



IndEx-2 system

A dual inducible, customisable cell line platform

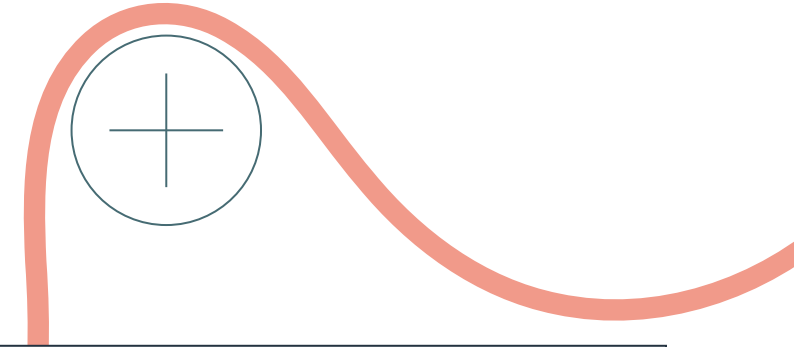
W: <https://rouken.bio/>

E: hello@rouken.bio

LI: <https://www.linkedin.com/company/roukenbio/>



The importance of target antigen density



1. Immune cell therapies
2. Immune cell engagers
3. Antibody-Drug Conjugates (ADCs)
4. Conventional mAbs

B. Preclinical Considerations for the Vector Component of CAR T Cells

The design of the CAR vector and the process by which the transgene is delivered to the T cells are critical in determining product safety and activity. Genetic material encoding the CAR has been delivered to T cells using multiple vector types, including gammaretroviral and lentiviral vectors, transposons, and naked mRNA (Ref. 10).

A major determinant of CAR T cell safety and efficacy is the antigen recognition domain used to confer target specificity. The antigen recognition domain may originate from monoclonal antibodies (mAbs), endogenous ligand/receptor pairs, or from other sources. Preclinical evaluation of the antigen recognition domain should include assessment of the specificity and affinity for the target antigen to evaluate the potential for on-target/off-tumor and off-target toxicities. **Undesired targeting of healthy/normal tissue that express the intended target antigen (on-target/off-tumor)**, as well as unintended targeting of other antigens expressed on healthy/normal tissue is a safety concern that may be evaluated using both in vitro and in vivo studies. Examples include: (1) tissue

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What would be the ideal system?



Common genetic background



**Large dynamic range of expression
~100s to 100,000s of receptors**



Finely titratable

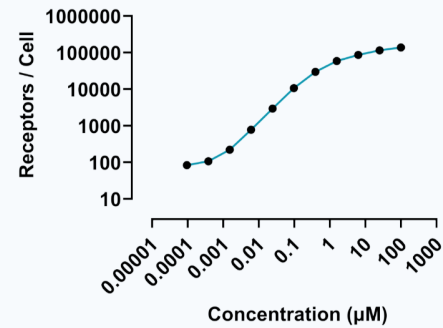
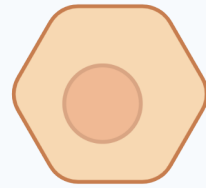


Availability of negative control

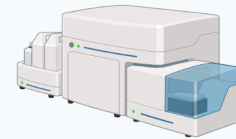
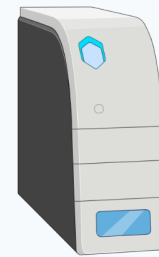
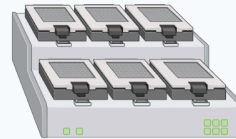
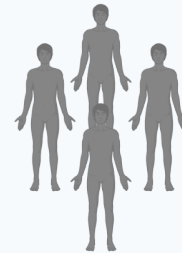
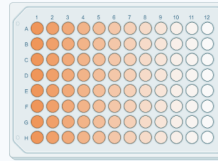


RoukenBio's solution: The IndEx-2 Assay Platform

Inducible expression of antigens



Functional assays



Threshold determination

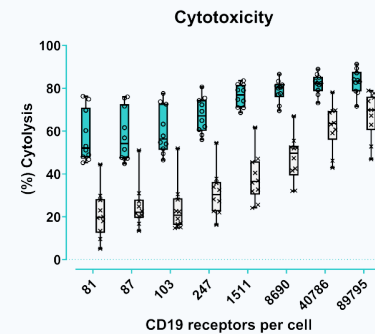
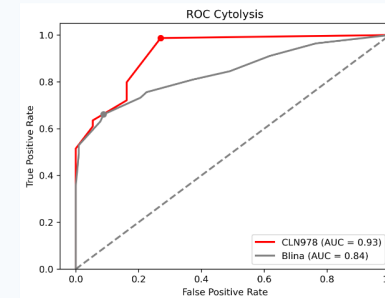
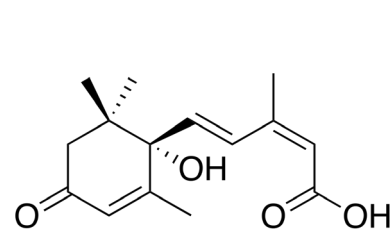
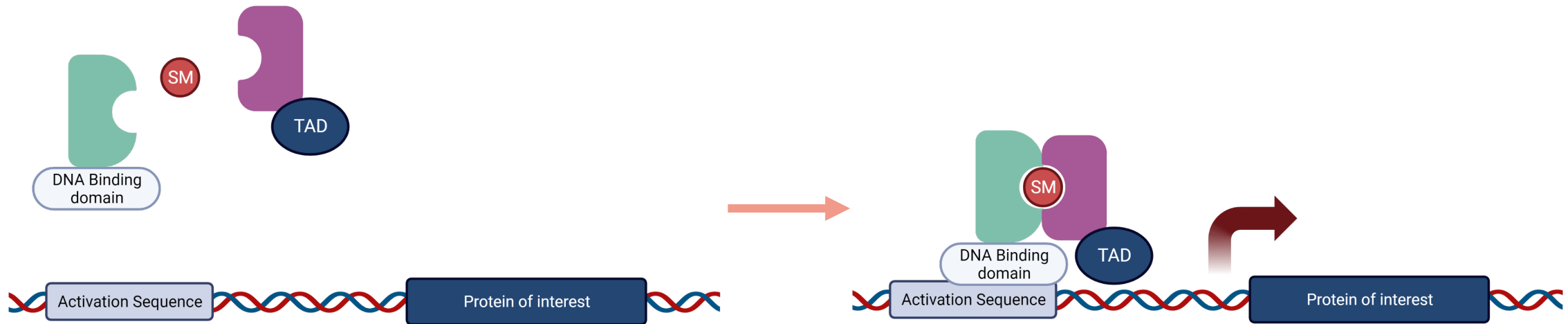


