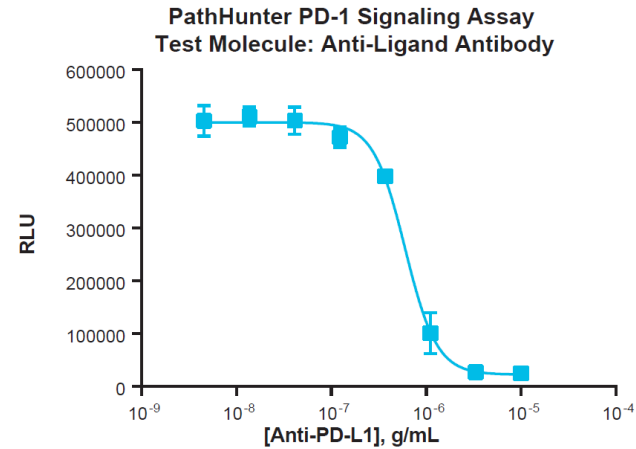


# Flexible Assays Adaptable to Drug Candidates with Different MOAs

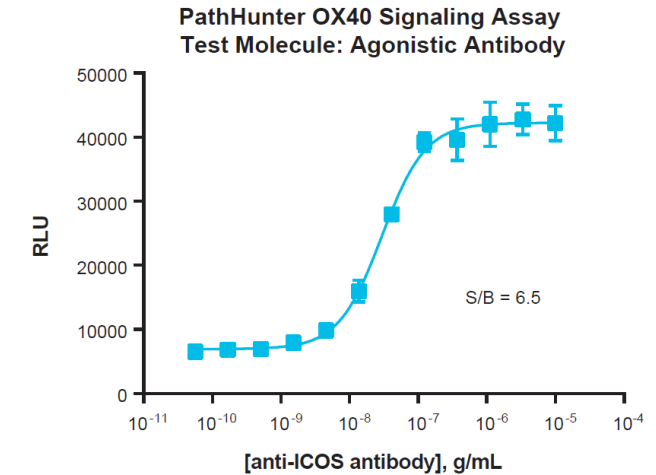
## Anti-receptor



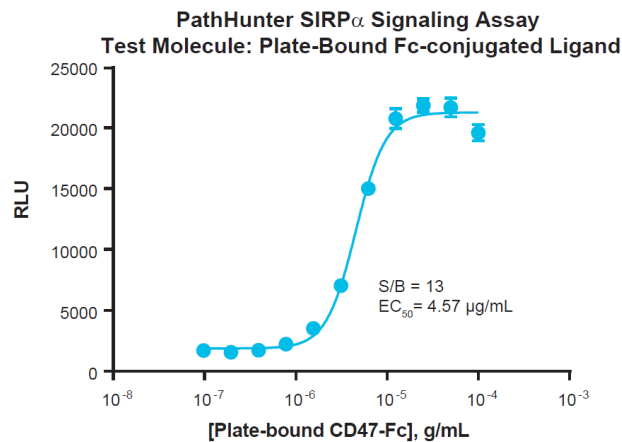
## Anti-ligand



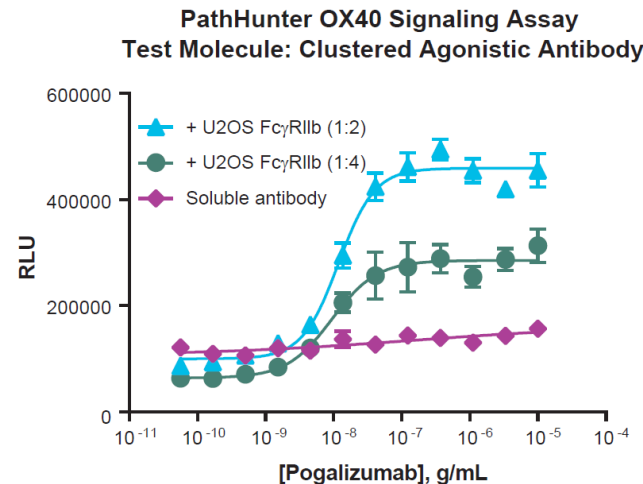
## Agonistic Antibody



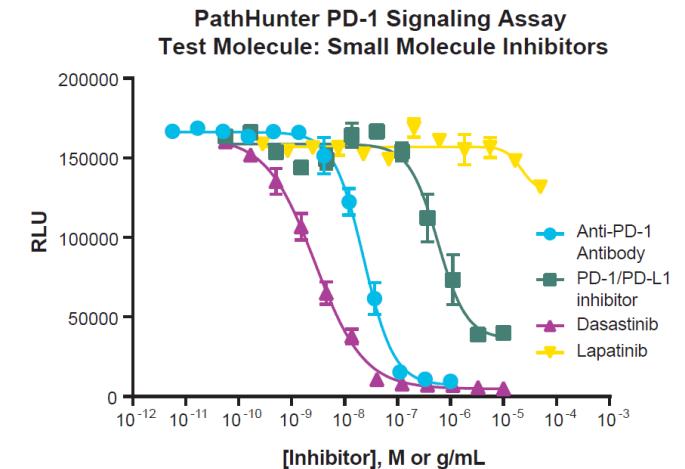
## Plate-bound Ligands



## Clustering and Crosslinking

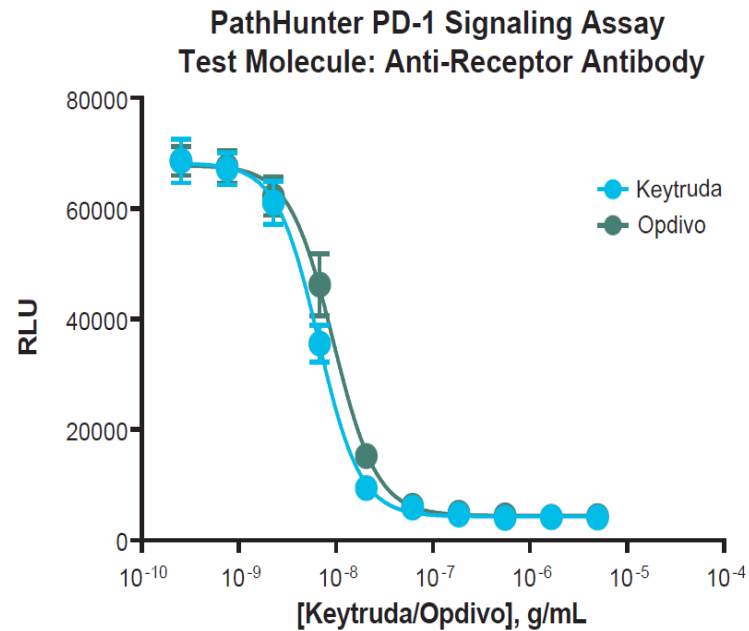


## Small Molecule Inhibitors

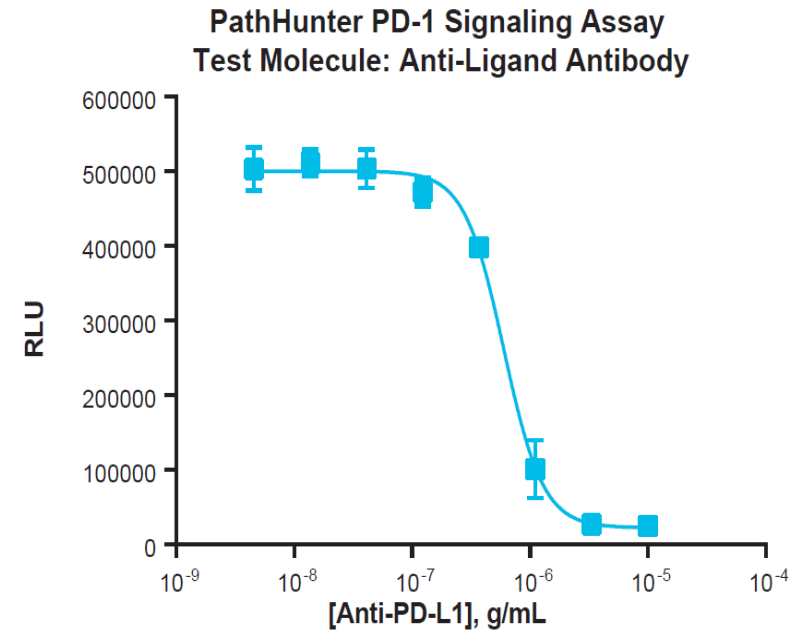


# Flexible Assays Adaptable to Drug Candidates with Different MOAs

## Anti-receptor



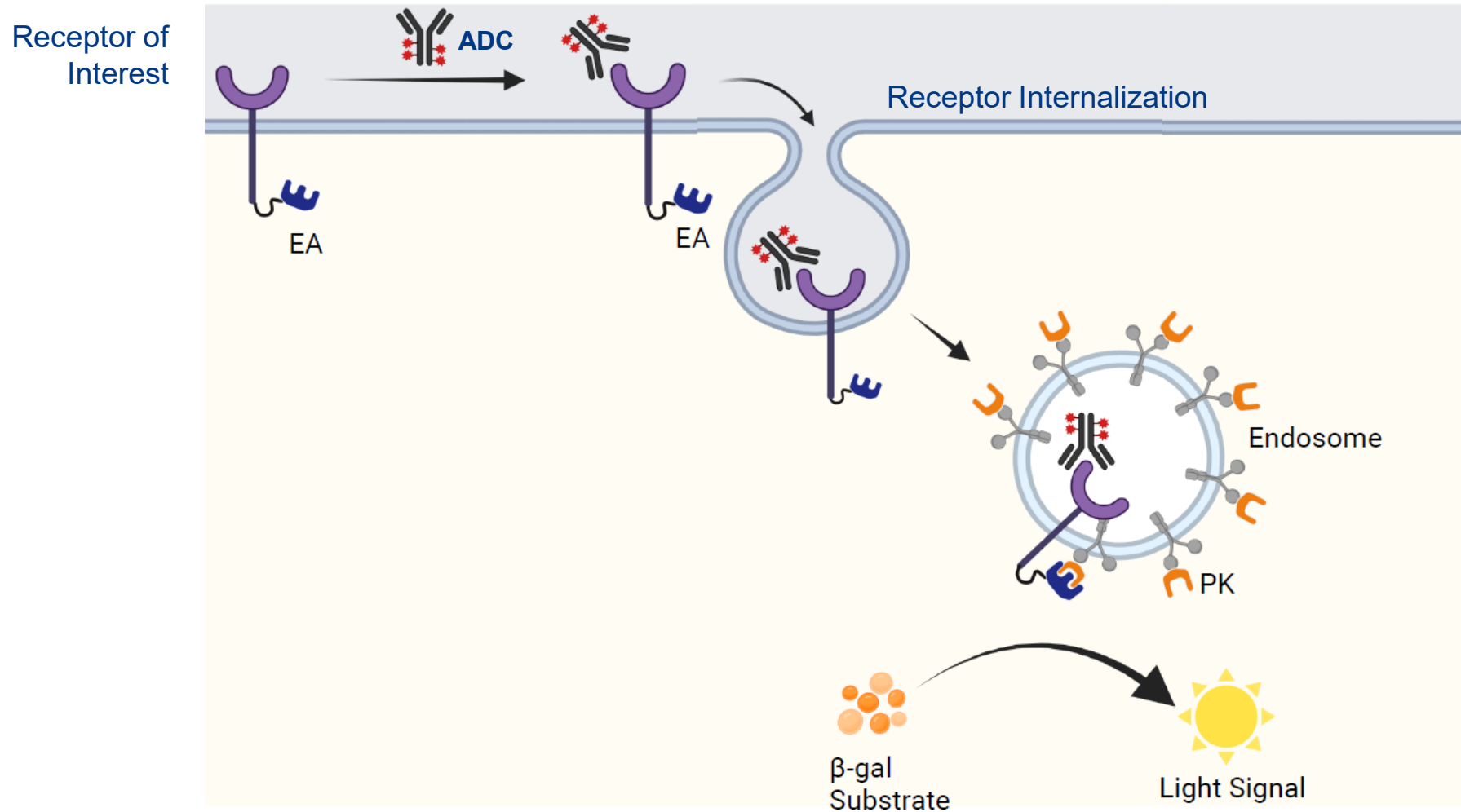
## Anti-ligand



# Immuno-oncology Cell-based Assays

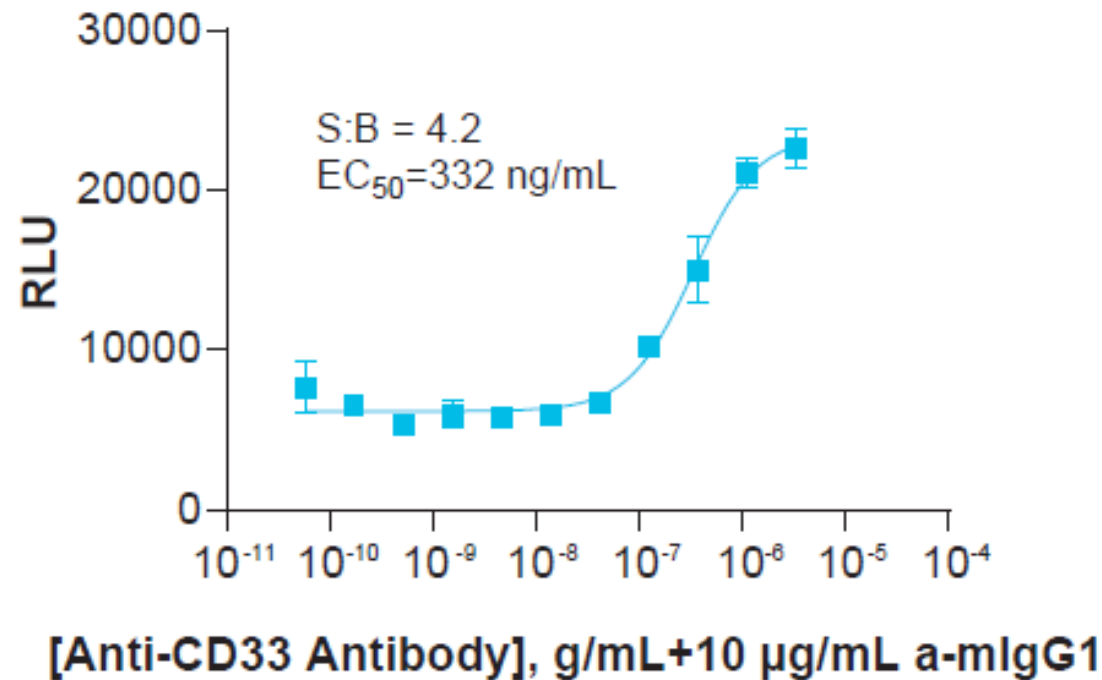
