



Greater than the sum of its parts:

**Development of a new class of target cell lines to evaluate
Fc-mediated Cytotoxicity**

Dr. Jordi Rodó Morera
Global Innovation & Scientific Lead
Svar Life Science AB

SVAR LIFE SCIENCE

WHO WE ARE

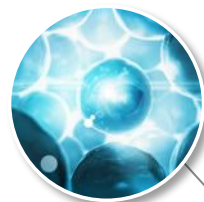


- ✓ **BASED IN MALMÖ, SWEDEN**
additional sites in France and in the US
- ✓ **GLOBAL PARTNER**
well-established provider of Life Science solutions
- ✓ **150 EMPLOYEES**
versatile cross-functional organization
- ✓ **+30 YEARS OF LIFE SCIENCE EXPERIENCE**
bioassay development, bioanalysis and clinical diagnostics
- ✓ **ISO13485 certified manufacturing of ELISA assays;**
Custom manufacturing of cell-based assays;
GLP, GCP and GMP compliant

2 Technology platforms

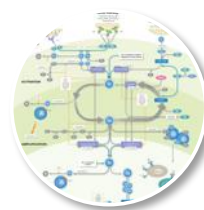
*i*LITE® CELL-BASED SOLUTIONS

Assay Ready Cells
& Custom development



COMPLEMENT SYSTEM SOLUTIONS

Functional & biomarker
ELISA assays (IVD & RUO)



...combined with 3 Service platforms



CRO SERVICES

BIOMARKER DISCOVERY SERVICES (non-GxP)

- Olink® technology
- Simoa® technology

BIOANALYTICAL SERVICES (GLP, GCP)

- TK, PK, and PD Assays
- Immunogenicity Assays
- Biomarker Assays

QC TESTING SERVICES (GMP)

- GMP Bioassays
- Potency testing



CUSTOM SERVICES

- Custom assay development
- Manufacturing Services



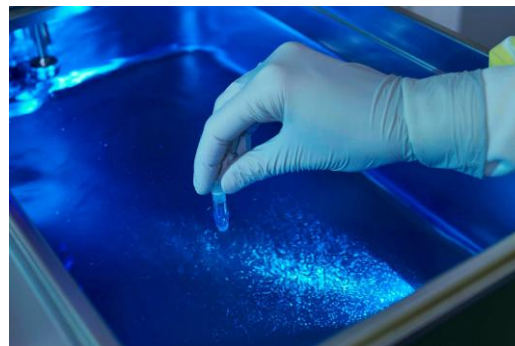
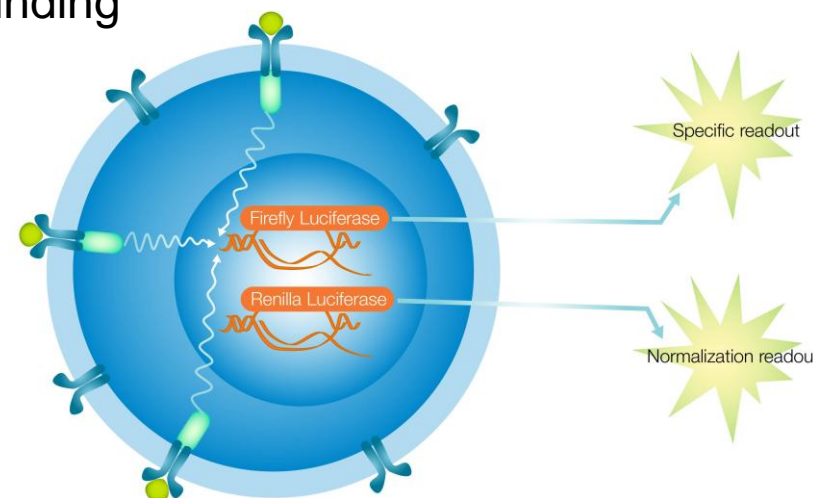
DIAGNOSTIC SERVICES

- Rare disease Clinical Testing
- Urgent testing
- Research Partner

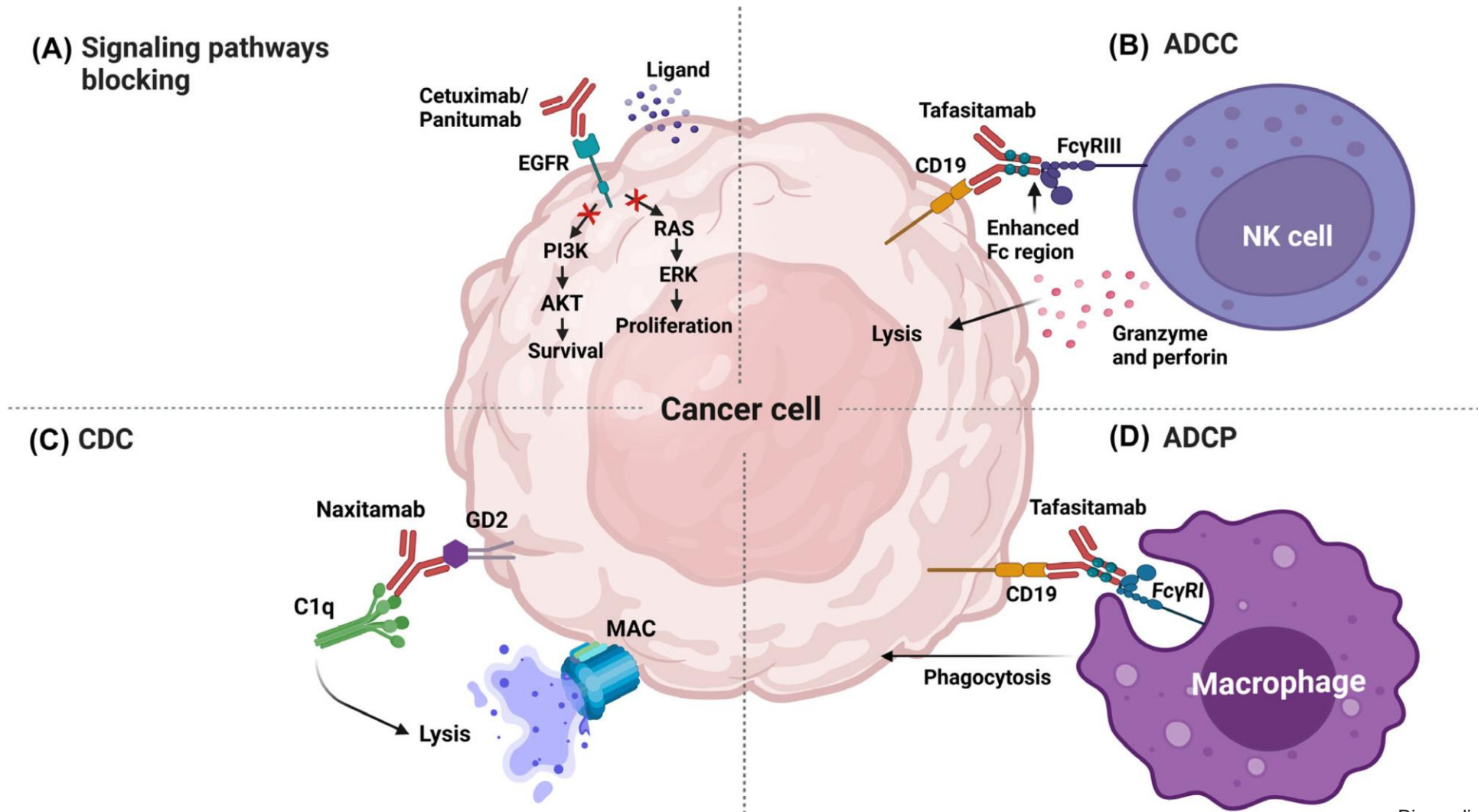
iLite® Cell-Based Assays

Features and Benefits

- **Functionality** of the compound **can be determined**, not just binding interaction
- Reflects the **Mechanism of Action (MoA)** of a potential drug
- Can be designed & customized for specific uses
- Cells provided in an **Assay Ready Cell** format
 - **Decreasing timelines and workload**
 - Resulting in **superior performance**
 - **Reducing** assay variation