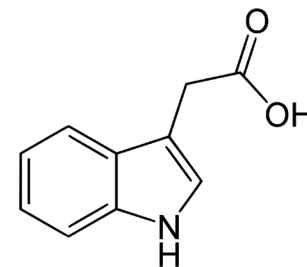
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IndEx-2, a CiP-based system for inducible titratable expression



Abscisic acid



Indole-3-acetic acid

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Titratable expression with large dynamic range

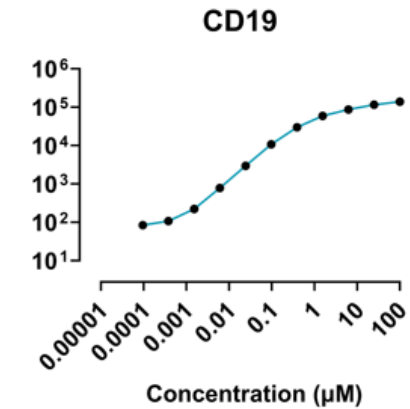
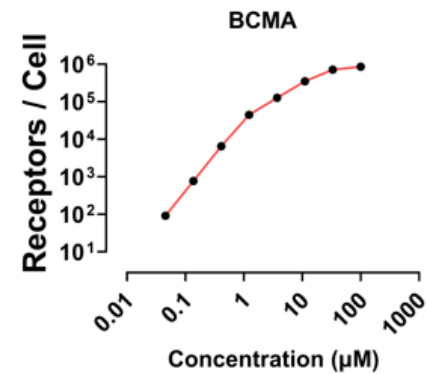
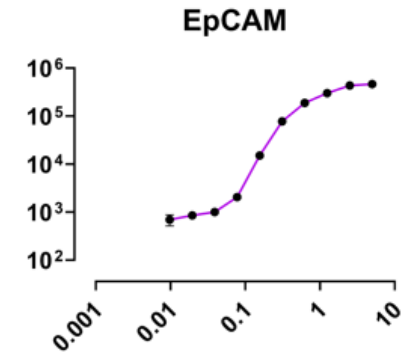
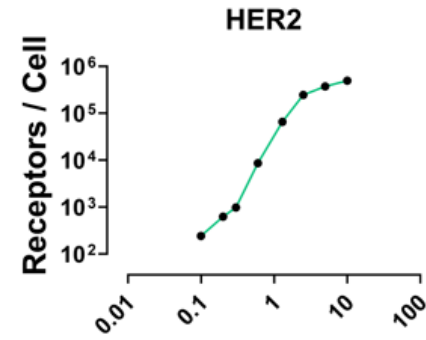
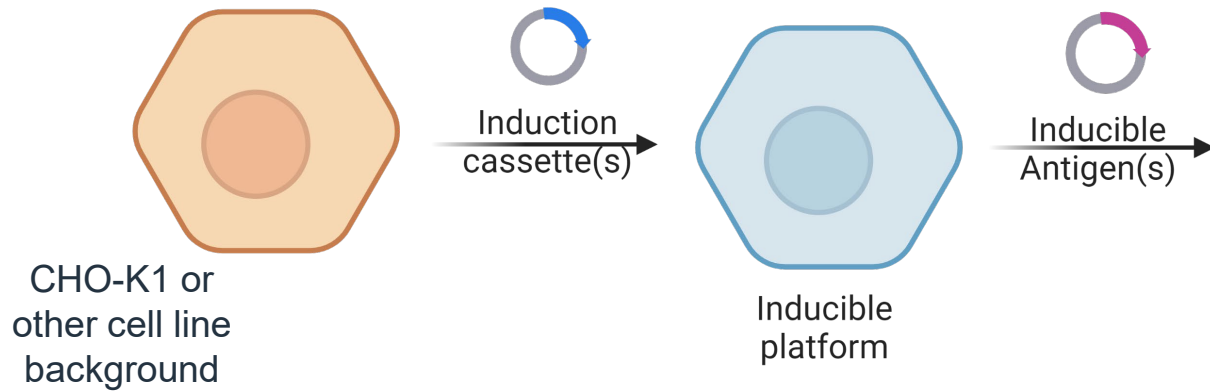


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Case Studies

Case Study 1 - HER2-CAR-T

- Main consideration:
 - On-target/Off-tumour toxicity

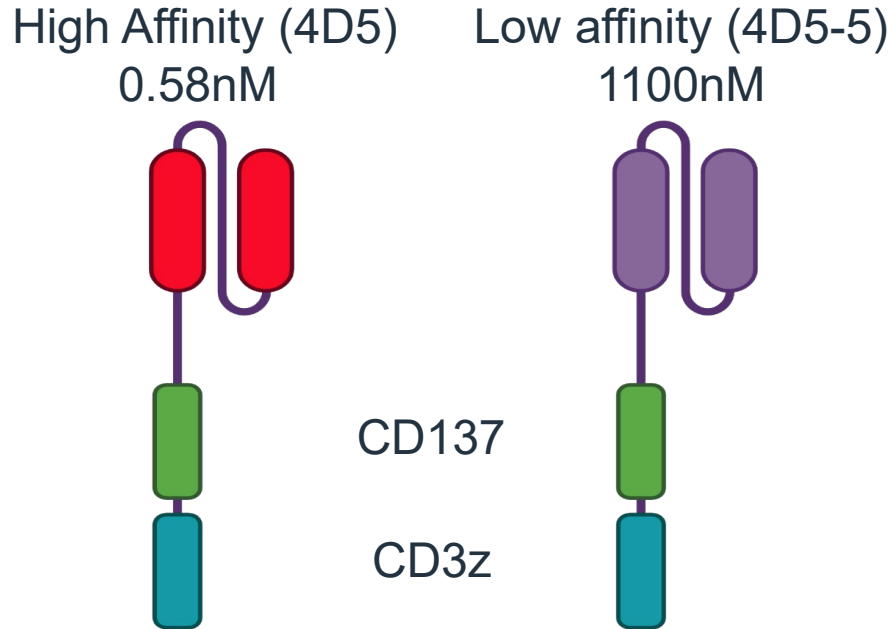
Case Study 2 – CD19 T cell engager

- Main consideration:
 - Immune escape



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Case Study 1 – Comparing high and low affinity CARs