- Immune Checkpoint Receptor
- Internalization Assays for ADCs
- Assays for Bi-specific Antibody Engagement
- Cytokines, Chemokines, and Interleukin Assays
- KILR® Cytotoxicity Platform

# Receptor Internalization Assay for Antibody Drug Conjugates (ADC)

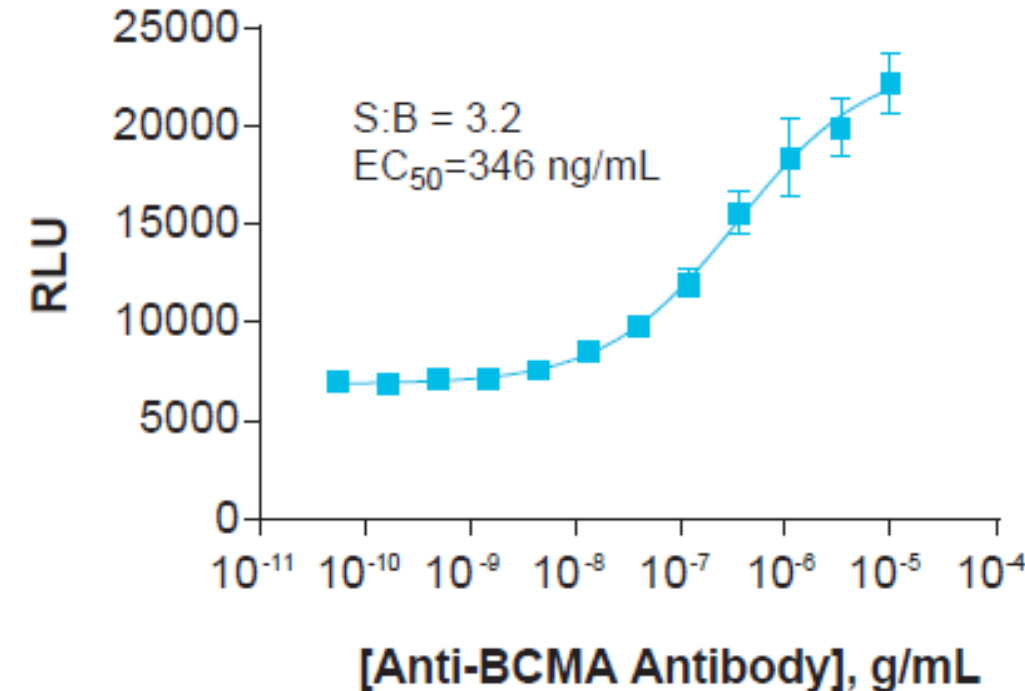




CD33 Receptor Internalization Assay



BCMA Receptor Internalization Assay

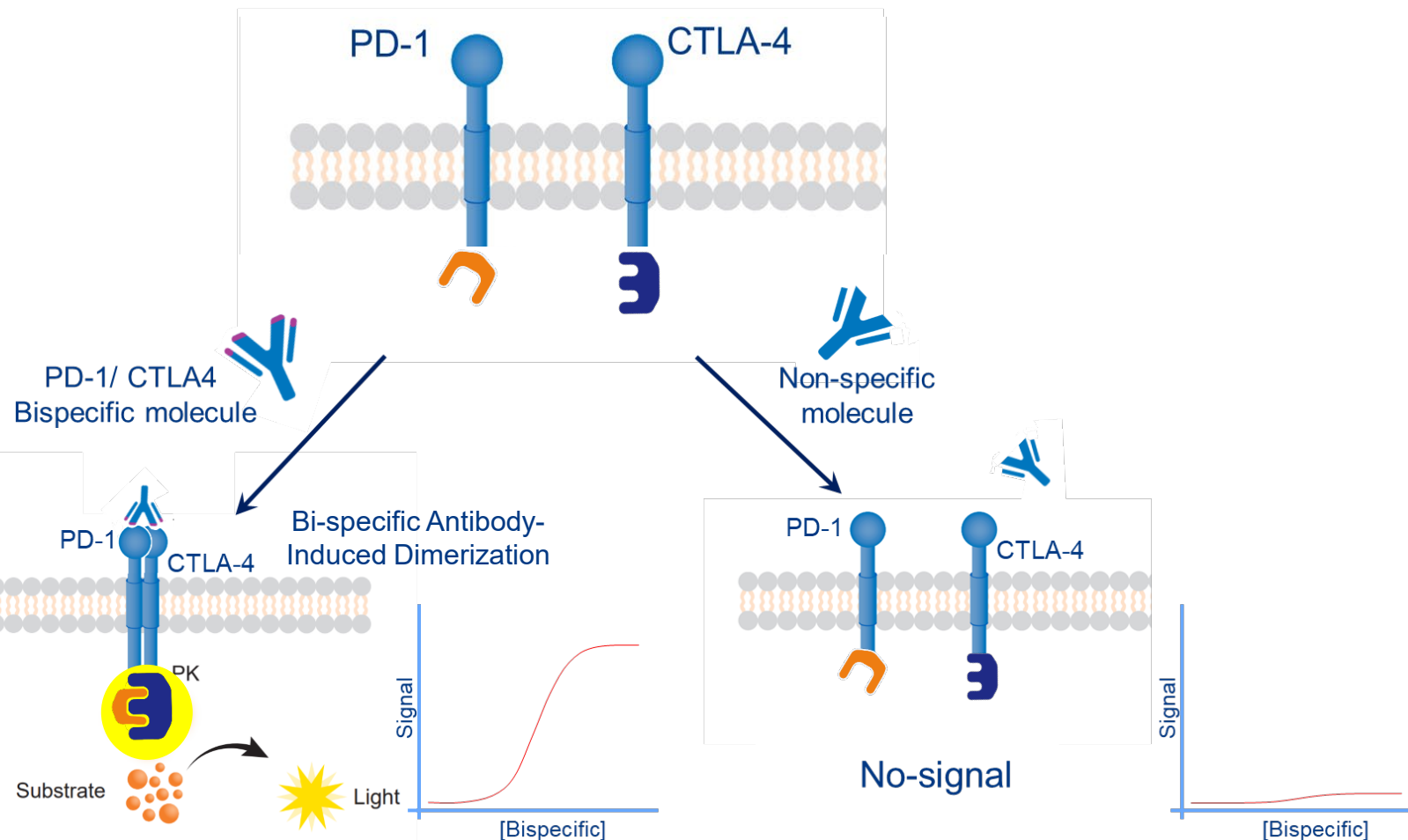


Robust internalization assays for characterizing ADCs

# Immuno-oncology Cell-based Assays

- Immune Checkpoint Receptor Assays
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## Example: PD-1/CTLA4 Receptor Dimerization Cell Line