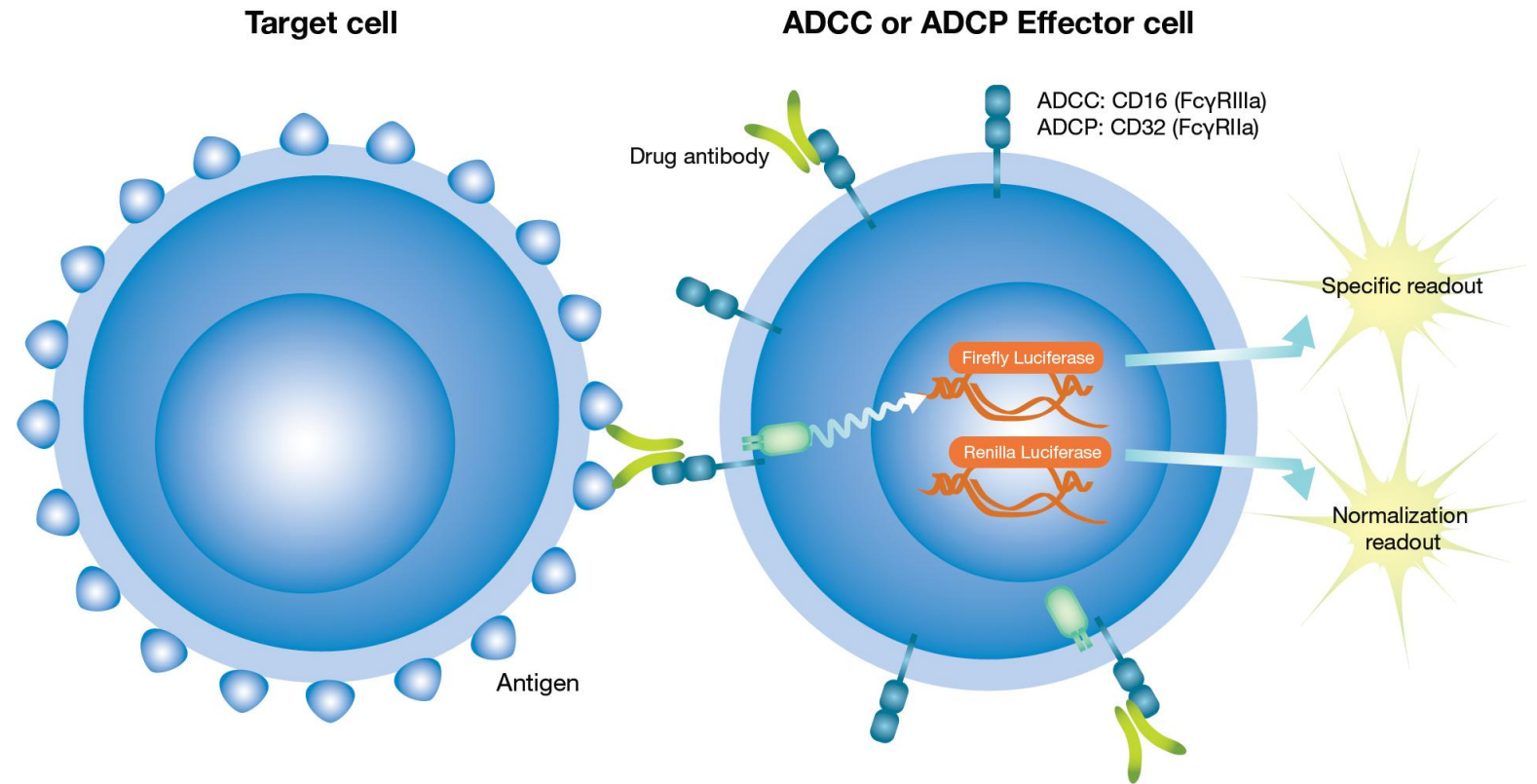


Fc-mediated mechanism of action

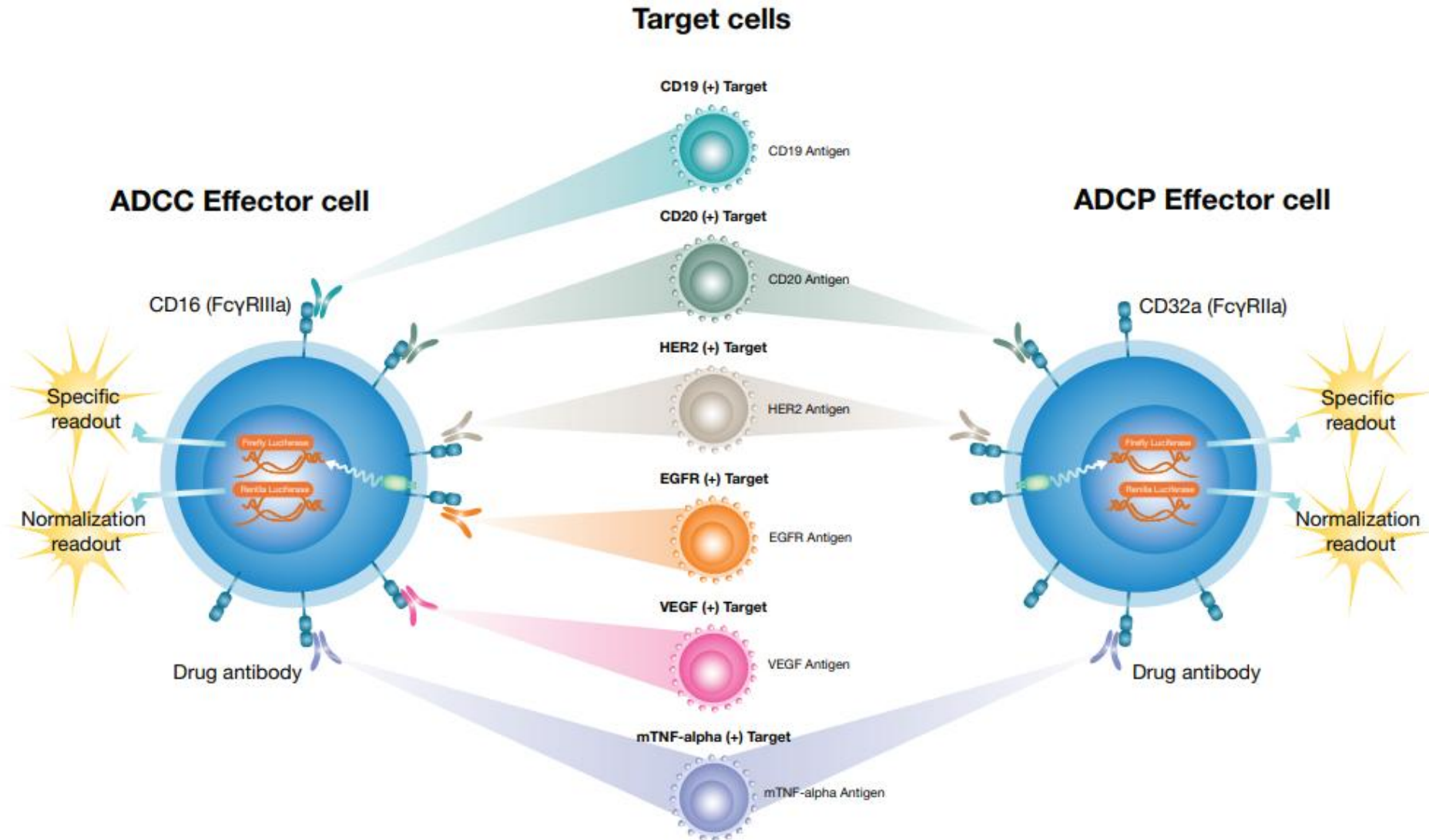


ADCC/ADCP Activity Assays

The *iLite* platform uses engineered cells to function as Target cells and act as the antibody presenting part in the bridging mechanism.

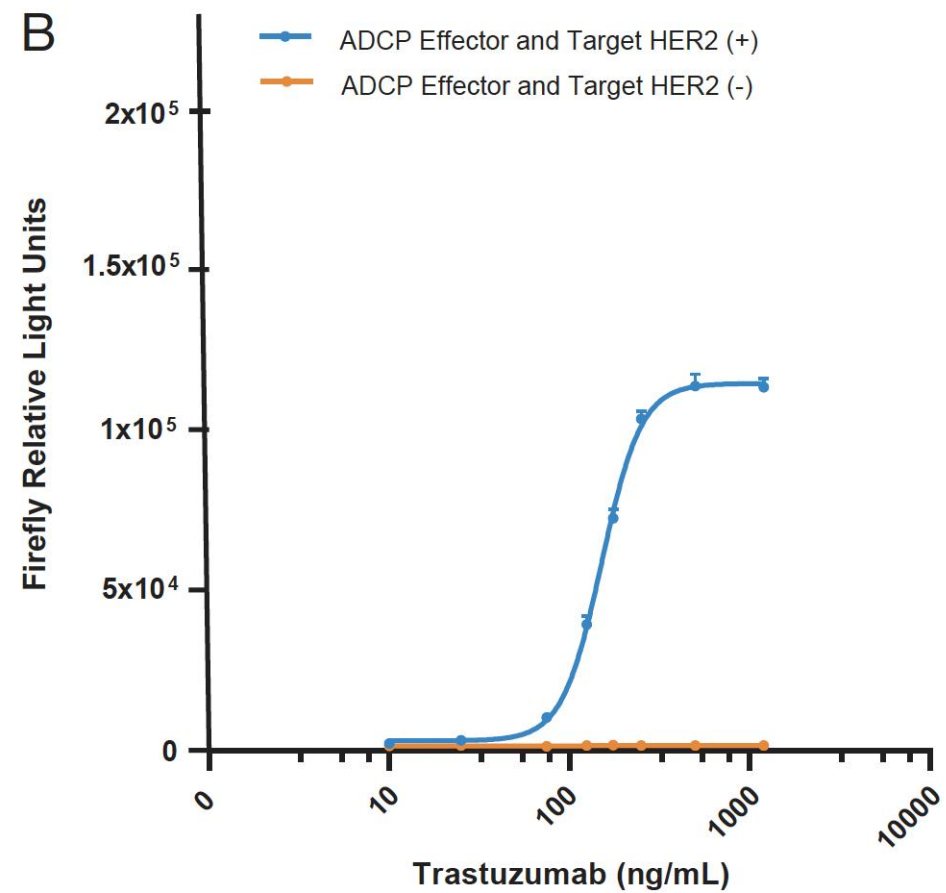
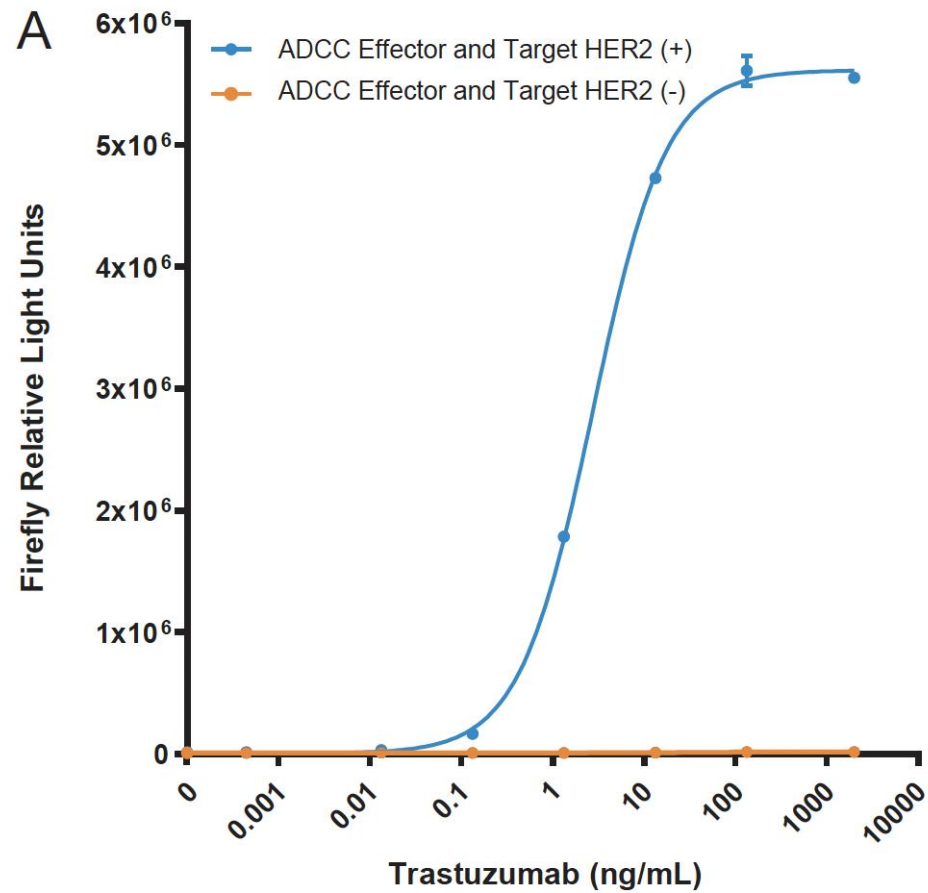