Therapeutics, Targets, and Chemical Biology

Cancer
Research

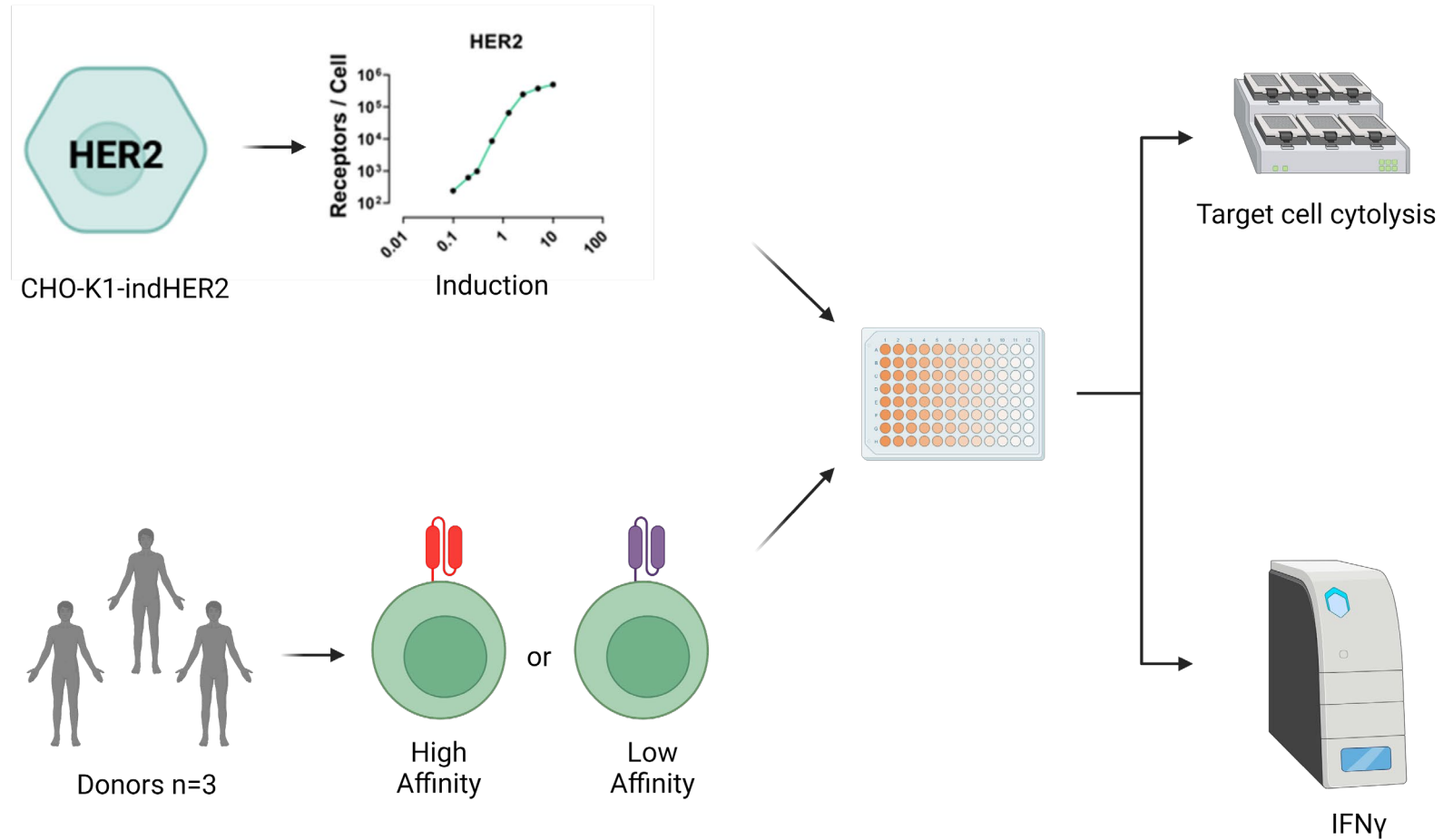
Affinity-Tuned ErbB2 or EGFR Chimeric Antigen Receptor T Cells Exhibit an Increased Therapeutic Index against Tumors in Mice

Xiaojun Liu¹, Shuguang Jiang¹, Chongyun Fang¹, Shiyu Yang¹, Devvora Olalere¹, Edward C. Pequignot¹, Alexandria P. Cogdill¹, Na Li², Melissa Ramones², Brian Granda², Li Zhou², Andreas Loew², Regina M. Young^{1,3}, Carl H. June^{1,3,4}, and Yangbing Zhao^{1,3,4}