### Other Available Assays

PD-1/LAG3

PD-1/CTLA4

PD-1/TIGIT

PD-1/CEACAM1

mPD-1/mLAG3

mPD-1/mCTLA4

PD-1/PD-L1

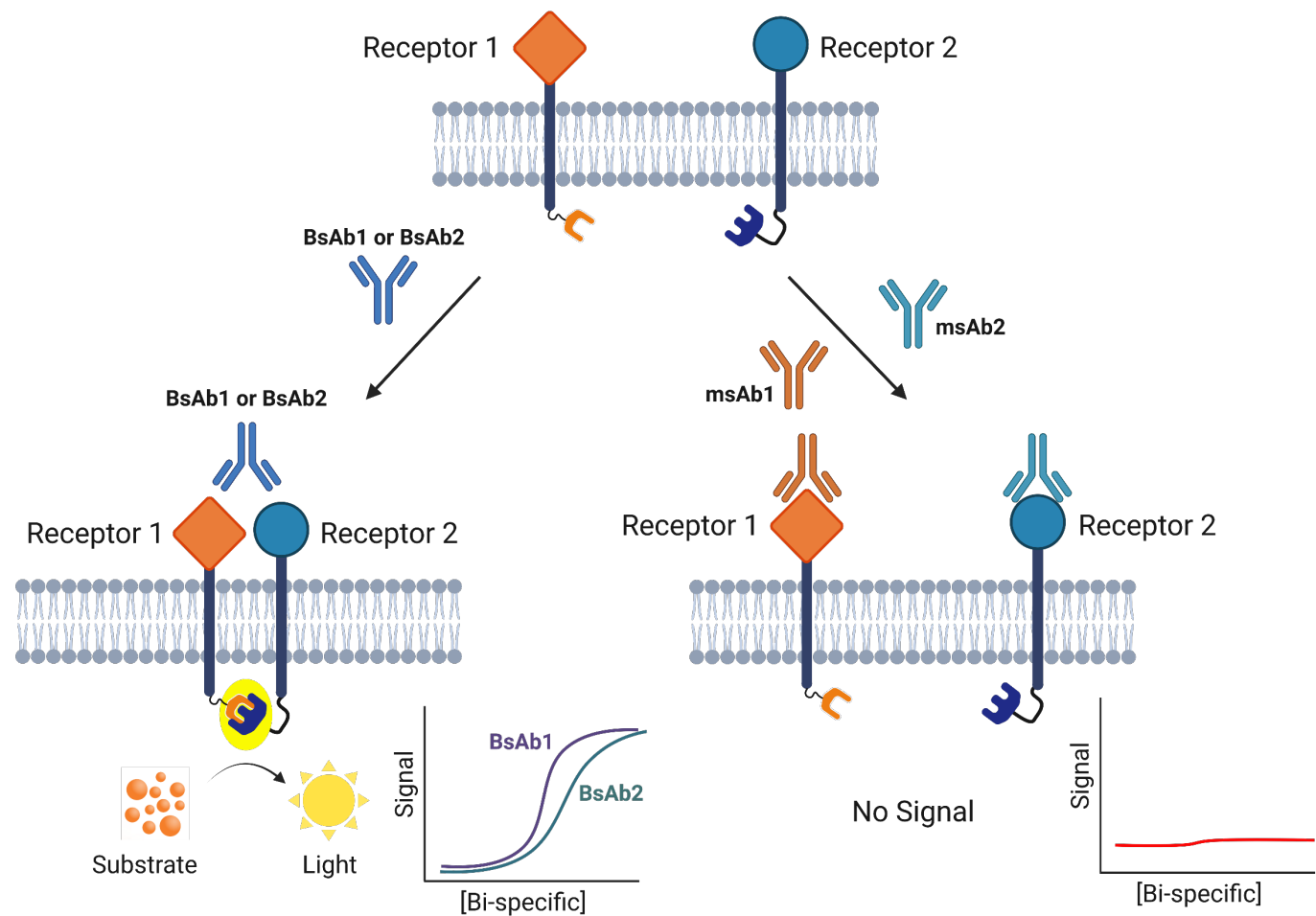
PD-L1/CTLA4

PD-L1/TIM3

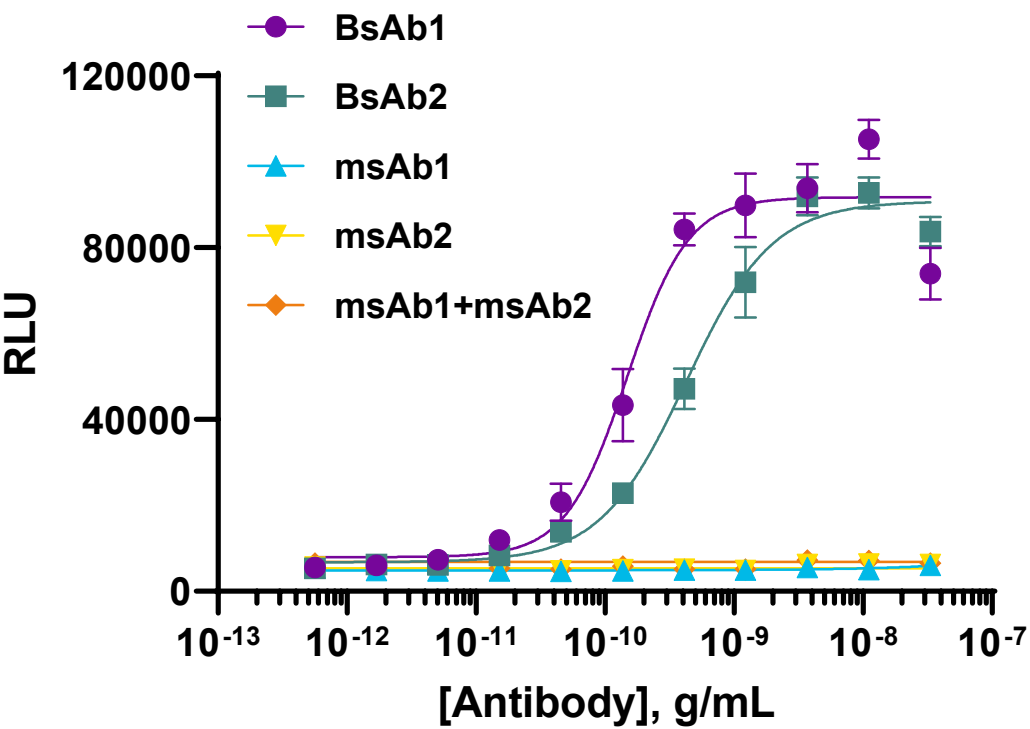
TIM3/CEACAM1

mPD-1/mTIGIT

# Bi-specific Antibodies (BsAbs) Assays for Immuno-Oncology



Immune Checkpoint BsAb Assay



msAb – Mono-specific antibody, BsAb – Bi-specific antibody



# Bi-specific Antibodies (BsAbs) Assays for Immuno-Oncology

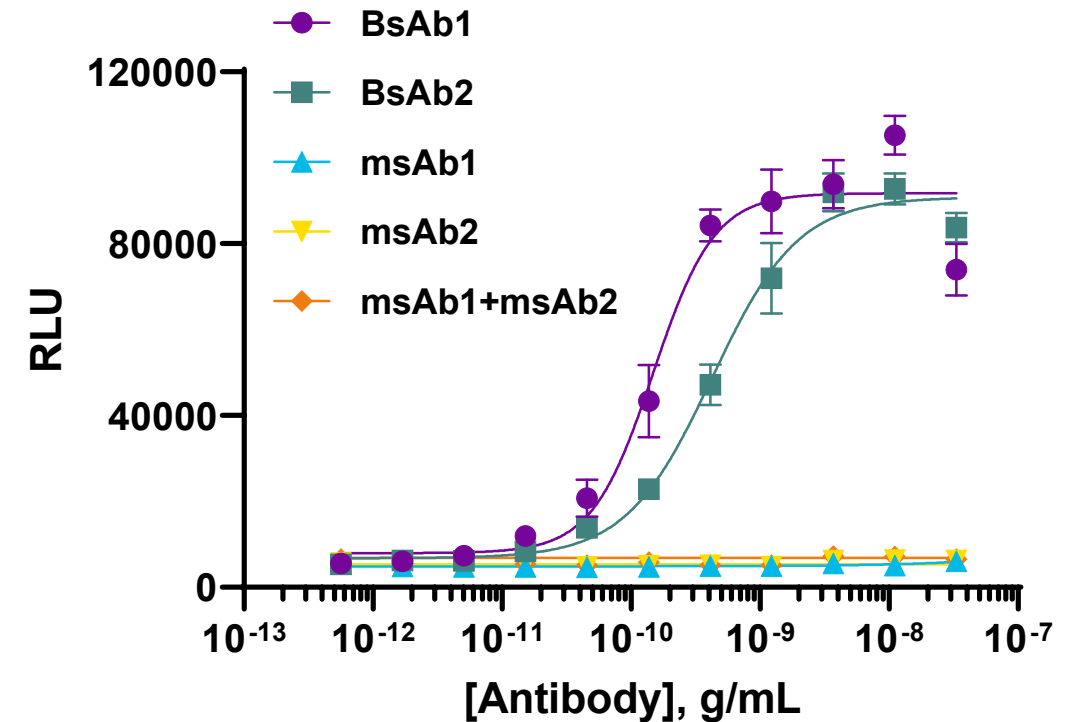
Assays for BsAbs to Immune Checkpoint Targets