iLite[®] Fc-Effector Bioassay Platform



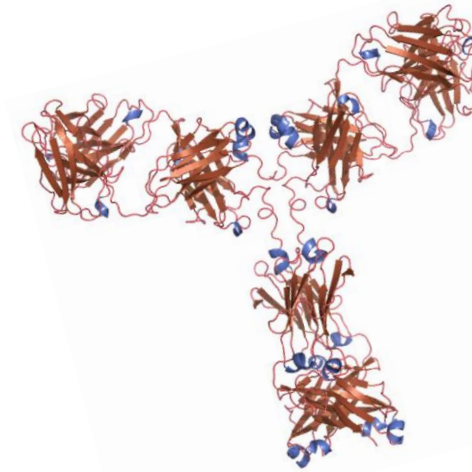
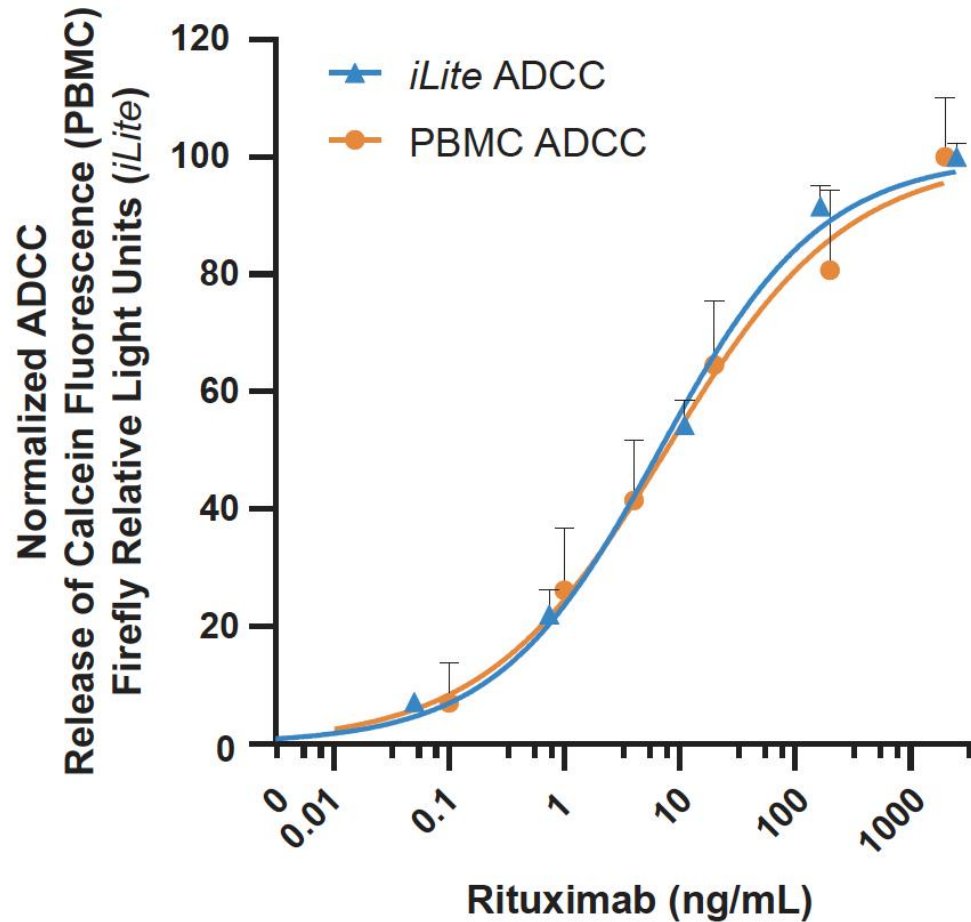
iLite[®] Fc-Effector Bioassay Set up

ADCC/ADCP - HER2(+/-) & Trastuzumab



iLite[®] Fc-Effector Bioassay Set up

ADCC - *iLite* vs. PBMC Calcein release assay

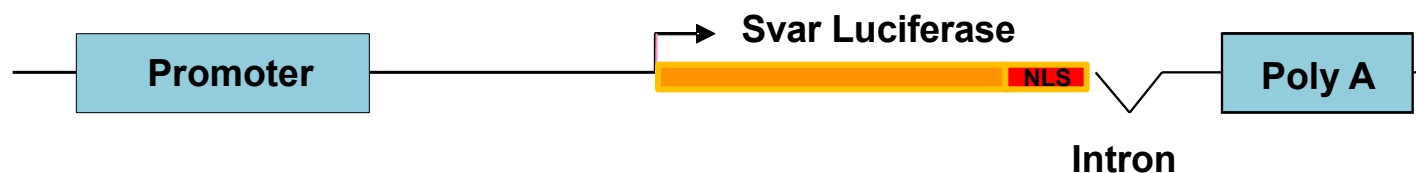


The background of the slide features abstract, flowing patterns of blue and yellow particles, resembling a microscopic view of cells or a dynamic scientific process. The particles are concentrated in the corners and edges, leaving a clear central area for text.

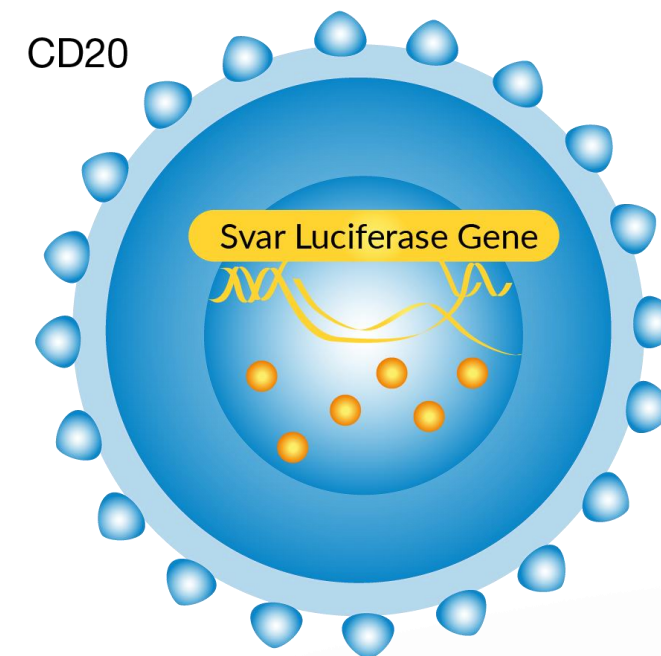
DEVELOPMENT OF A NEW CLASS OF TARGET CELL LINES TO EVALUATE FC-MEDIATED CYTOTOXICITY

iLite[®] CDC Bioassay

DEVELOPMENT OF TARGET CELL LINES

Construction of a Luciferase reporter-gene construct

Construction of a Luciferase reporter-gene construct (SL) fused to a Nuclear localization signal (NLS)



iLite[®] CDC Bioassay

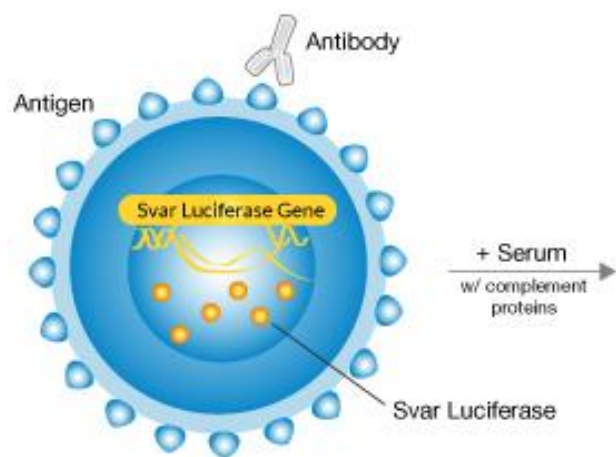
FEATURES & BENEFITS

- **Functionality can be determined** – does the antibody REALLY induce a cell lysis
- Mimics the **Mechanism of Action** (MoA) of a potential drug while having a more quantifiable endpoint.
- **By replacing antigen expression – Flexibility and adaptability** of the platform – create customized cell
- Cells are provided in an **Assay Ready Cell** format
 - **Decreasing time-consuming cell procedures**
 - Resulting in **superior performance and reproducibility**

iLite[®] CDC Bioassay

FEATURES & BENEFITS

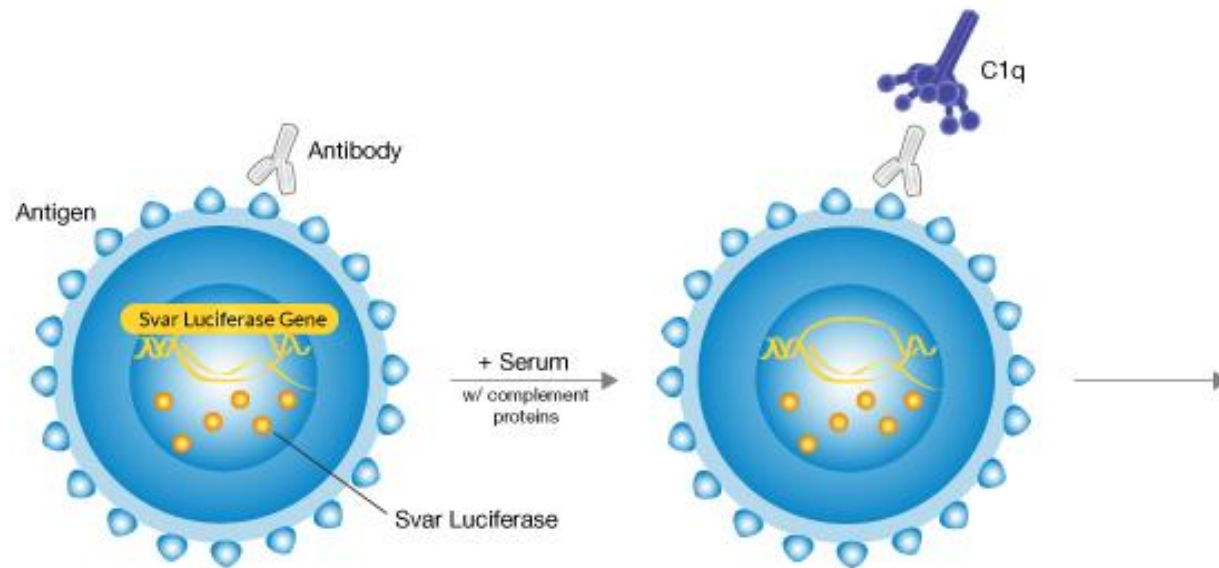
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iLite[®] CDC Bioassay