Cancer Res; 75(17) September 1, 2015

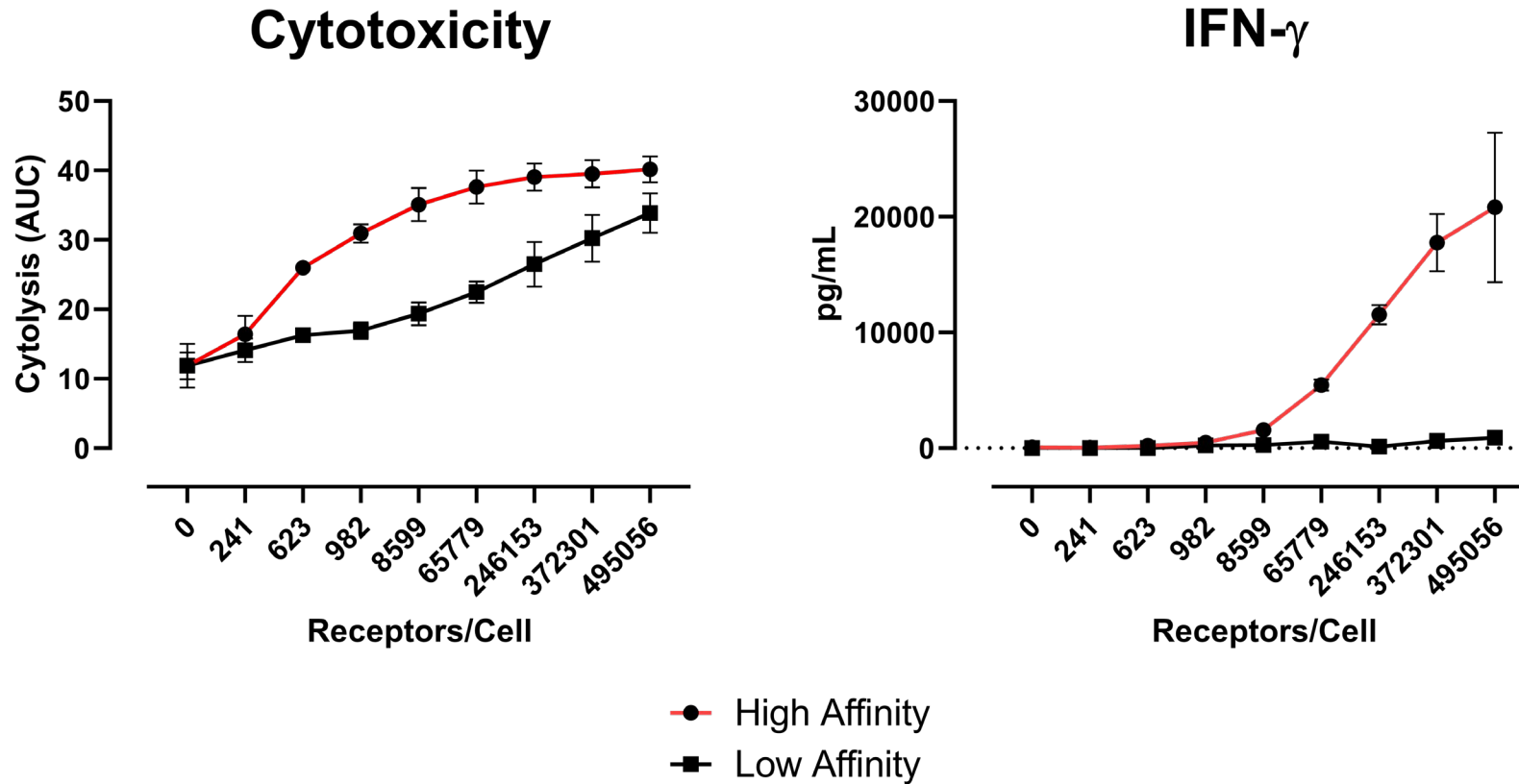


Experimental set-up





High affinity CAR-T have higher efficacy at lower levels of HER2 expression



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Case Study 2 – Using the system to assess TCE receptor activation thresholds