Highly specific response

Suitable for screening

Robust MOA-reflective dimerization assays for characterizing bi-specific antibodies

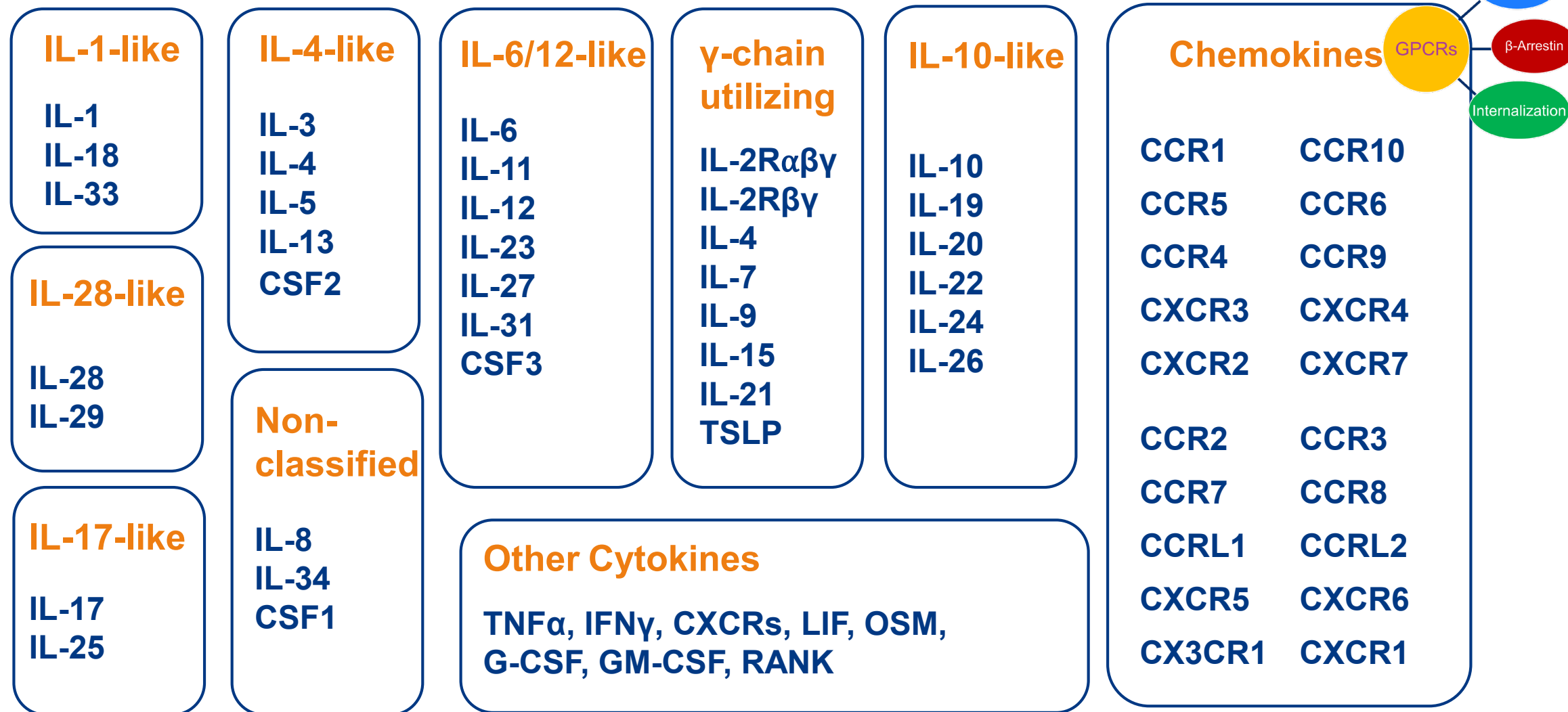
## Immune Checkpoint BsAb Assay



# Immuno-oncology Cell-based Assays

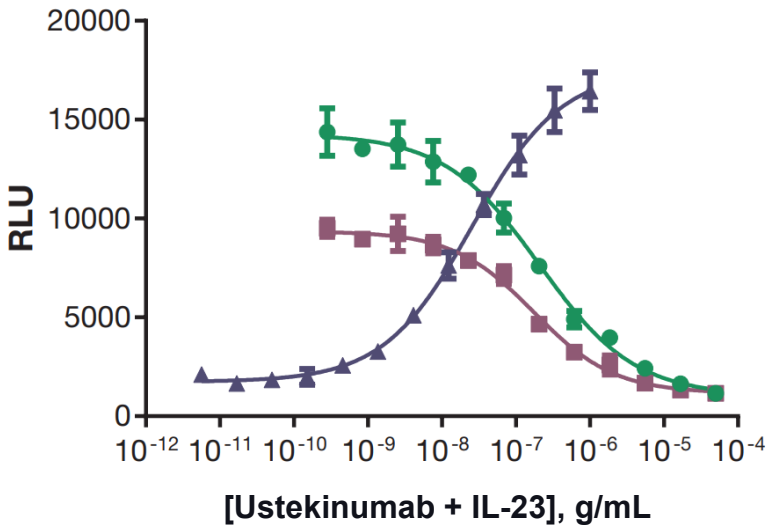
- Immune Checkpoint Receptor Assays
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- Assays for Bi-specific Antibody Engagement
- Cytokines, Chemokines, and Interleukin Assays
- KILR® Cytotoxicity Platform

Functional assays for >85% human interleukins and cytokines

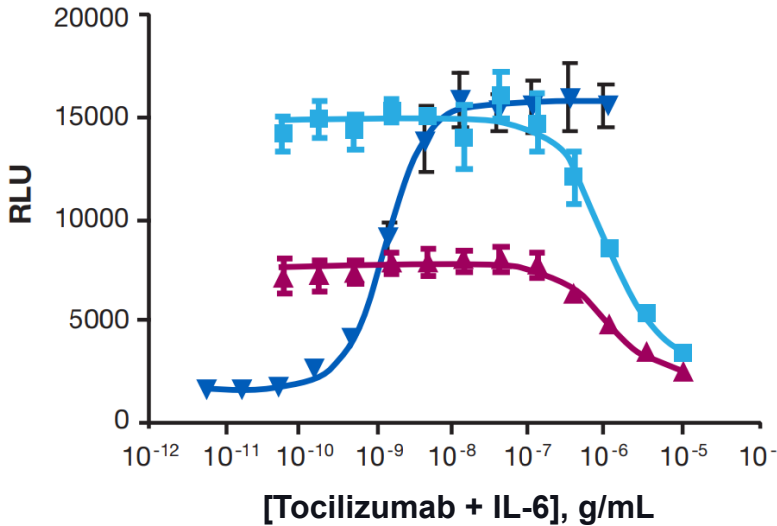


# Functional Assays for Therapeutic Antibodies and Inhibitors for Interleukin Receptors

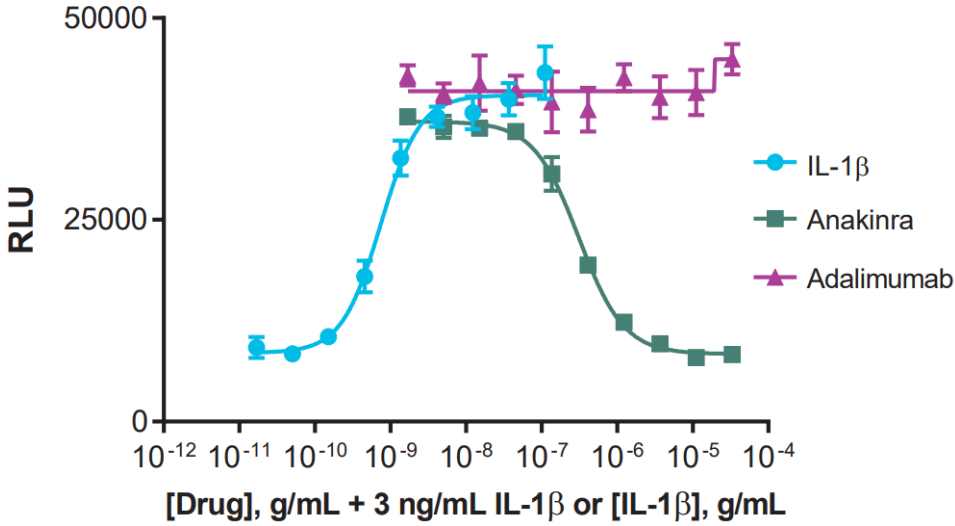
Ustekinumab (Stelara®)  
IL-12RB1/IL-23R Assay