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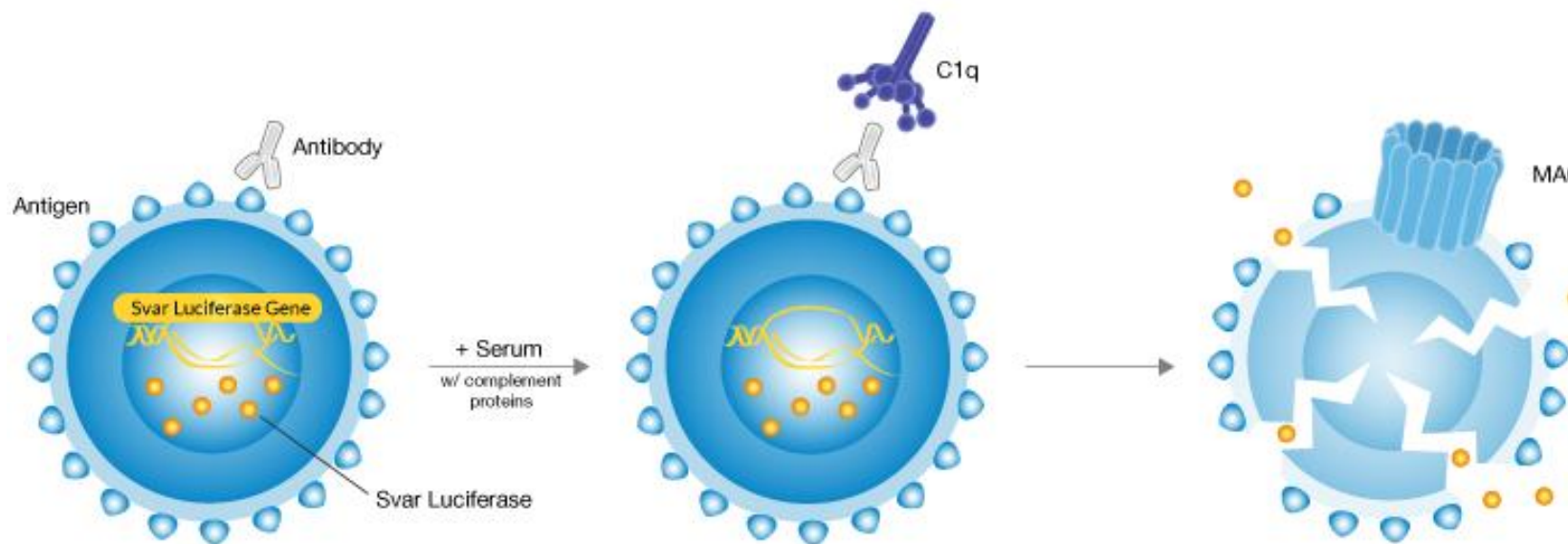
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iLite[®] CDC Bioassay

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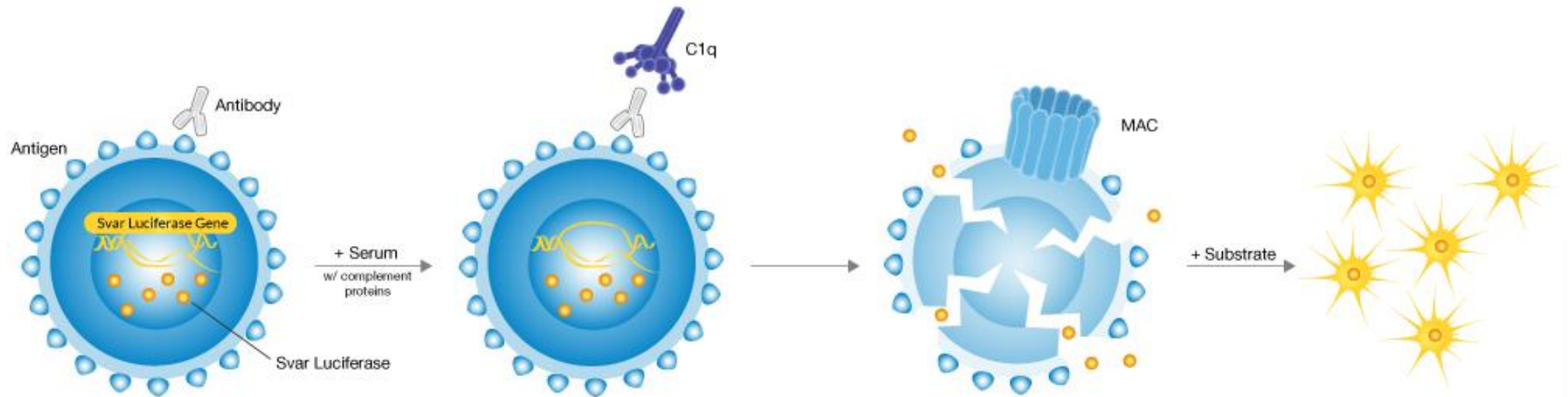
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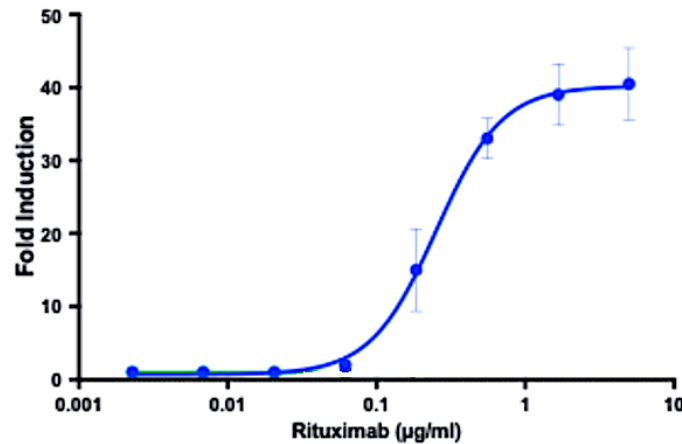
iLite[®] CDC Bioassay

FEATURES & BENEFITS

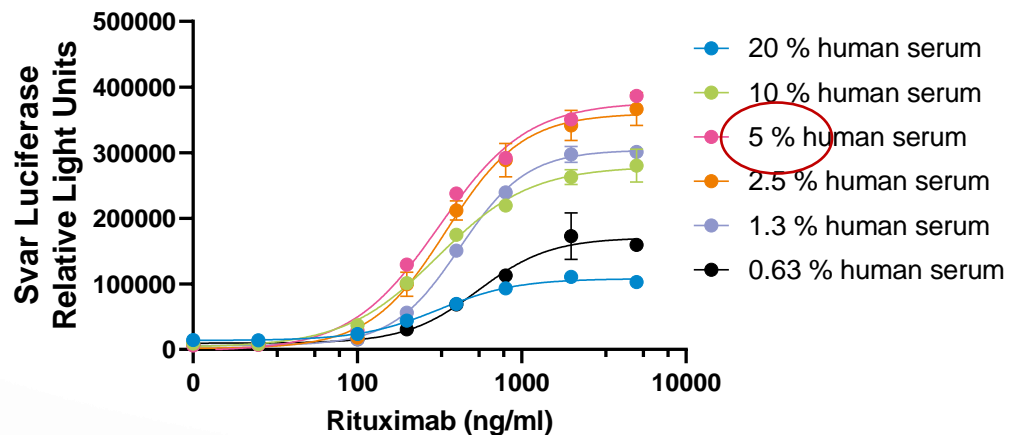
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DEVELOPMENT OF TARGET CELL LINES

Selection of a “Complement-Competent” target cell line

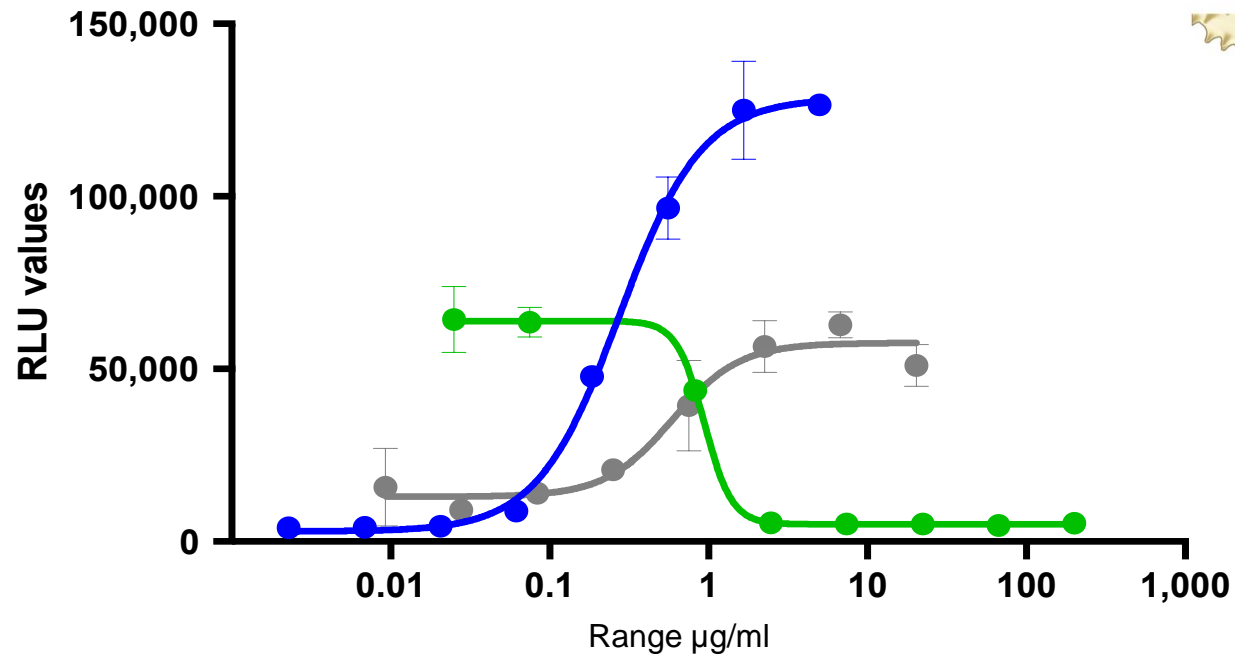
- Source of complement: Human Serum
- Assay time 1h or 5h
- Substrate for Svar Luciferase - Coelenterazine



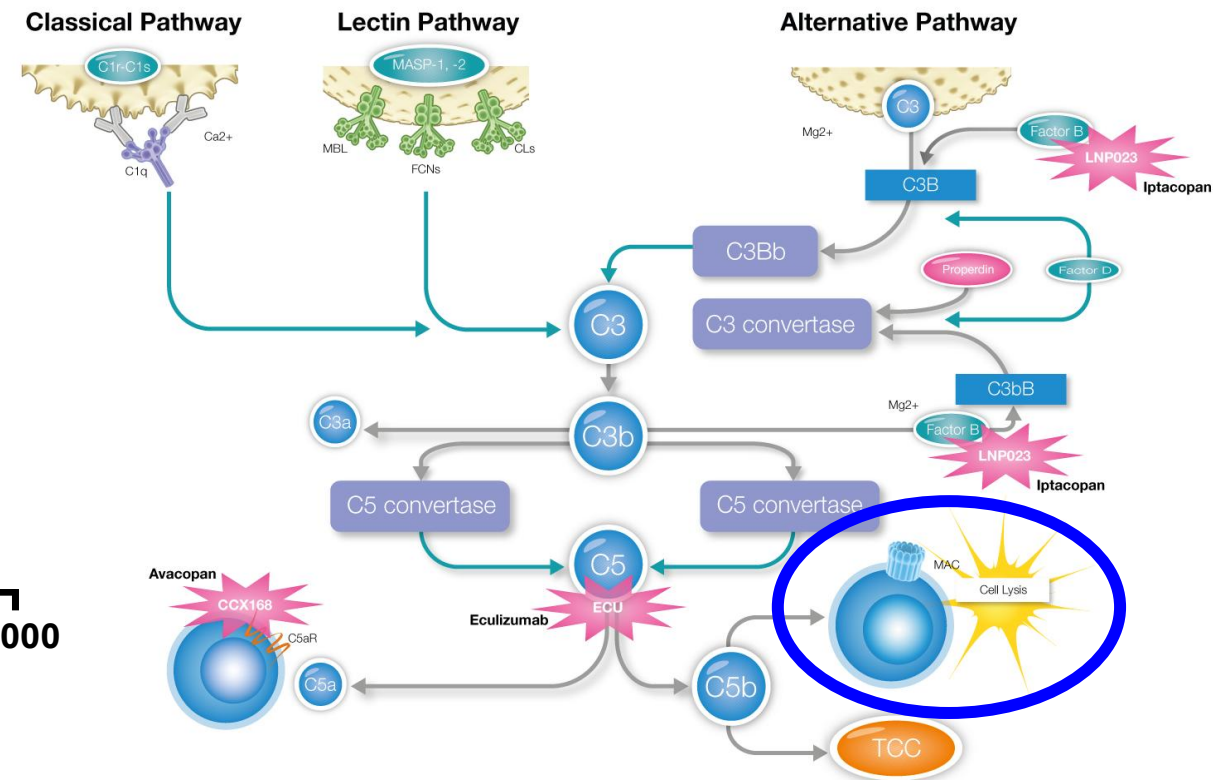
Human serum	20 %	10 %	5 %	2.5 %	1.3 %	0.63 %
FOLD ACTIVATION	8	32	50	45	38	20
HILLSLOPE	1.8	1.6	1.6	1.9	2.1	2
EC50	313	309	314	347	420	543