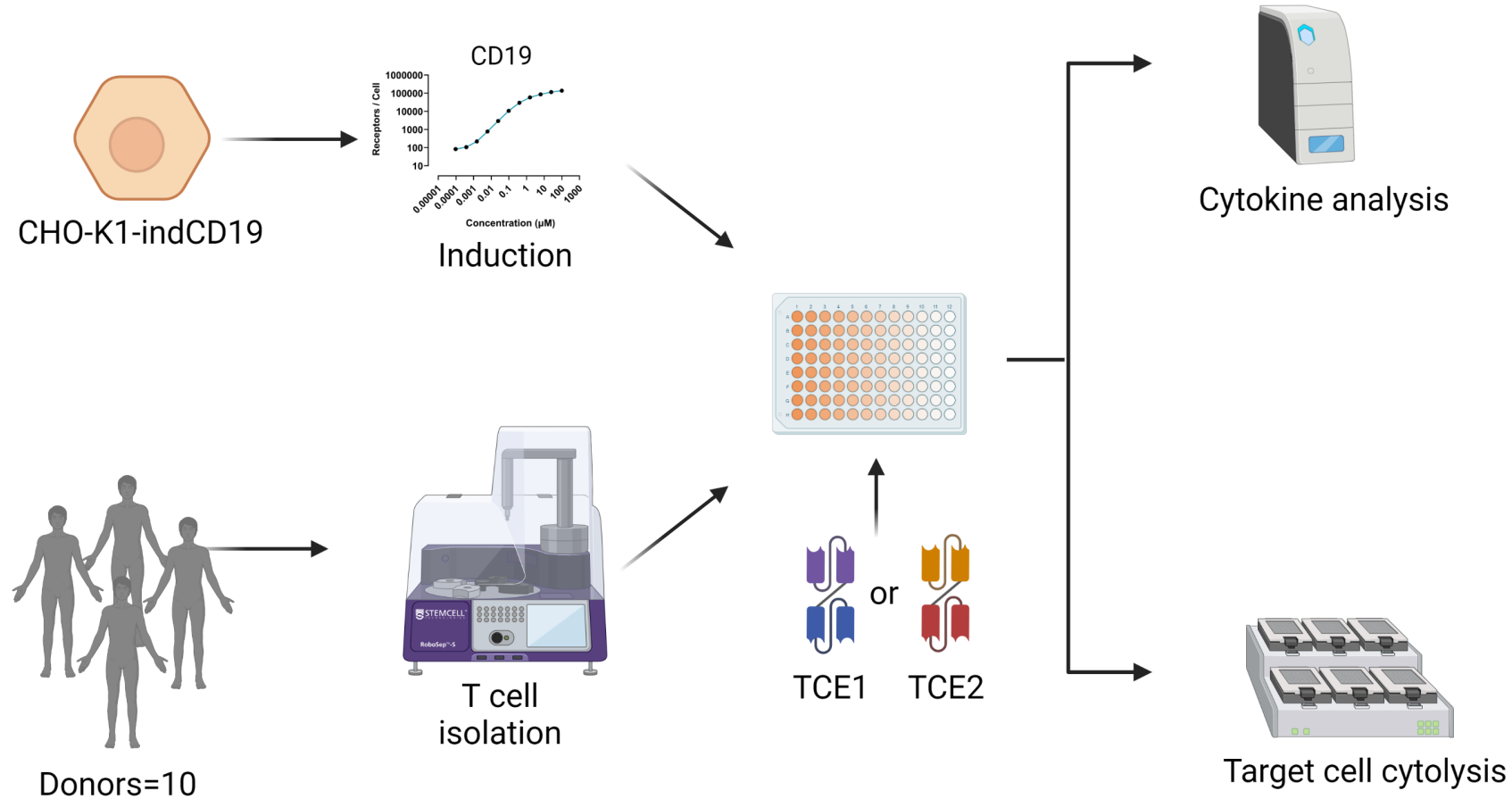
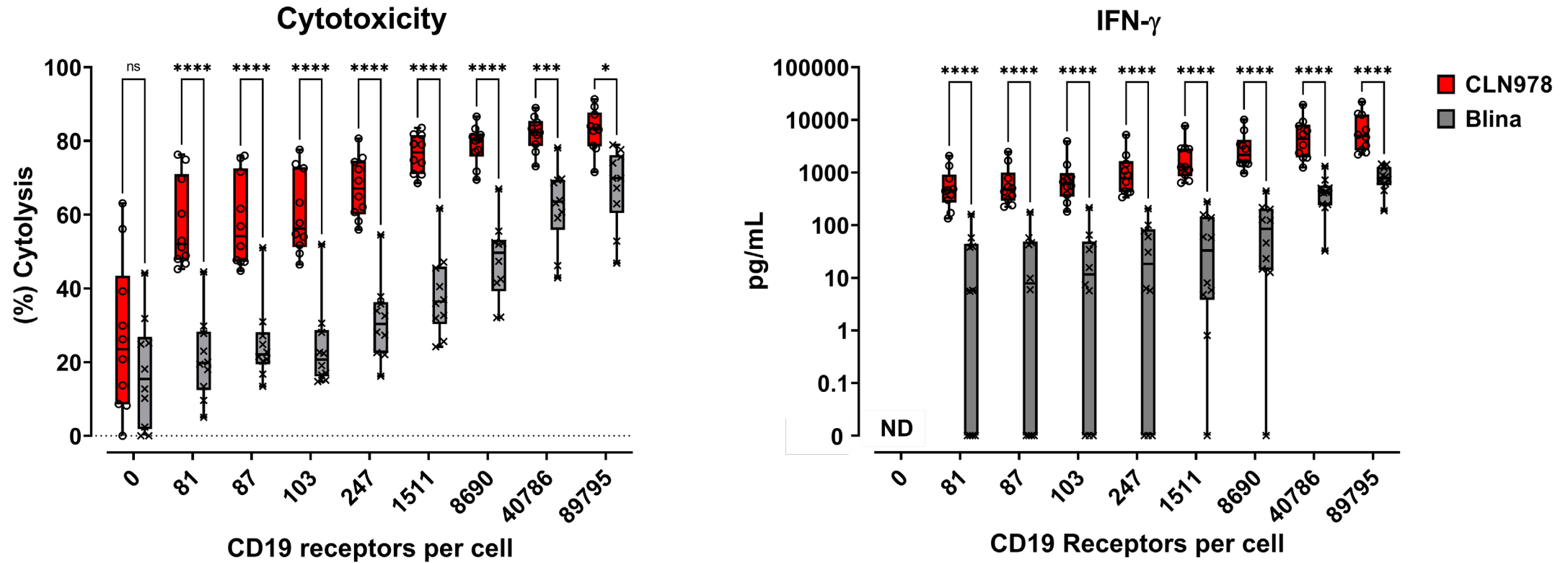