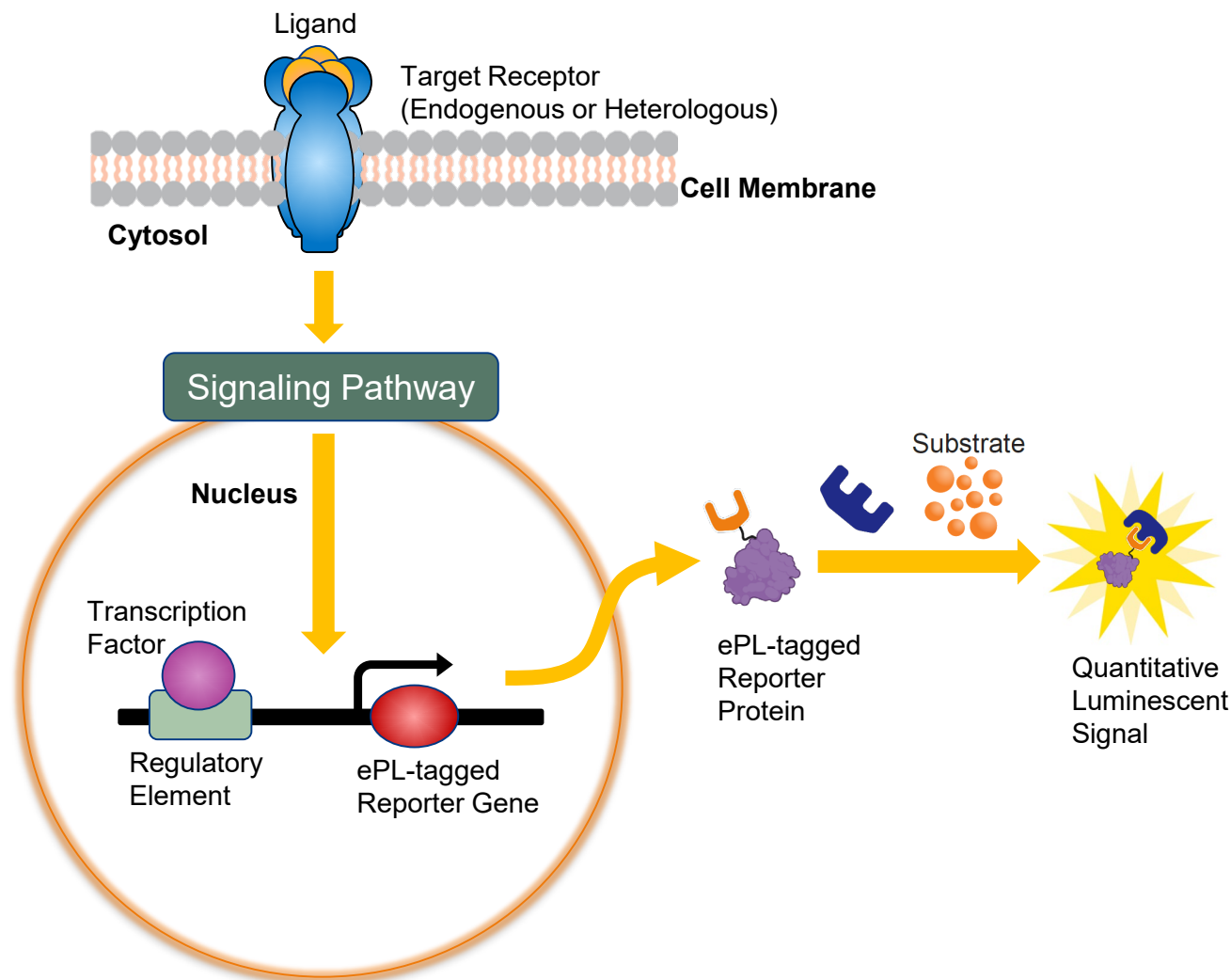
Tocilizumab (Actemra®)  
IL-6R/IL-6ST Assay



Anakinra (Kineret®)  
IL-1R/IL-1RAP Assay







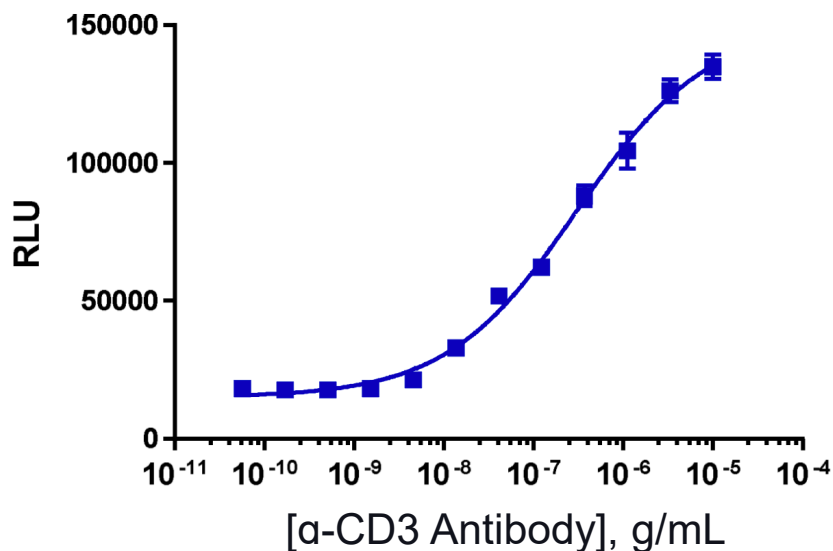
*Signaling Pathway  
Reporter*

**STAT3**  
**STAT5**  
**NF- $\kappa$ B**  
**NFAT**

# Signaling Pathway Reporter Assays

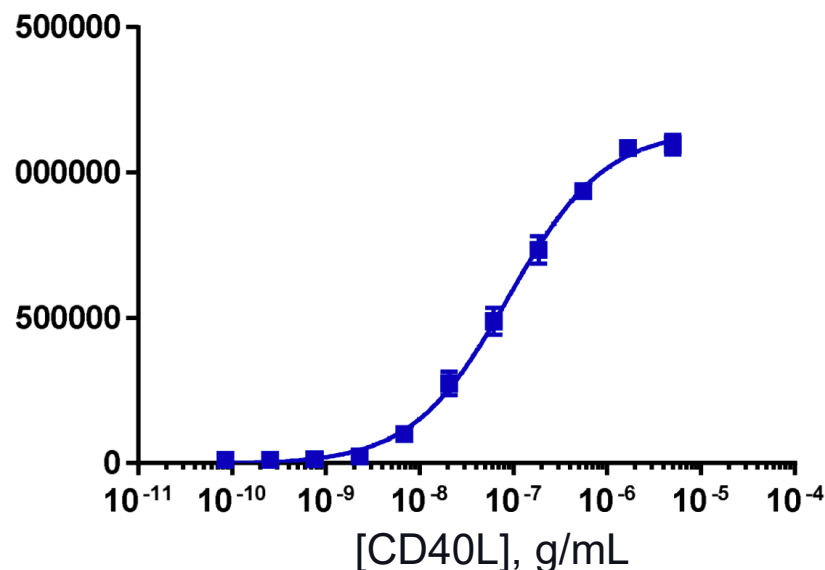
Robust Assay Performance with Endogenous Receptor Activation

## NFAT Reporter



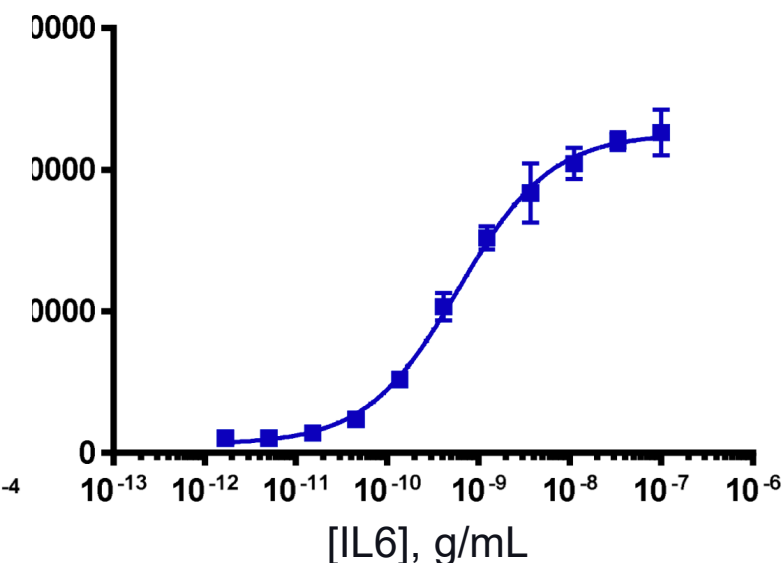
EC<sub>50</sub> (ng/mL): 302.5  
S:B: 7.6

## NF-κB Reporter



EC<sub>50</sub> (ng/mL): 88.7  
S:B: 102.3

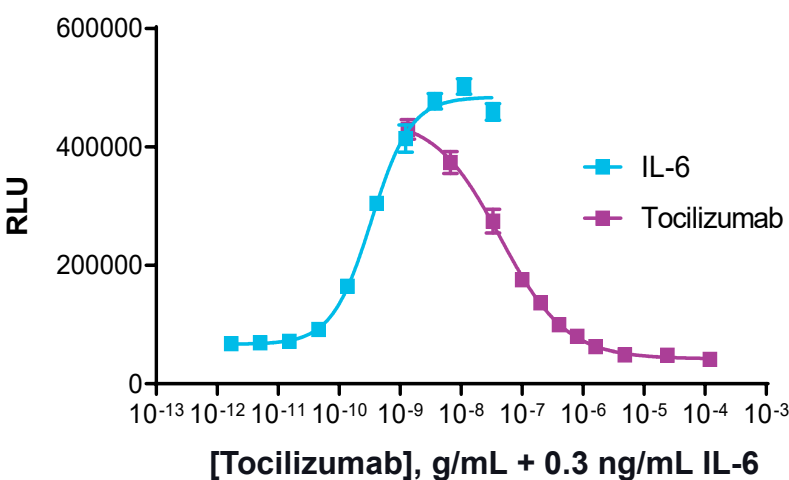
## STAT3 Reporter



EC<sub>50</sub> (ng/mL): 0.61  
S:B: 21.3

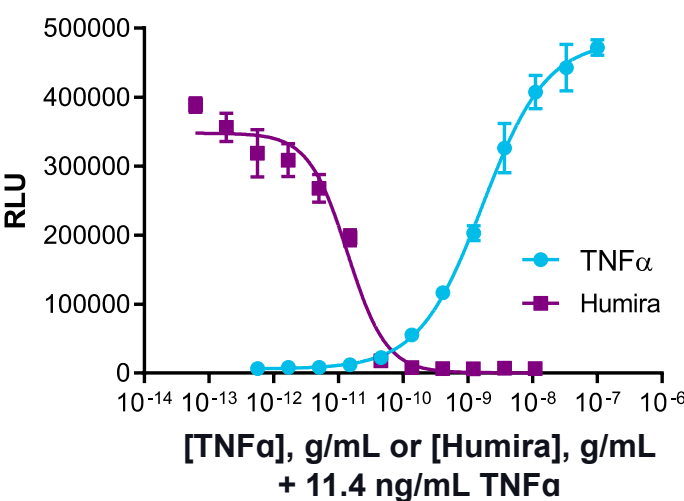
# Quantify The Activation And Inhibition Of Signaling Pathways