DEVELOPMENT OF THE TARGET CELL LINES

Case: Effect of the C5 inhibitor Eculizumab

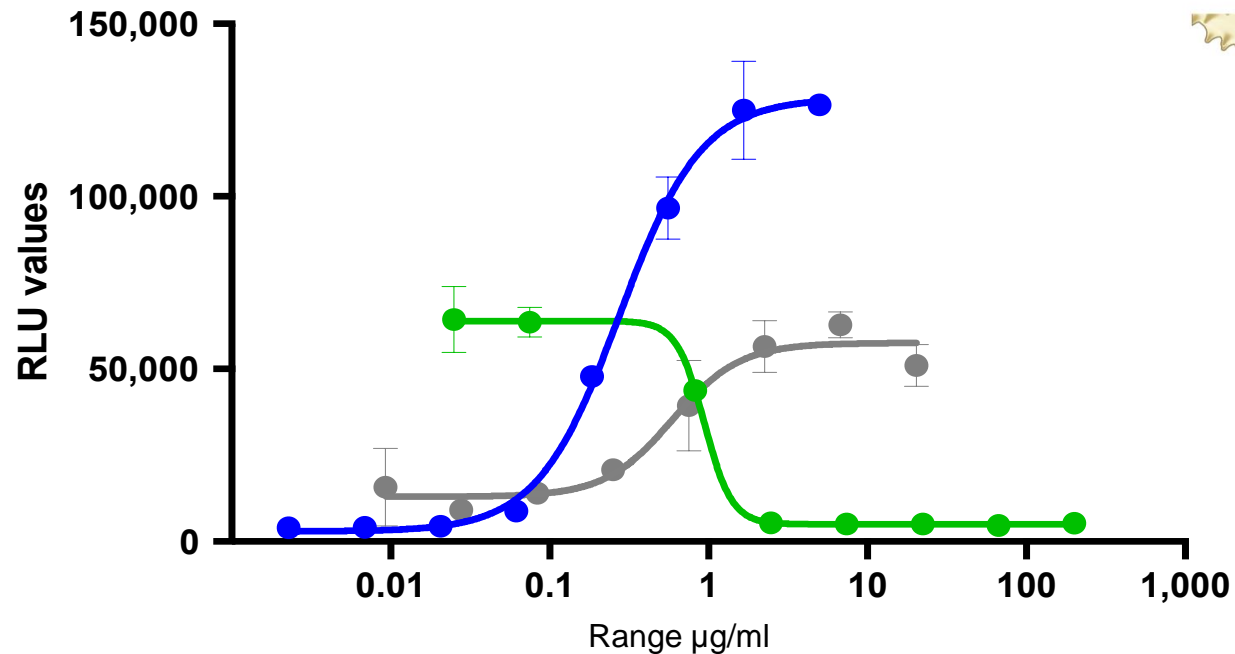


- Rituximab range (RTX) + human serum (HS)
- Eculizumab range + 0,2µg/ml RTX + HS
- Anti-Eculizumab range + 2µg Eculizumab + 0,2µg/ml Rituximab + HS

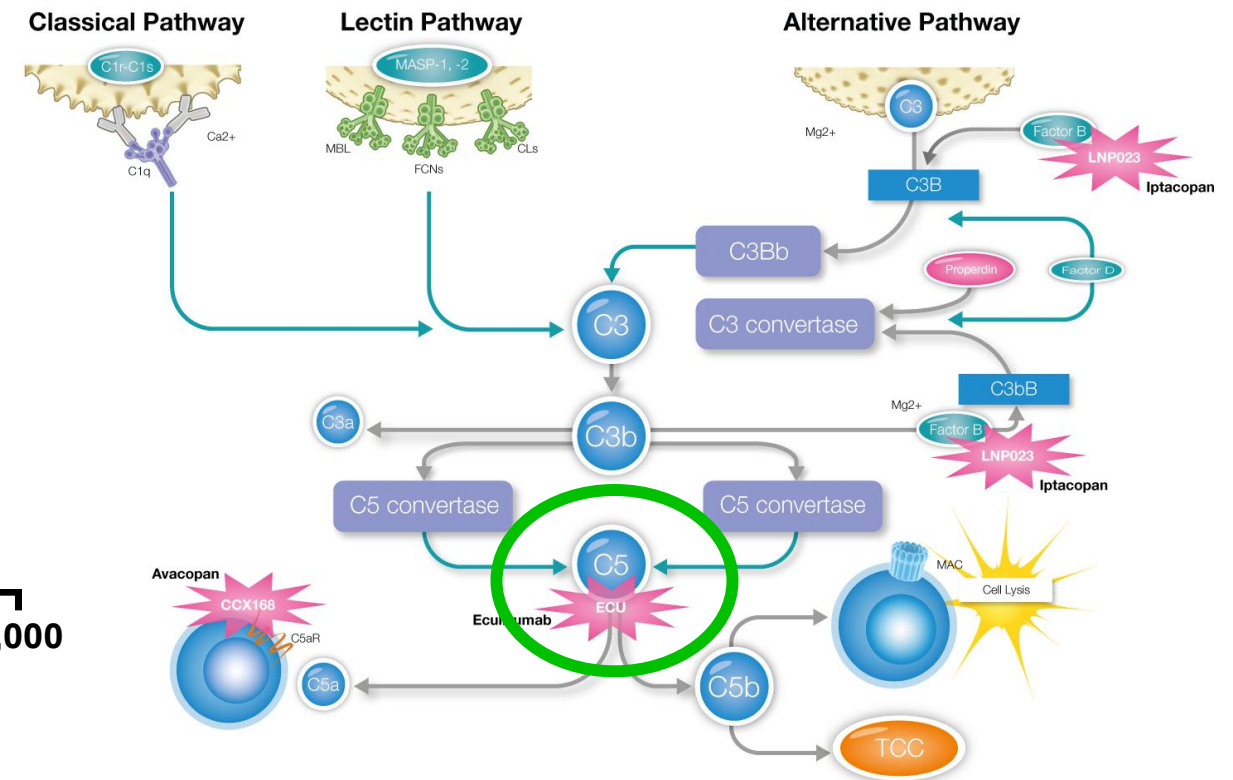


DEVELOPMENT OF THE TARGET CELL LINES

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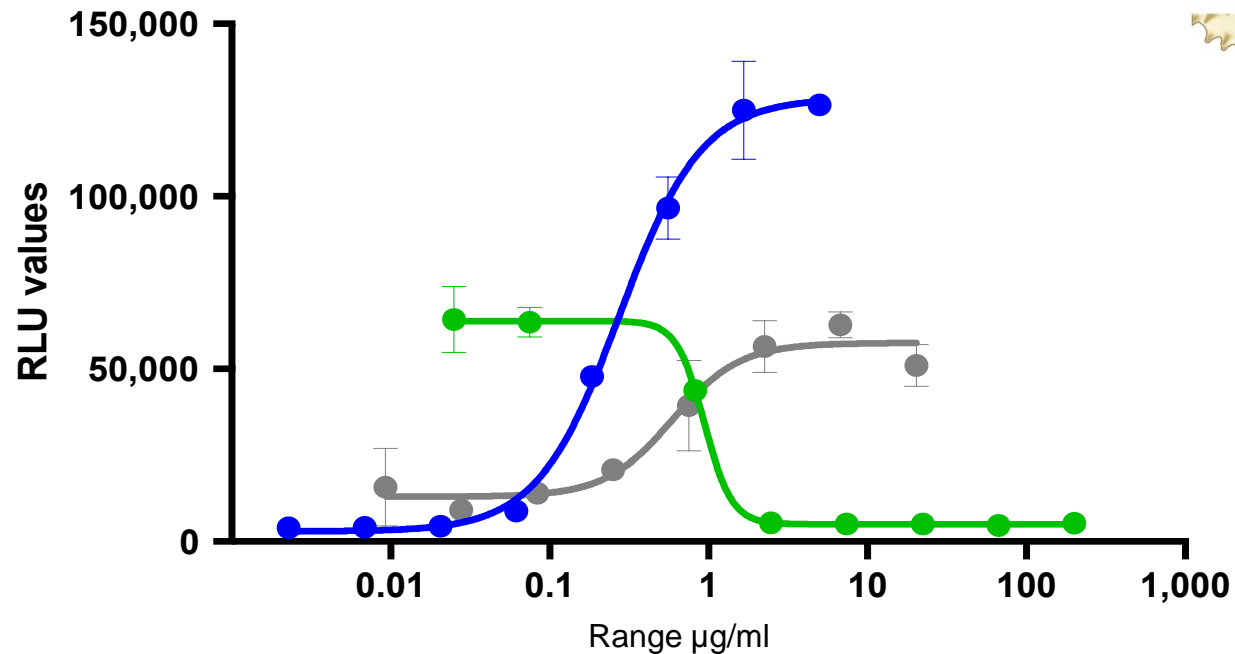