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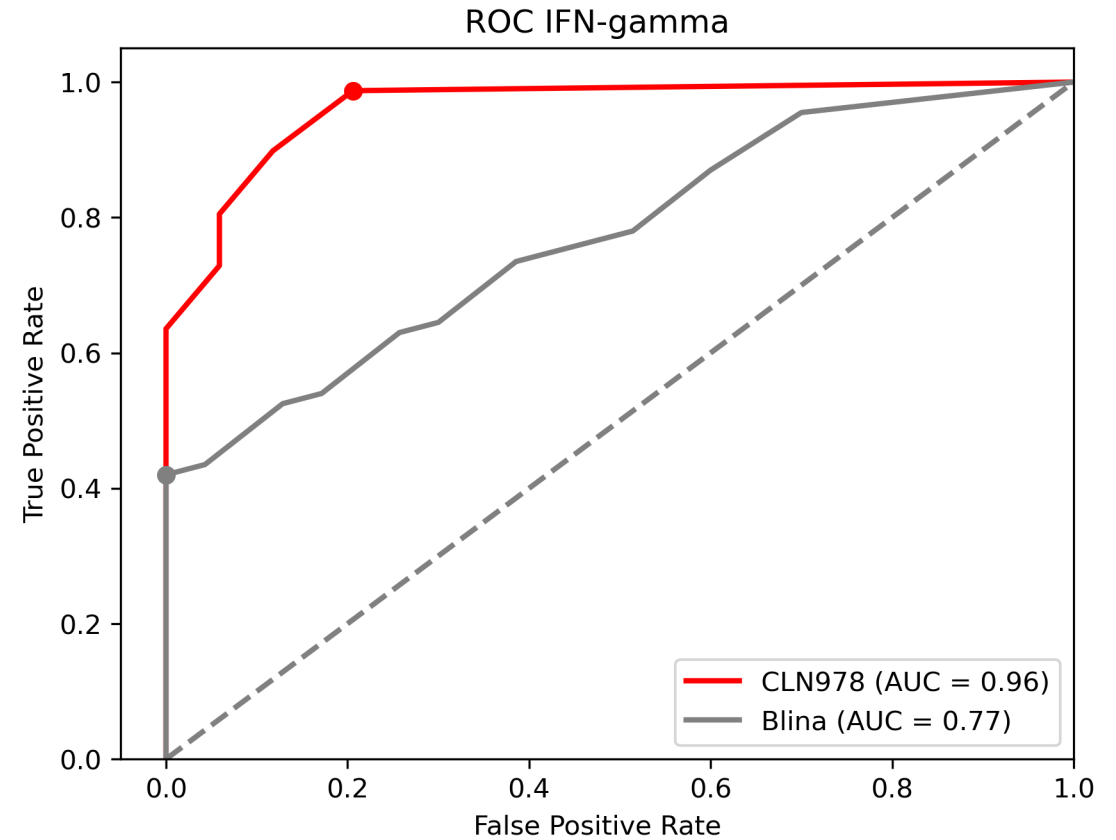
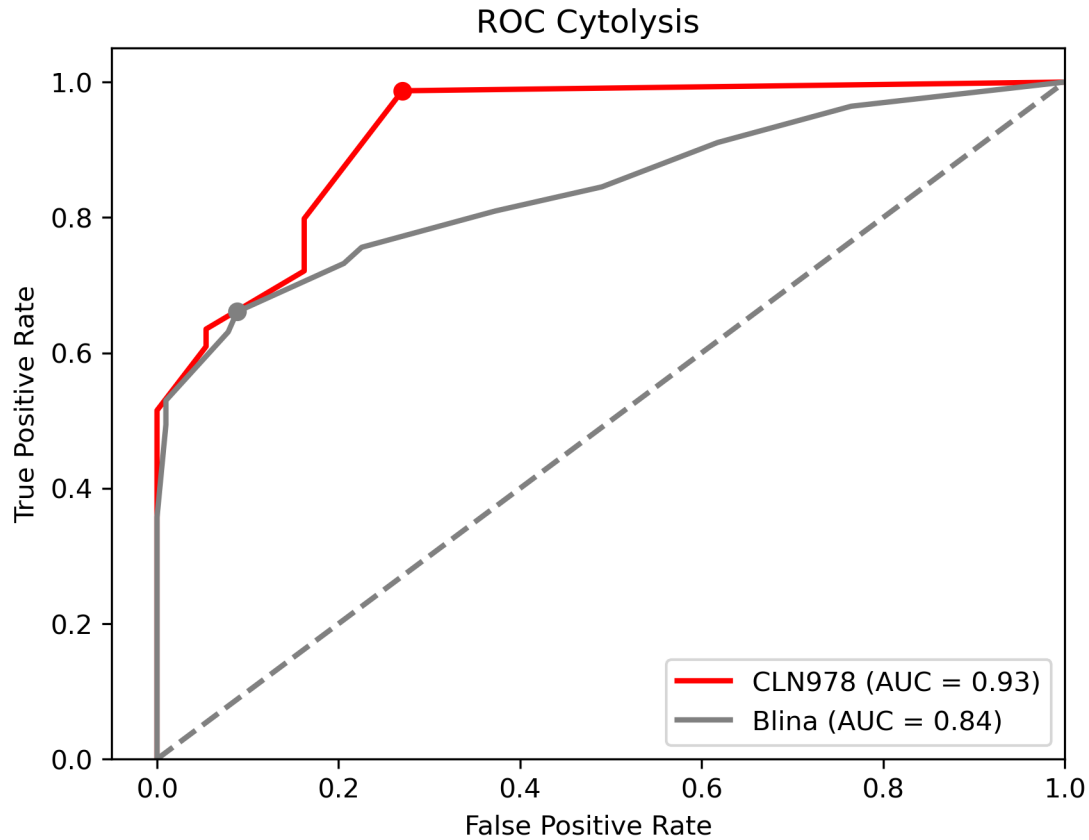


CLN-978 demonstrates efficacy at lower levels of CD19 relative to blinatumomab



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Estimation of Activation Threshold





Dual IndEx-2

CD19 + CD22 dual inducible
expression

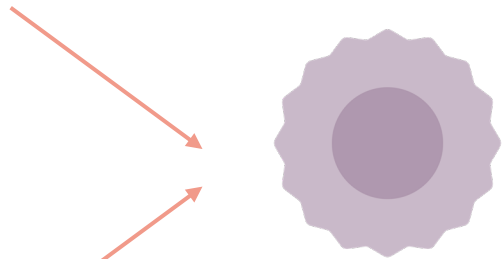


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Dual expression IndEx-2 – CD22 & CD19

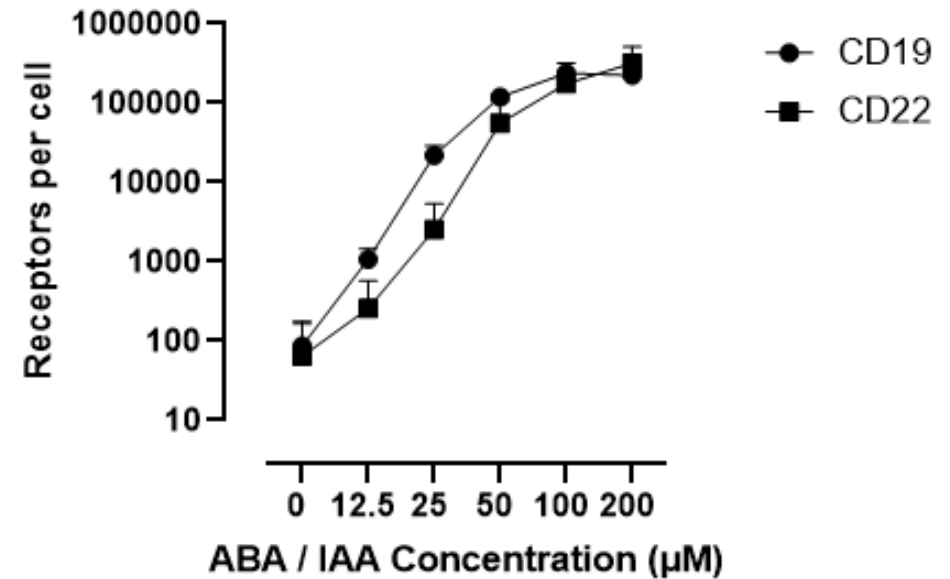
Abscisic acid
(ABA) → **CD19**
induction