## IL6 Reporter Assay (STAT3)



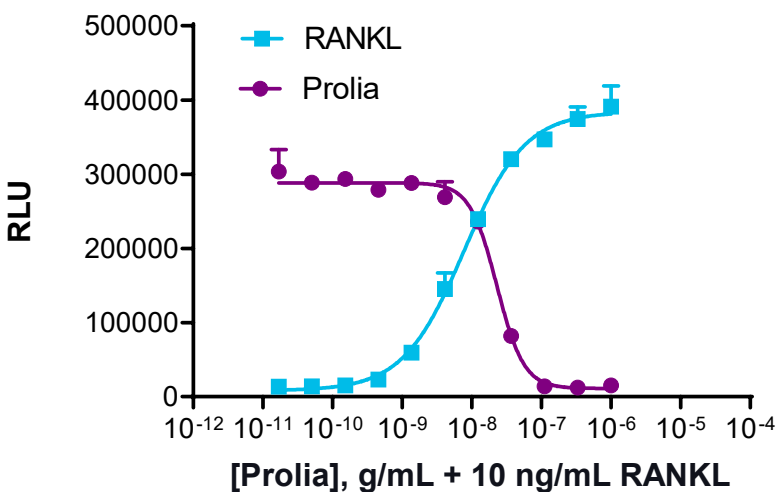
	EC <sub>50</sub> /IC <sub>50</sub>	S/B
IL-6	0.33 ng/mL	6.2
Tocilizumab	44 ng/mL	11.6

## TNFα Reporter Assay (NF-κB)



	EC <sub>50</sub> /IC <sub>50</sub>	S/B
TNFα	1.7 ng/mL	72.3
Humira	13 pg/mL	64

## RANK Reporter Assay (NF-κB)



	EC <sub>50</sub> /IC <sub>50</sub>	S/B
RANKL	7.7 ng/mL	27
Prolia	23 ng/mL	21

# Immuno-oncology Cell-based Assays

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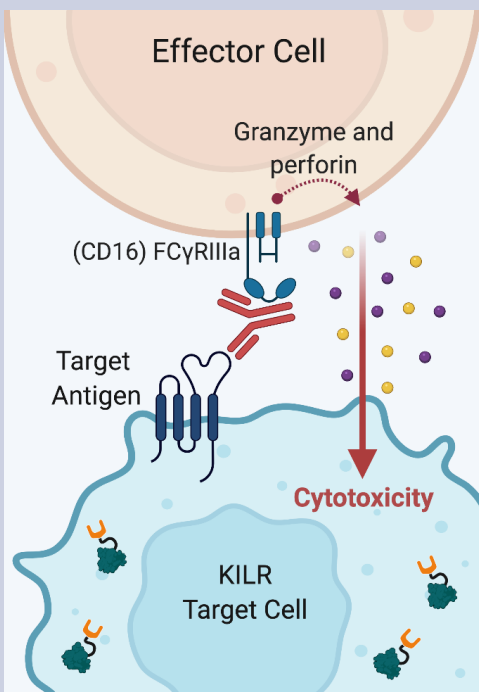


# Multiple Cytotoxicity Mechanisms can be Evaluated by KILR® Platform

## ADCC

### Antibody-Dependent Cellular Cytotoxicity

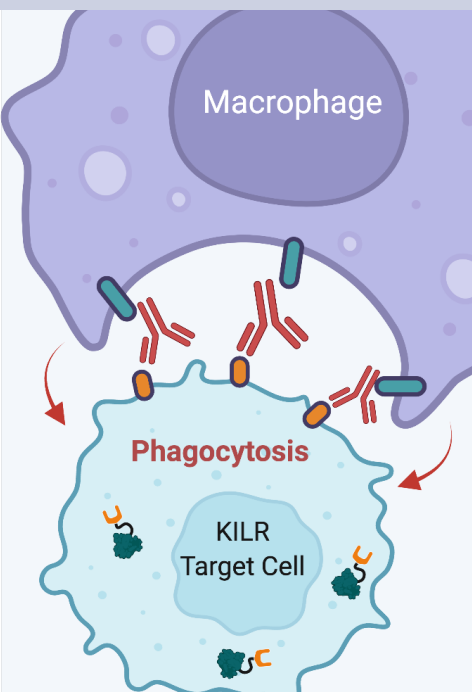
Effector cells engage with the target cells through the antibody and kill



## ADCP

### Antibody-Dependent Cellular Phagocytosis

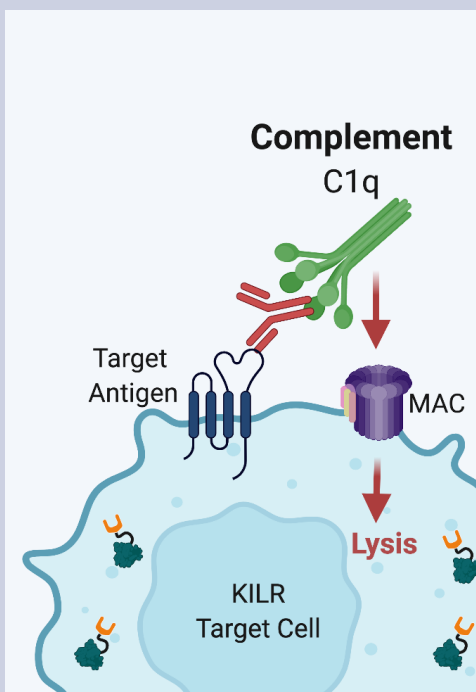
Fc-dependent phagocytosis and lysosomal degradation



## CDC

### Complement Dependent Cytotoxicity

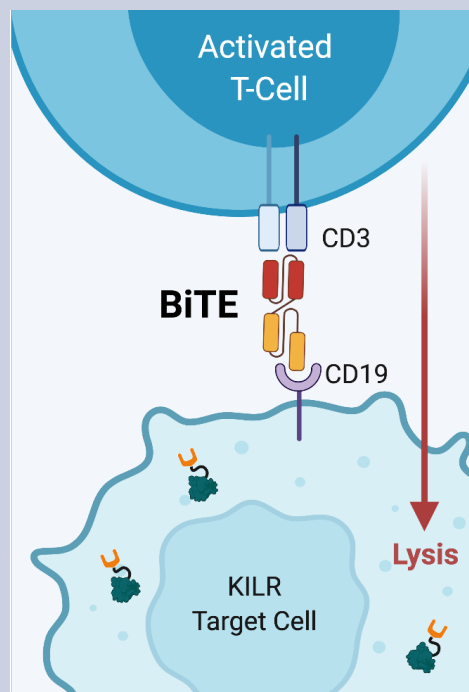
Activated complement system leads to formation of membrane attack complex (MAC)



## TCR

### T-cell Redirection (TDCC) with Bi-specific Antibody

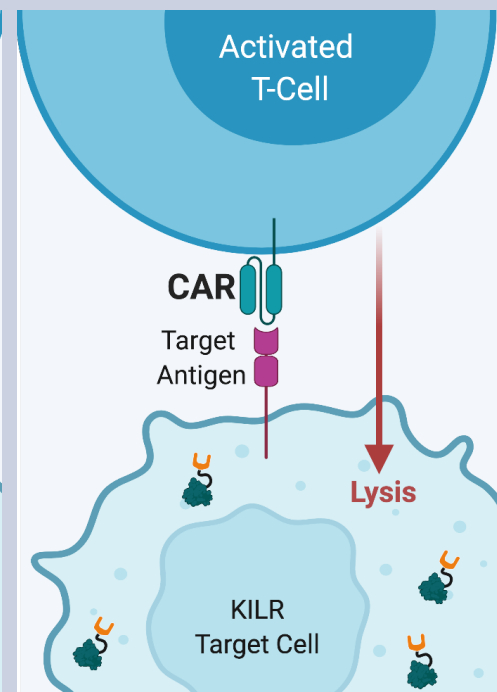
Bi-specific antibody engages T-cell with cancer cells for killing



## CAR-T

### Chimeric Antigen Receptor (on) T-cells

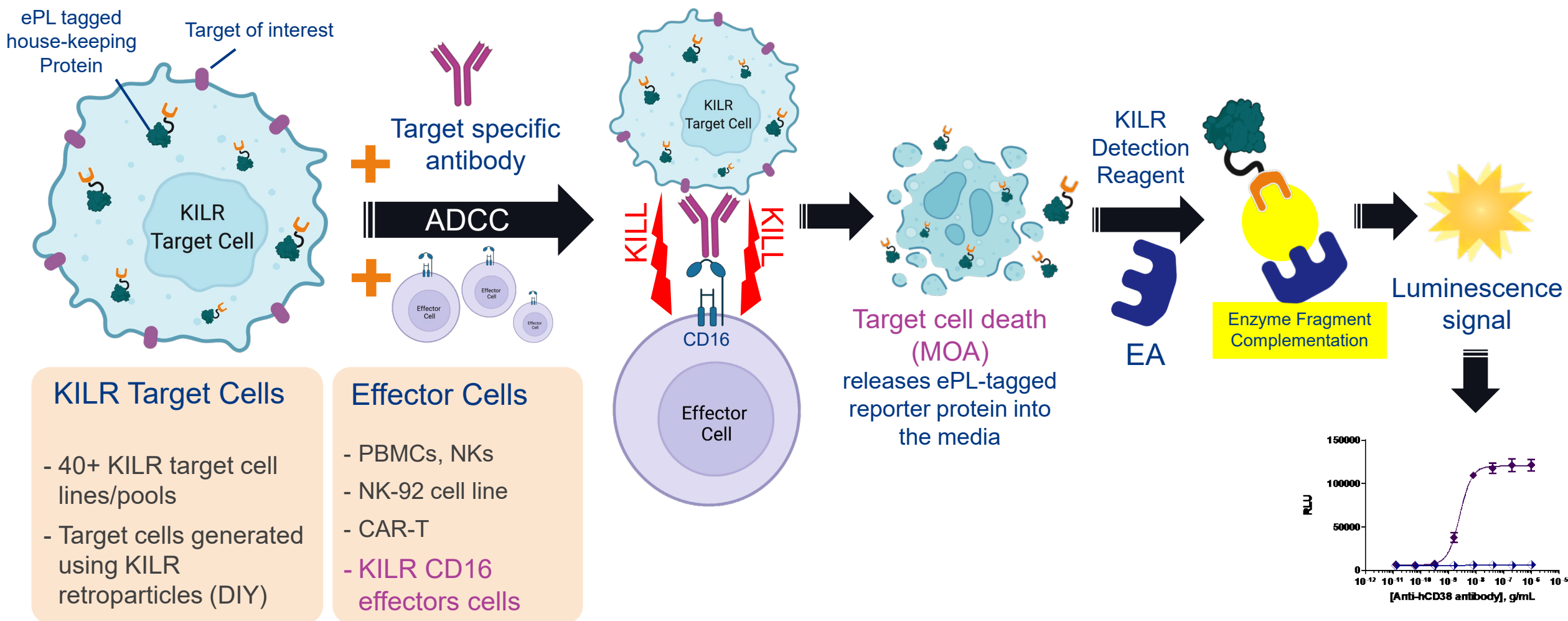
Engineered T-cells recognize and kill cancer cells