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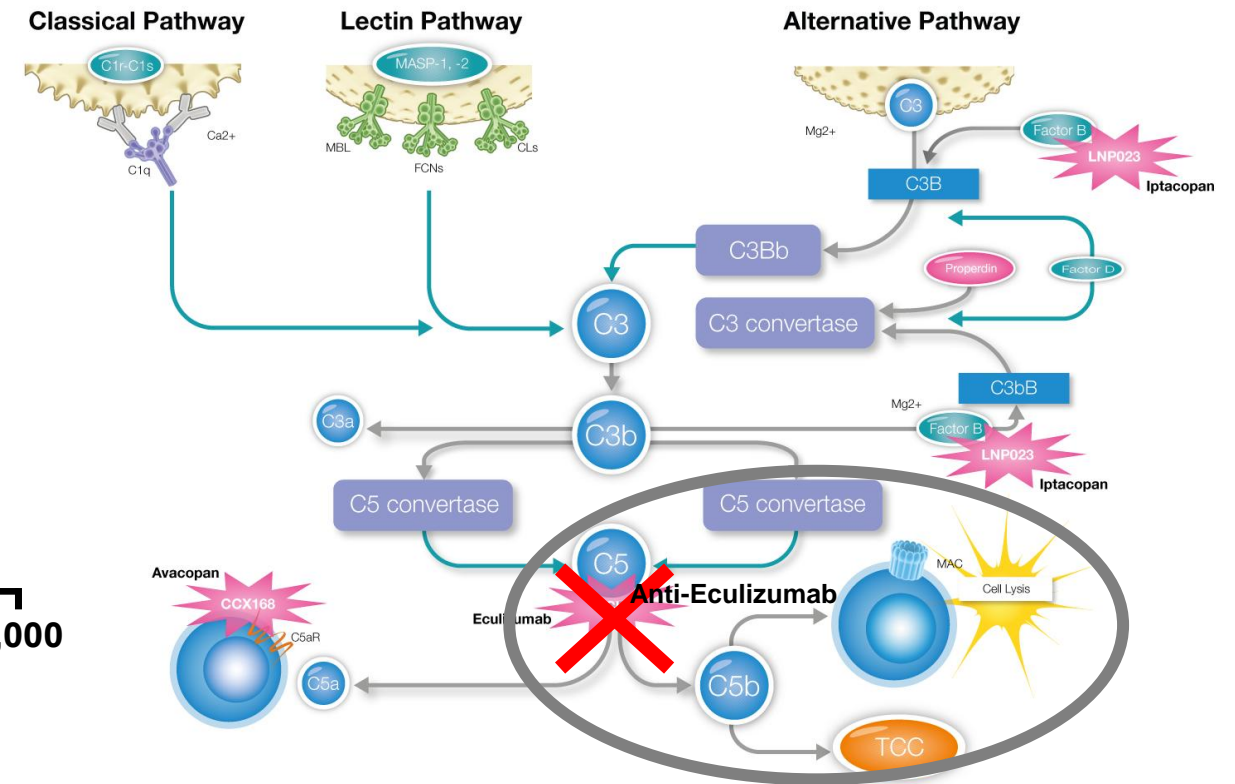


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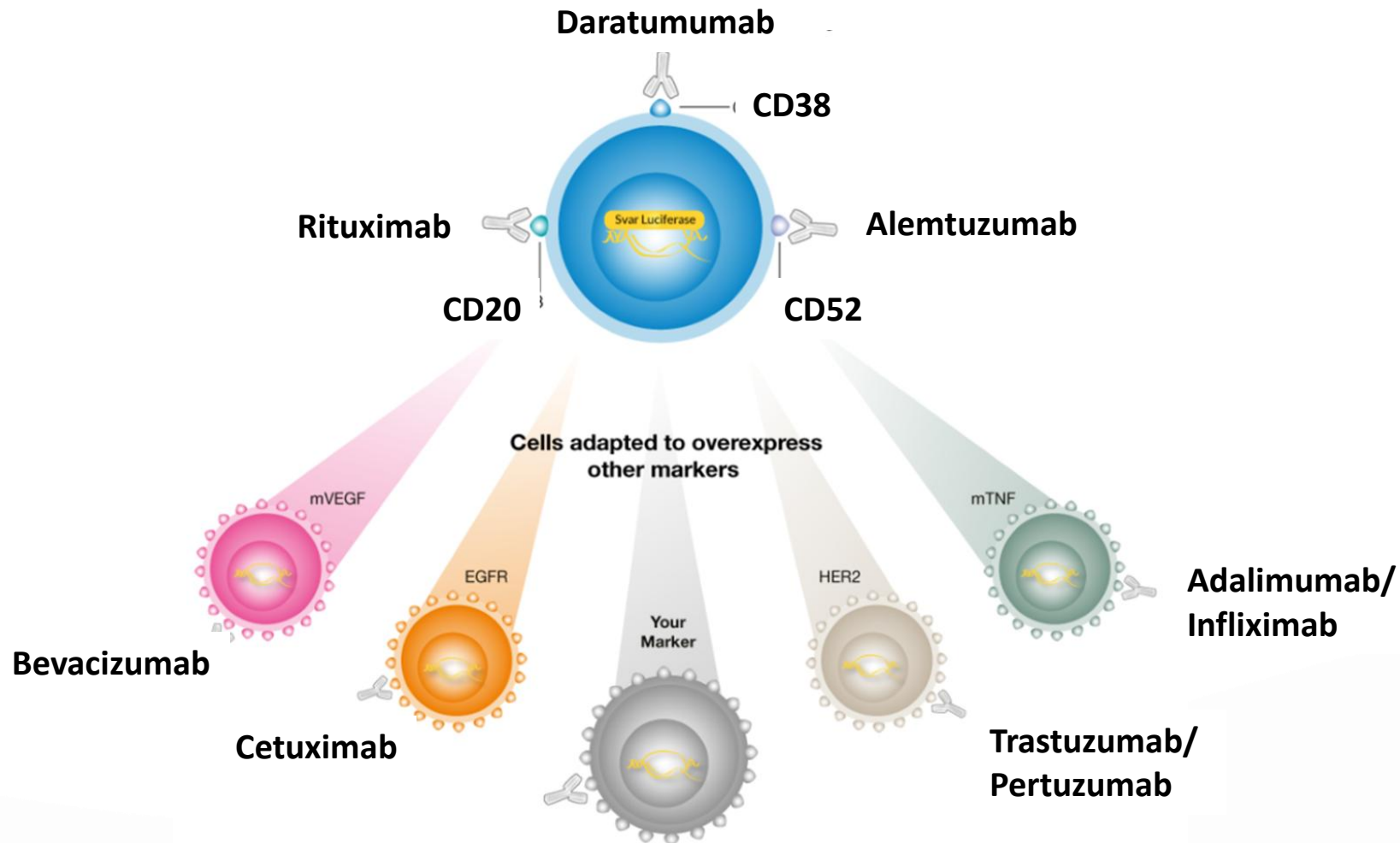


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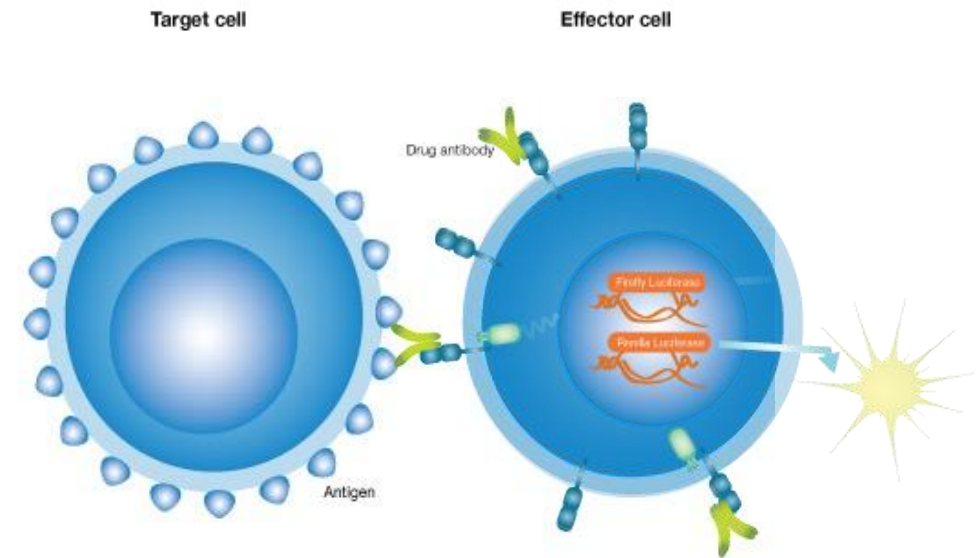
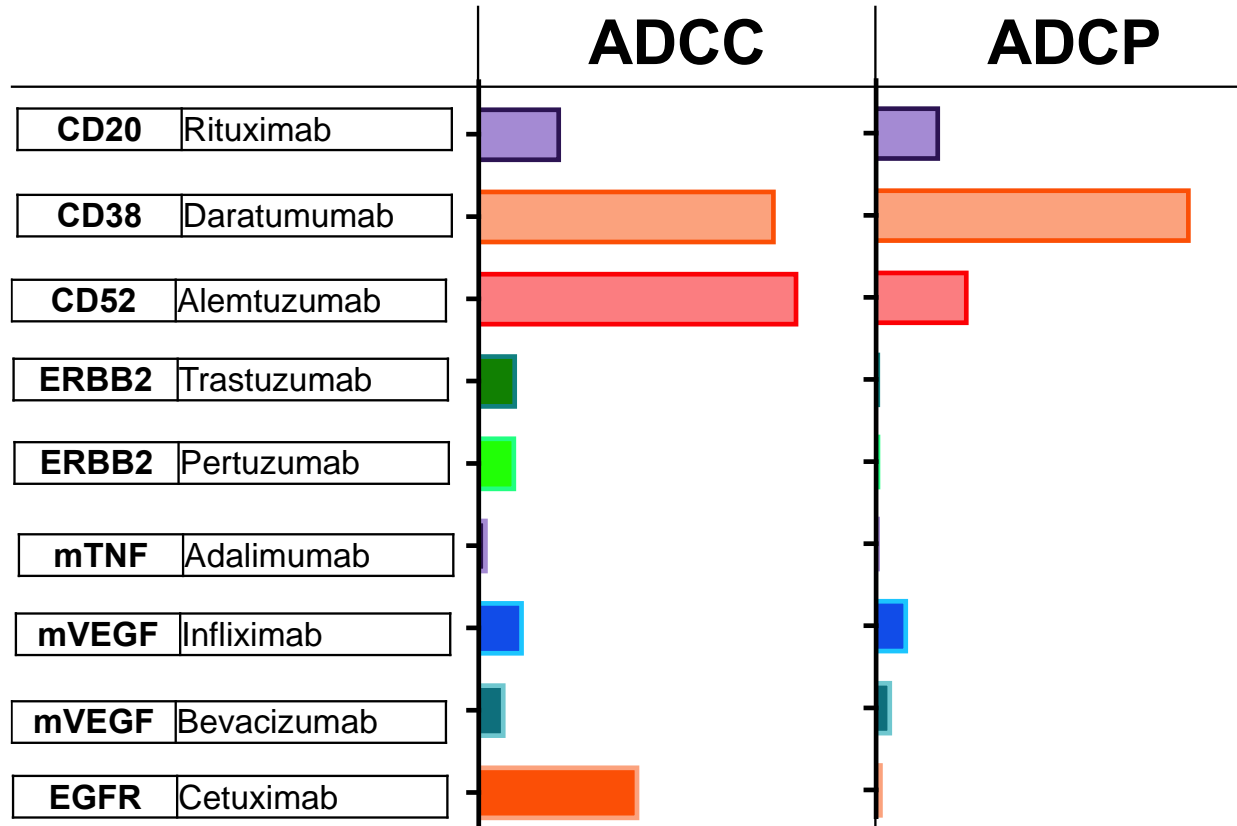
Platform Technology