Indole-3-acetic acid
(IAA) → **CD22**
induction



CHO-K1 IndEx-2
CD22/CD19

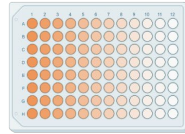
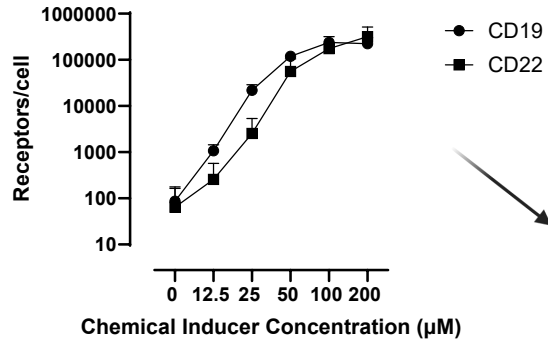
Dual Inducible Quantification



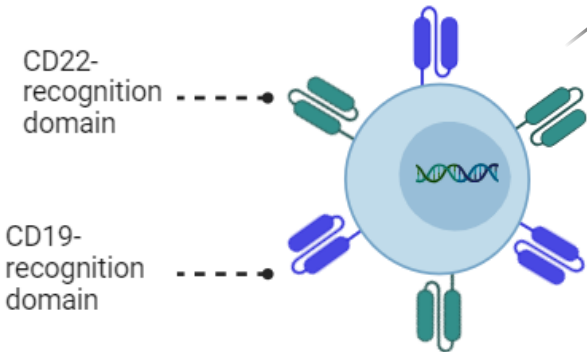
+

Dual expression 'OR' CAR-T – CD22 & CD19

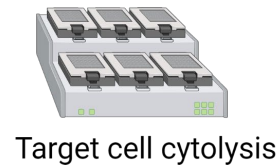
CHO-K1 IndEx-2
CD22/CD19



CD19 / CD22
Dual "OR" CAR T cell



Cytokine analysis



Target cell cytotoxicity

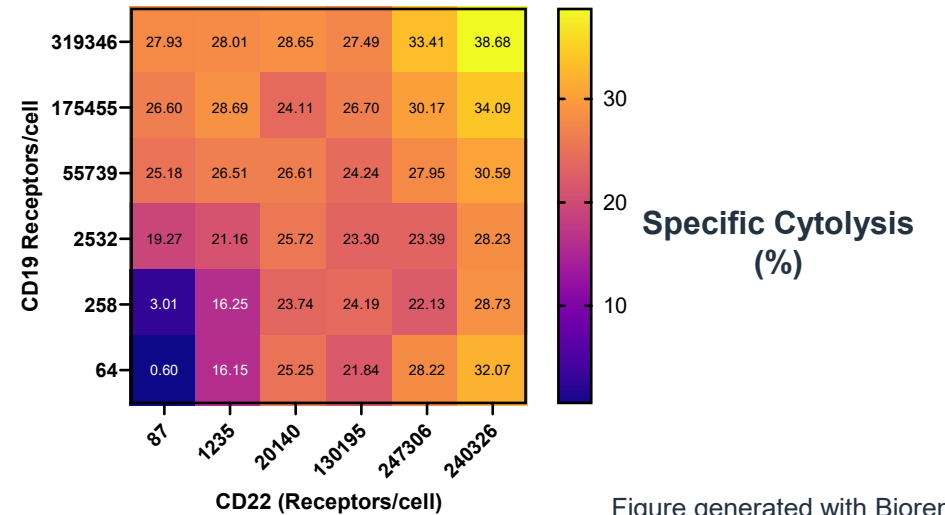
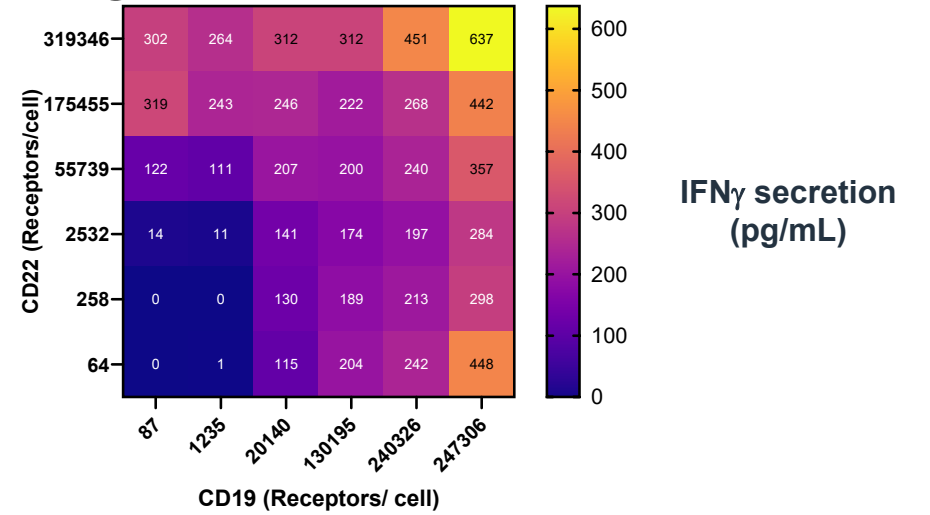
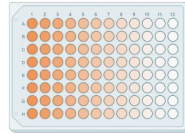
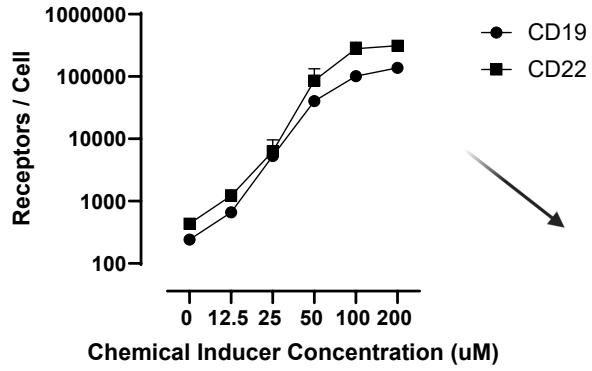