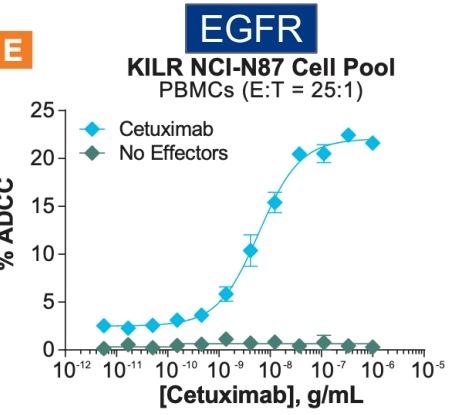
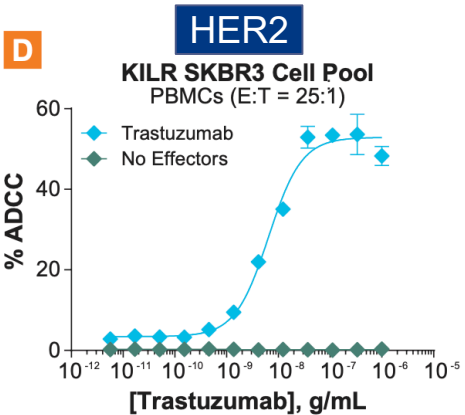
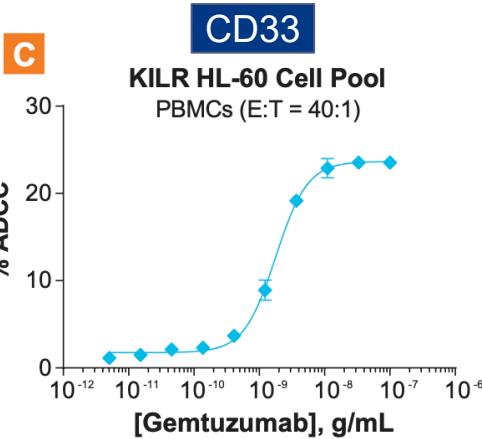
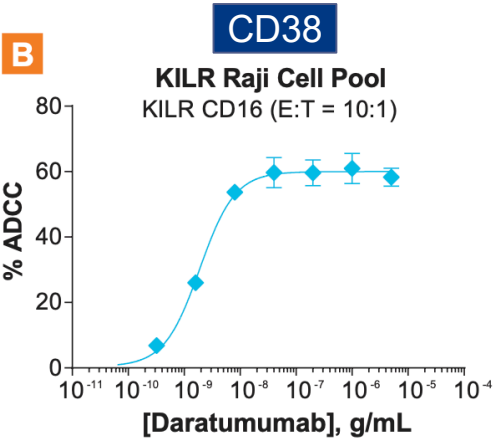
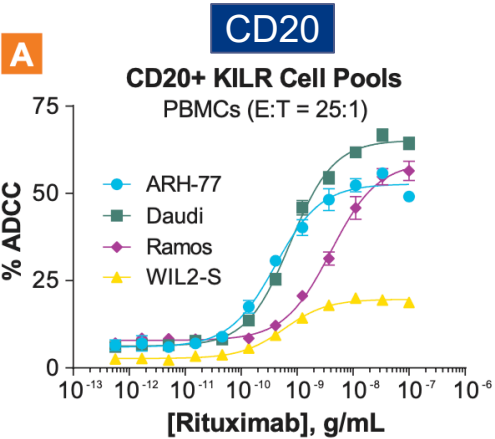
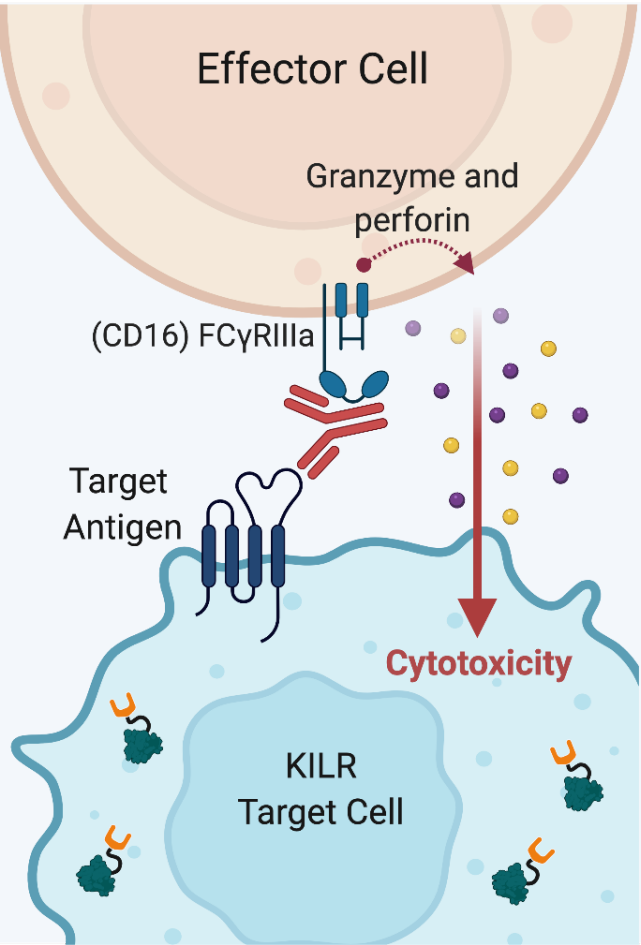
 KILR Reporter Protein

# KILR® Platform Offers Flexible Format and MOA-Reflective Assay Read-out

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



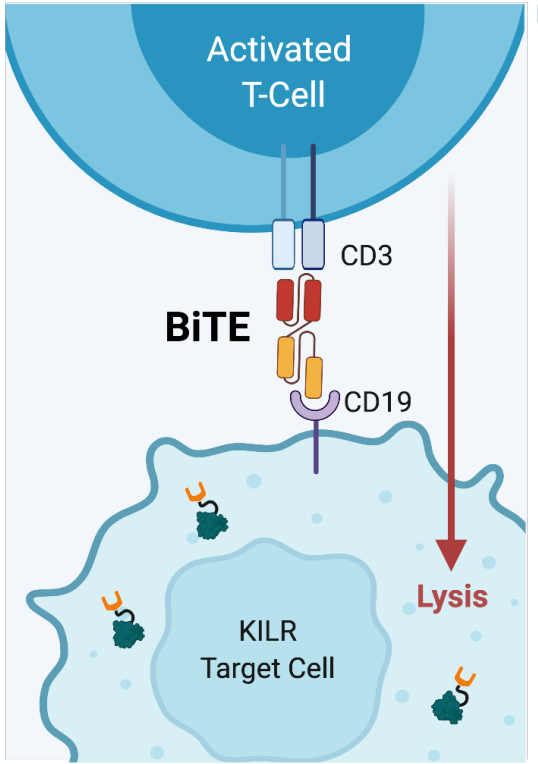
# ADCC Demonstrated Using KILR<sup>®</sup> Assay with a Variety of Antibodies, Antigens, and Cell Types



KILR ADCC Assay is compatible with multiple effector cell types producing robust assays

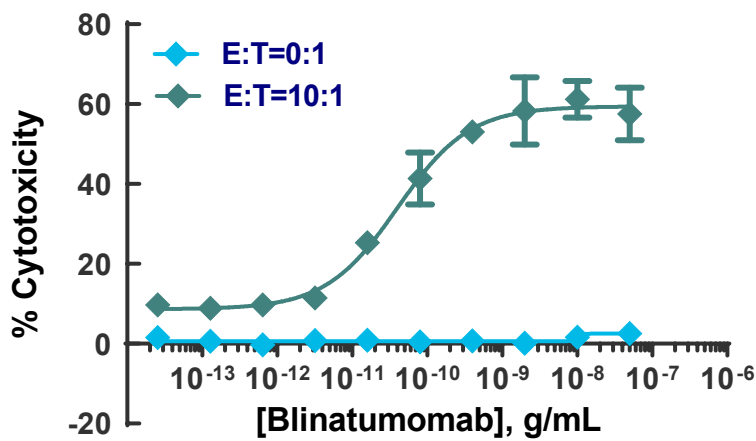
## T-cell Engagement

T-cell Engagers, such as BiTEs engage T-cell with a cancer cells to mediate killing