FAST GENERATION OF CUSTOM TARGET CELLS



1 TARGET CELL LINE TO ASSESS 3 MECHANISMS OF ACTION

Heterogeneity of the prevalent MoA of different therapeutic antibodies

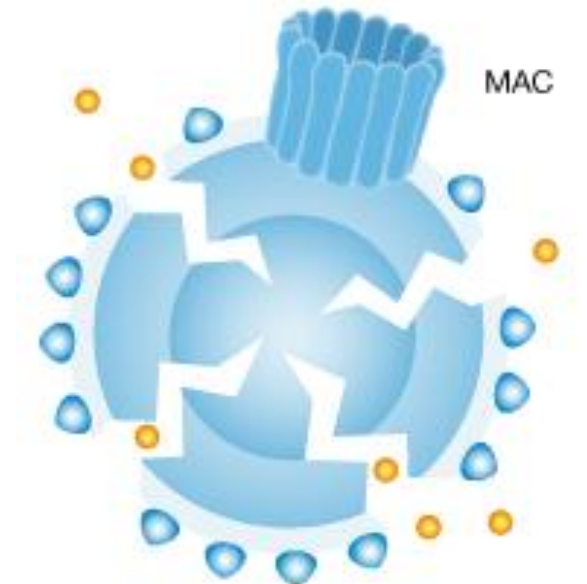
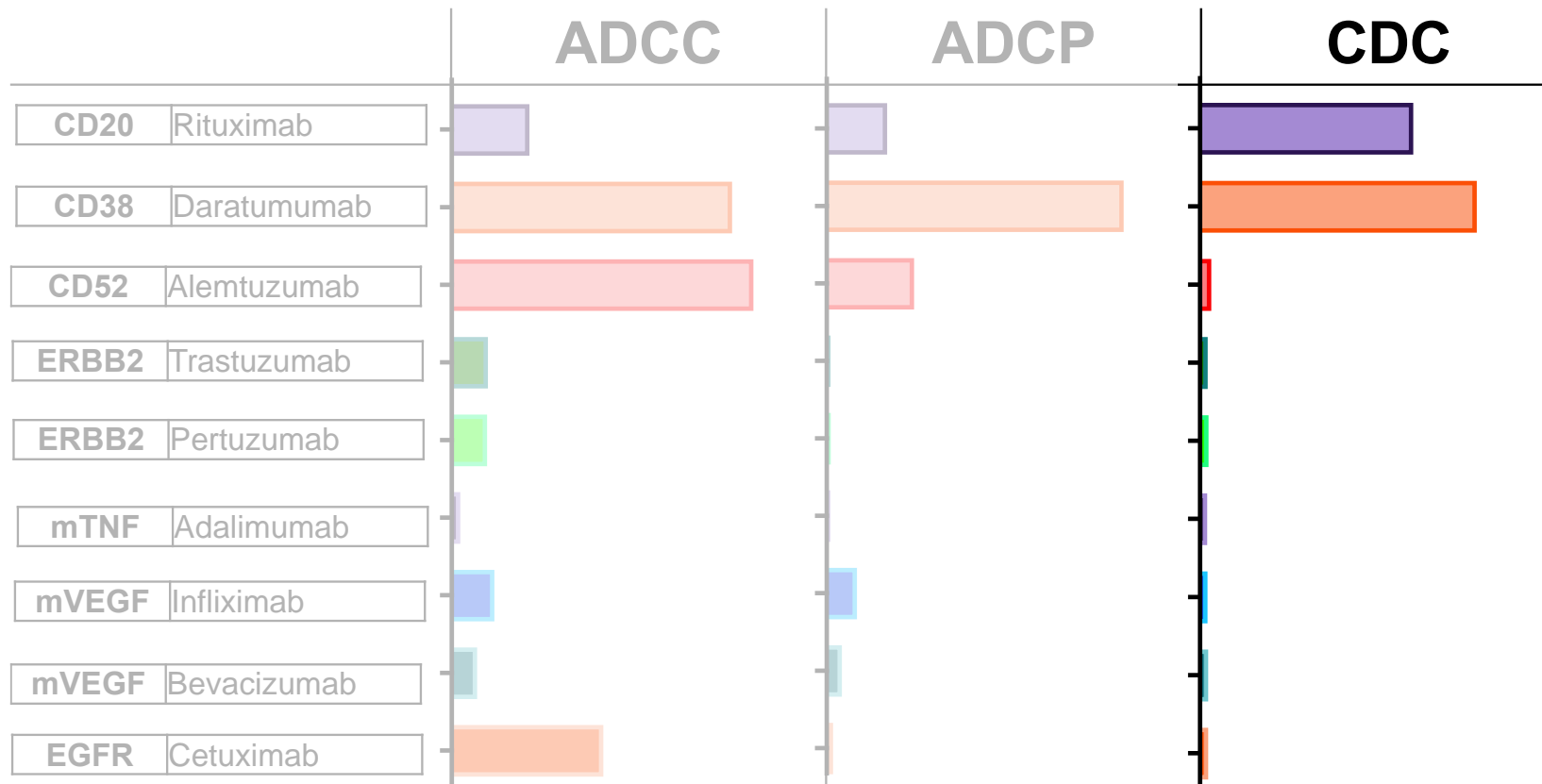


ADCC or ADCP Assays using iLite Effector cells (BM5001 and BM5004) tested in combination with different target cells (CD20/CD38/CD52/her2/mTNF/mVEGF/EGFR) and respective drug antibodies.

Cells (all in an Assay Ready Cell format) have been incubated for 5h with the respective antibodies before luminescence readout using Firefly luciferase substrates.

1 TARGET CELL LINE TO ASSESS 3 MECHANISMS OF ACTION

Heterogeneity of the prevalent MoA of different therapeutic antibodies

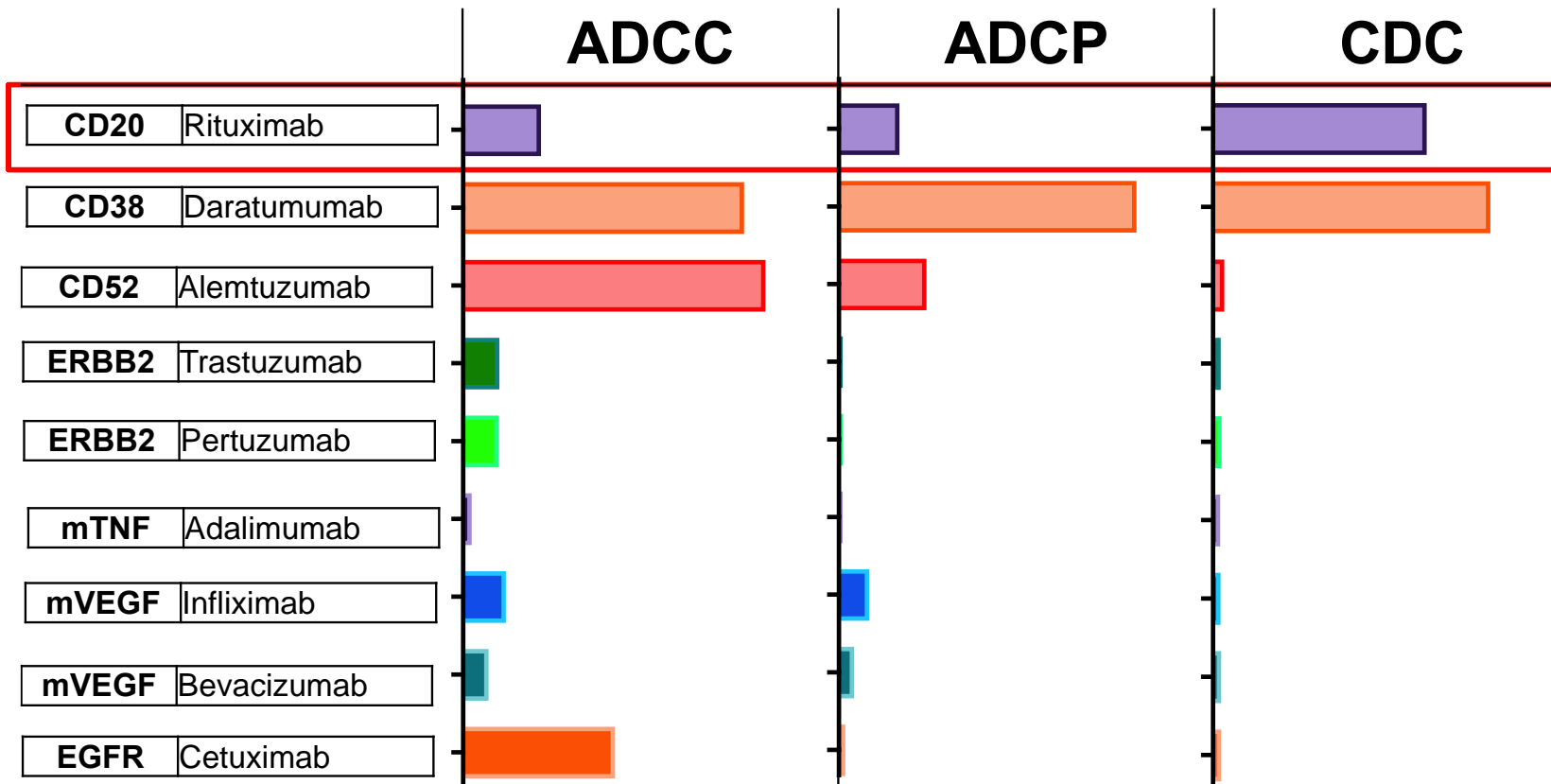


CDC using different ARC CD20+SL Target cells (CD20/CD38/CD52/her2/mTNF/mVEGF/EGFR).

Cells are incubated 5h in presence of Human serum as complement source and the respective drug antibodies. Data were assessed using a SvarLuc specific substrate (Coelenterazine).

1 TARGET CELL LINE TO ASSESS 3 MECHANISMS OF ACTION

Heterogeneity of the prevalent MoA of different therapeutic antibodies

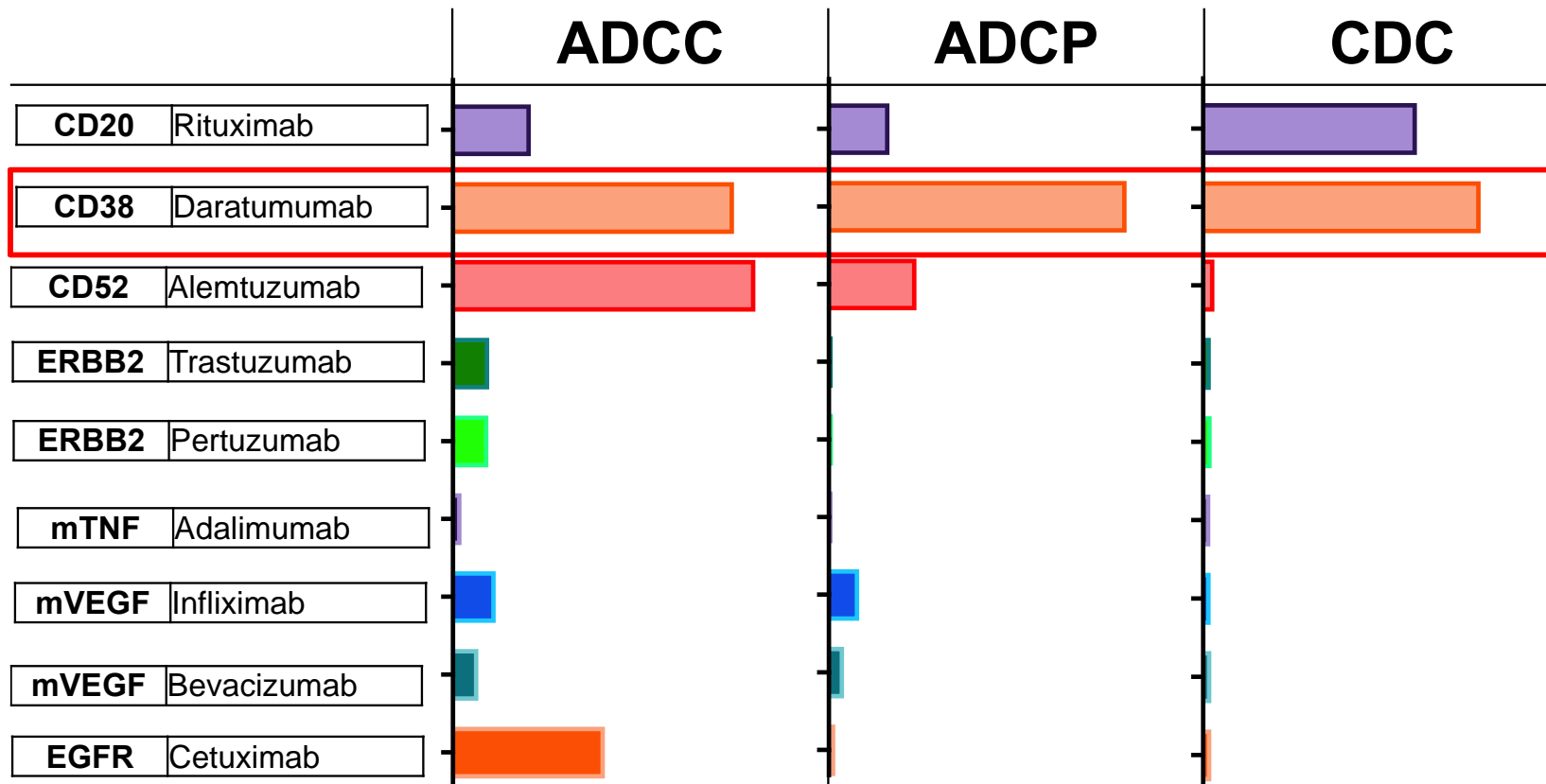


The direct effects of rituximab include complement-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity

Cerny et al. 2002
Anticancer Drugs 2002 Nov; 13
Suppl2:S3-10

1 TARGET CELL LINE TO ASSESS 3 MECHANISMS OF ACTION

Heterogeneity of the prevalent MoA of different therapeutic antibodies

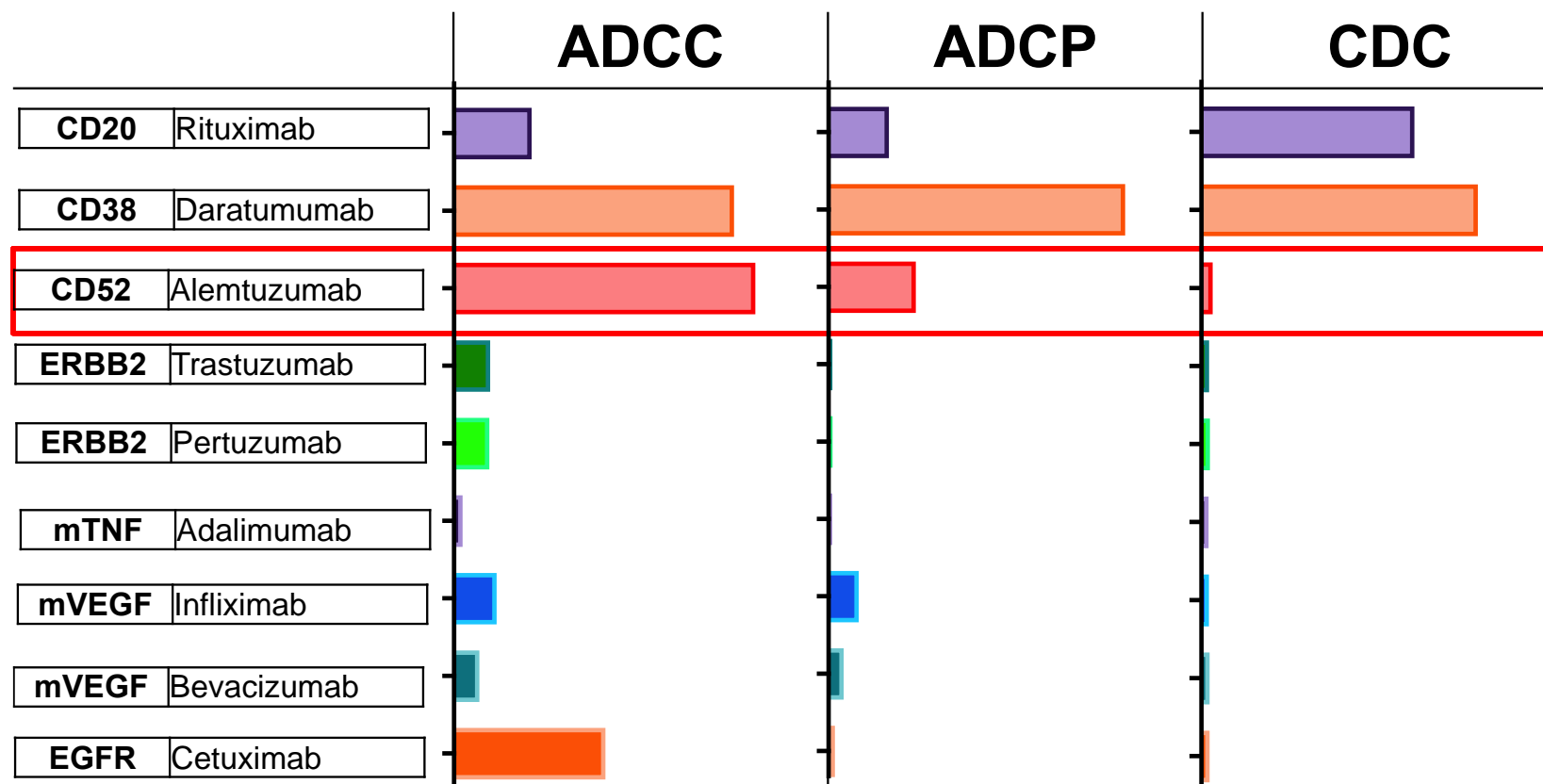


Preclinical studies have shown that daratumumab induces cell death by CDC, ADCC and ADCP

Sanchez et al.
J Hematol Oncol 2016; 9: 51

1 TARGET CELL LINE TO ASSESS 3 MECHANISMS OF ACTION

Heterogeneity of the prevalent MoA of different therapeutic antibodies



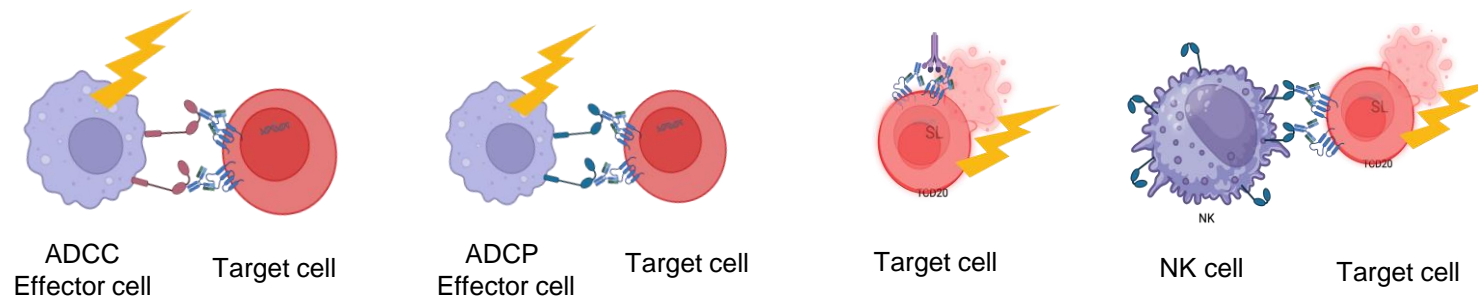
Alemtuzumab has been reported to mediate cell lysis by CDC and ADCC *in vitro*.

Experiments in hCD52-expressing mice suggested a predominant role for ADCC as opposed to CDC in lymphocyte depletion.

Hu et al. Immunology, 2009 Oct; 128(2): 260-270

Platform Technology

ONE TARGET CELL LINE TO ASSESS 3 MOAS



TARGET	ANTIBODY	<u>Surrogate Bioassay (Signaling)</u>		<u>Target Cell Killing Bioassay</u>	
		ADCC (BM5001)	ADCP (BM5002)	CDC	ADCC (NK)
CD20	Rituximab				
CD38	<u>Daratumumab</u>				
ERB2	Trastuzumab				
<u>mTNF</u>	Infliximab				
<u>mVEGF</u>	<u>Bevacizumab</u>				
EGFR	<u>Cetuximab</u>				

iLite® target cells

Summary

We established a target **cell line platform allowing the development of** fast, robust, and reproducible functional assays to detect Fc-mediated **ADCC, ADCP and CDC functionalities** or **other killing mechanisms** based on luciferase detection.

The same unique/single target cell line **is suitable for use in determining an antibodies** CDC activity and assessing ADCC **and** ADCP activity **in surrogate and killing assay setups**.

We show at the example of Rituximab initiated CDC that a C5 complement inhibitor (Eculizumab) is inhibiting killing of the cells. Potentially, this platform potentially allows to detect complement inhibition at many levels of complement activation.

The *iLite*® CD20+SL target cell platform allows fast customization of the target cells by adding surface antigens of interest thereby enabling the determination of Fc-mediated ADCC, ADCP and CDC of any antibody.

This versatile system is well-suited to detect (unwanted) Fc-mediated cytotoxic MoAs of therapeutic antibodies with agonistic, antagonistic or neutralising functions in functional assays using only 1 target cell line.





THANK YOU FOR YOUR ATTENTION!