## Initial Testing with Blinatumumab (T-cell Engager)

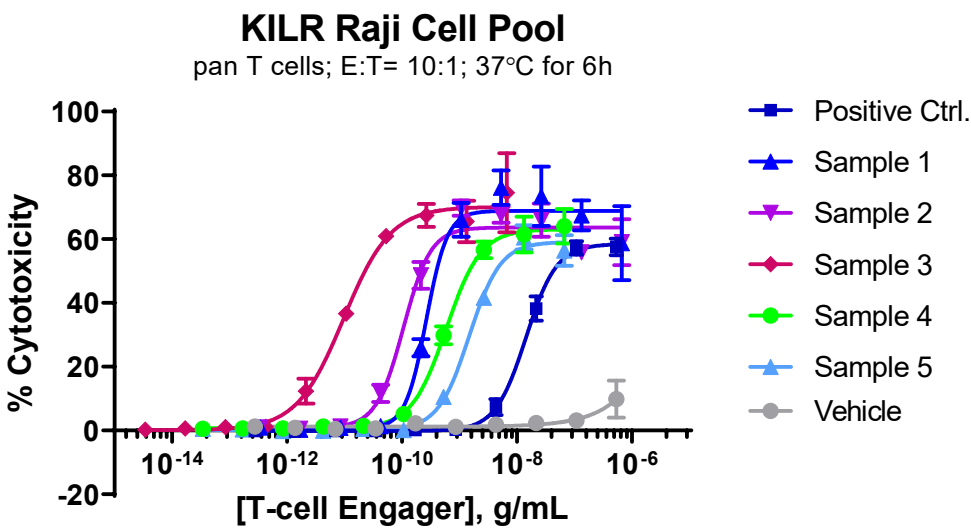
Effector Cells: **Pan T Cells**  
Target Cells: **KILR Raji Cell Pool**  
(E:T= 10:1); 24h incubation



Max Cytotoxicity, %	EC <sub>50</sub> , pg/mL
51.3	40.2

Results consistent with reported EC<sub>50</sub> of Blinatumomab = 10-100 pg/mL

## Profiling Data with T-cell Engagers (384-well format)



Reduced assay time, adapted to 384-well format; suitable for rank ordering

TDCC – T-Cell Dependent Cellular Cytotoxicity

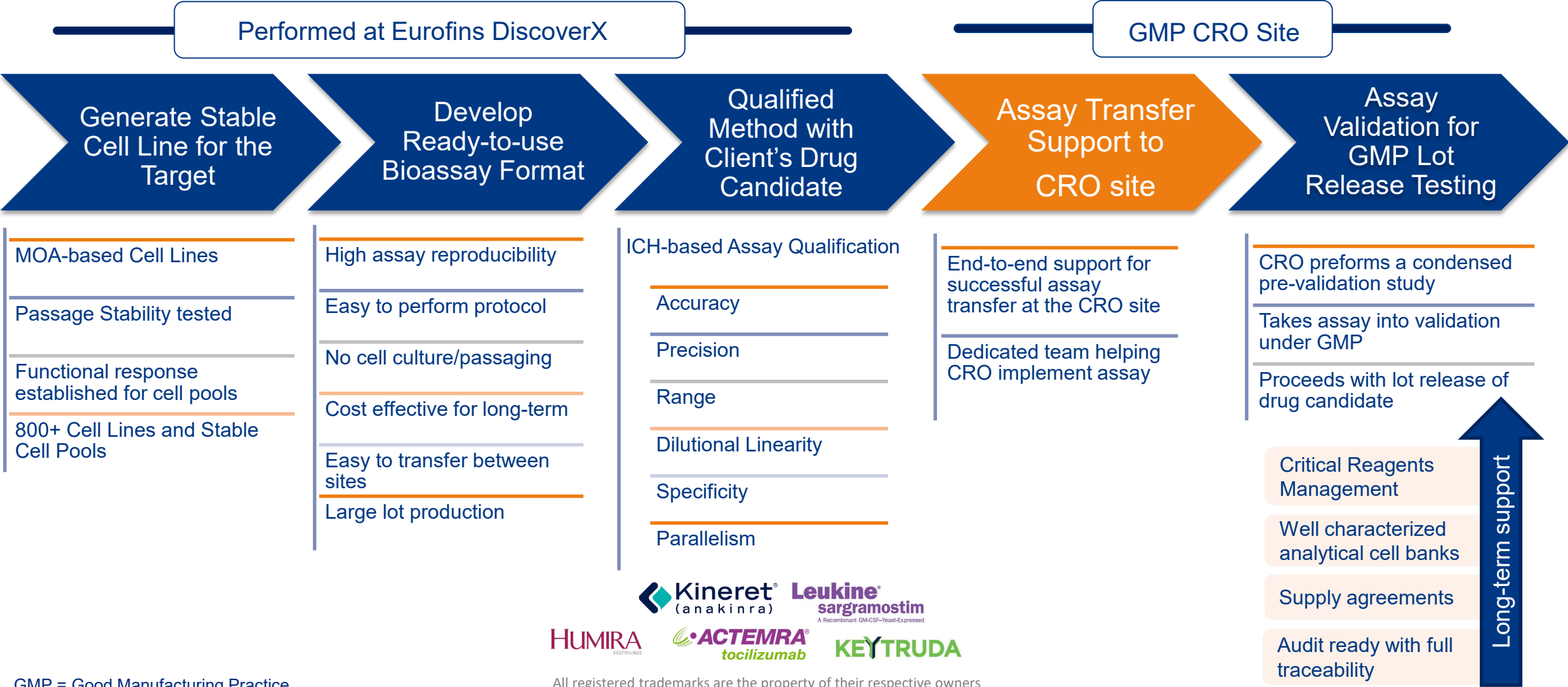


# End-to-End Support for QC Lot Release Testing

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GMP = Good Manufacturing Practice

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# Global Programs using DiscoverX Bioassays for Potency, Stability, and NAb Testing for Drug Release

<b>SIRPα</b> <hr/> Multiple programs in NA & EU for originator biologics <hr/> Potency & NAb	<b>PD-1</b> <hr/> Multiple programs in NA & APAC for originator & biosimilars <hr/> Potency	<b>BTLA</b> <hr/> US-based program for originator biologic <hr/> Not disclosed	<b>VEGF</b> <hr/> Multiple programs in NA, EU & APAC for biosimilars <hr/> Potency	<b>IGF1R</b> <hr/> Multiple programs in NA and APAC for originator biologic <hr/> Potency	<b>GLP1R</b> <hr/> Multiple programs in NA, EU & APAC for originator & biosimilars <hr/> Potency	<b>CNR1</b> <hr/> US-based program for originator biologic <hr/> Potency	<b>BRDKBR2</b> <hr/> US-based Program for originator biologic <hr/> Potency	<b>Insulin</b> <hr/> Multiple programs in NA, EU & APAC for biosimilars <hr/> Potency	<b>NPY2R</b> <hr/> EU based program for originator biologic <hr/> Potency
<b>IL-17R</b> <hr/> EU biopharma for biosimilar <hr/> Not disclosed	<b>IL-2R</b> <hr/> Multiple programs in NA and EU for originator biologic <hr/> Not disclosed	<b>IL-31R</b> <hr/> US-based pharma for originator biologic <hr/> Potency & NAb	 <p>NA = North America; EU = Europe; APAC = Asia Pacific</p>			<b>MC1R</b> <hr/> Multiple programs in NA & EU for originator biologic <hr/> Not disclosed	<b>FSHR</b> <hr/> APCA-based program for biosimilar <hr/> Potency	<b>PTHR1</b> <hr/> Multiple programs in NA, EU & APAC for biosimilars <hr/> Potency	<b>C5A</b> <hr/> NA based program for originator biologic <hr/> Potency
<b>IL-10R</b> <hr/> Multiple programs in NA for originator biologic <hr/> Potency	<b>IL-23R</b> <hr/> US-based biopharma for originator biologic and biosimilar <hr/> Potency	<b>IL-7R</b> <hr/> EU-based program for originator biologic <hr/> Potency				<b>CALCRL (RAMP3)</b> <hr/> NA-based pharma for originator biologic <hr/> Potency	<b>CXCR4</b> <hr/> EU-based pharma for originator biologic <hr/> NAb	<b>CXCR2</b> <hr/> US-based program for originator biologic <hr/> Not disclosed	<b>CD20 (ADCC)</b> <hr/> Multiple programs in NA and EU for biosimilars <hr/> Potency
<b>TSLPR</b> <hr/> EU-based pharma for originator biologic <hr/> Potency	<b>CSF2R</b> <hr/> Multiple programs in NA & EU <hr/> Potency	<b>CSF1R</b> <hr/> Multiple programs in NA & EU <hr/> Potency	<b>RANK</b> <hr/> Multiple programs in NA & APAC for biosimilars <hr/> Potency	<b>FGFR</b> <hr/> US-based biopharma for originator biologic <hr/> NAb	<b>ErbB2/ErbB3</b> <hr/> Multiple programs in NA, EU & APAC for biosimilars <hr/> Potency	<b>GM-CSF</b> <hr/> Multiple programs in NA & EU for originator biologic <hr/> Potency	<b>AXL</b> <hr/> US-based program for originator biologic <hr/> Not disclosed	<b>IL4</b> <hr/> Program in EU for biosimilar <hr/> Potency	<b>CD16 Effectors</b> <hr/> Multiple programs in NA & EU for ADCC <hr/> Potency

# Conclusions

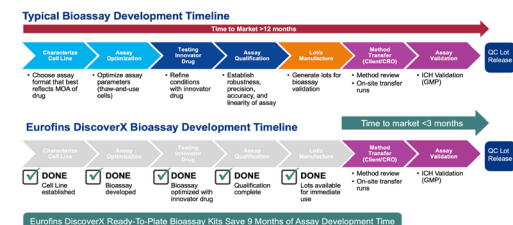
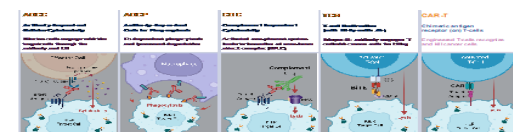
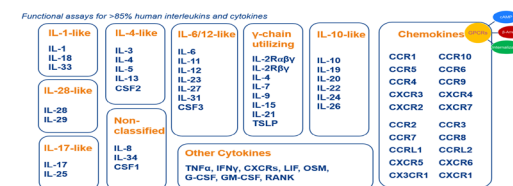
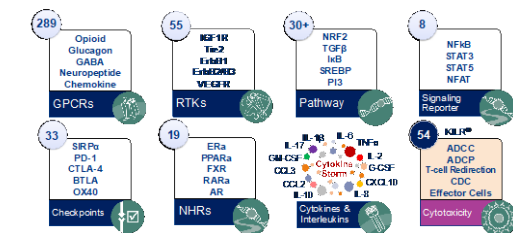
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ADCC, ADCP, CDC, T-cell Redirection and CAR-T

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