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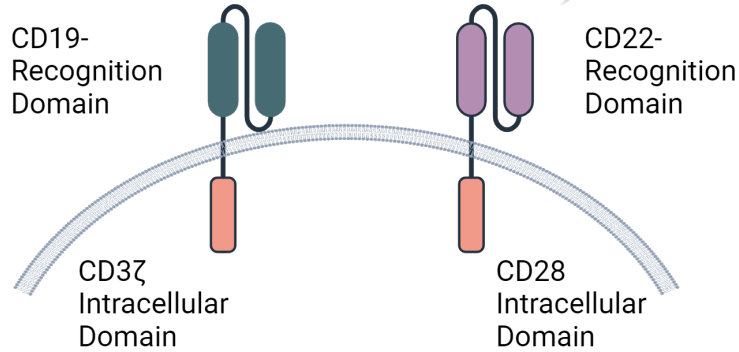


Dual expression 'AND' CAR-T – CD22 & CD19

CHO-K1 IndEx-2
CD22/CD19



CD19 / CD22 Dual
"AND" CAR T cell



Cytokine analysis

Target cell cytotoxicity

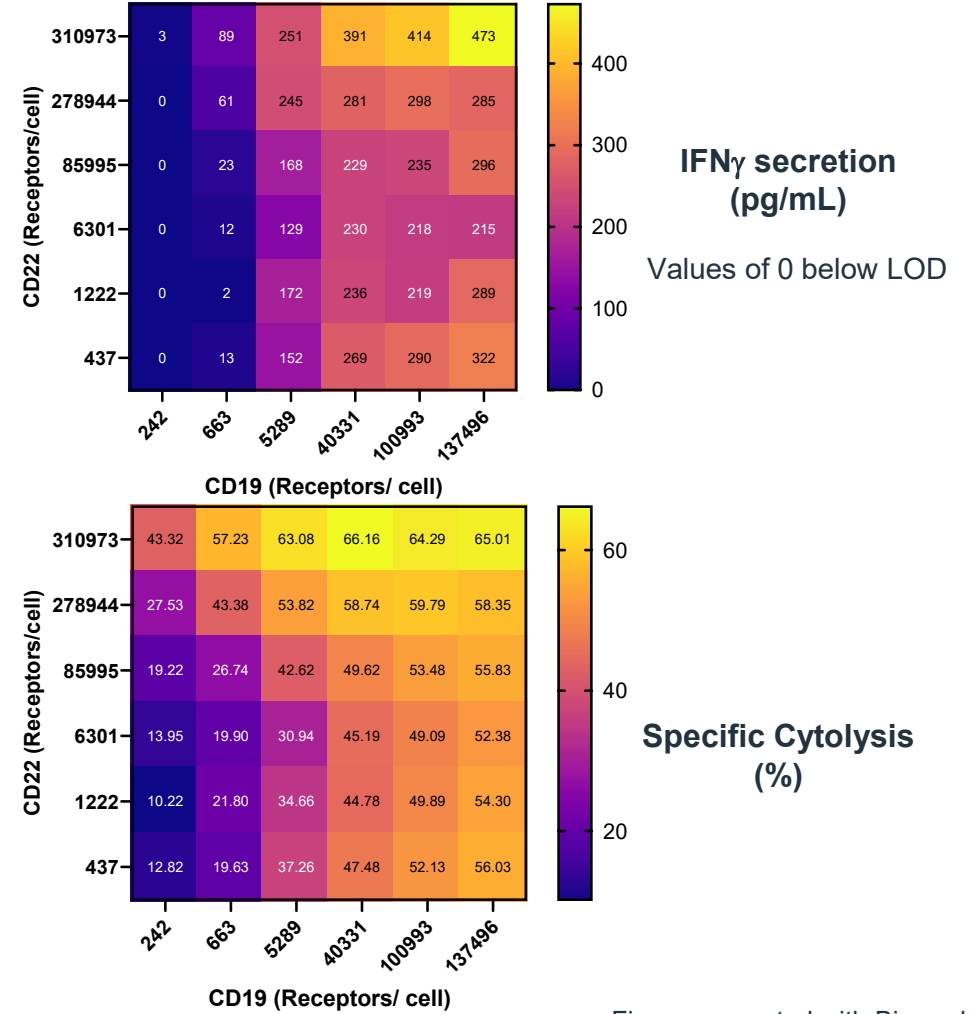


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Summary

The IndEx-2 System is:

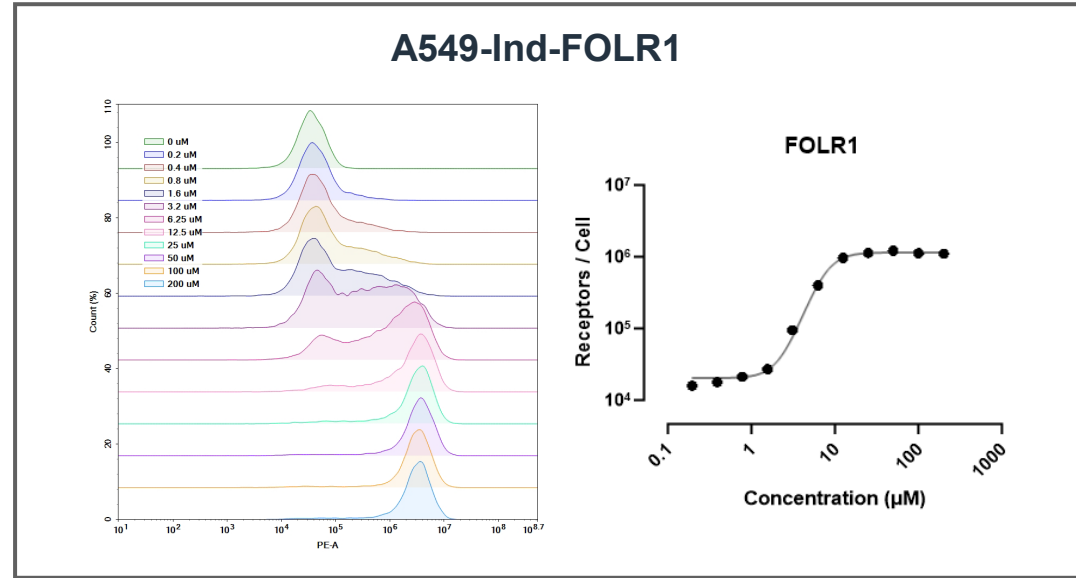
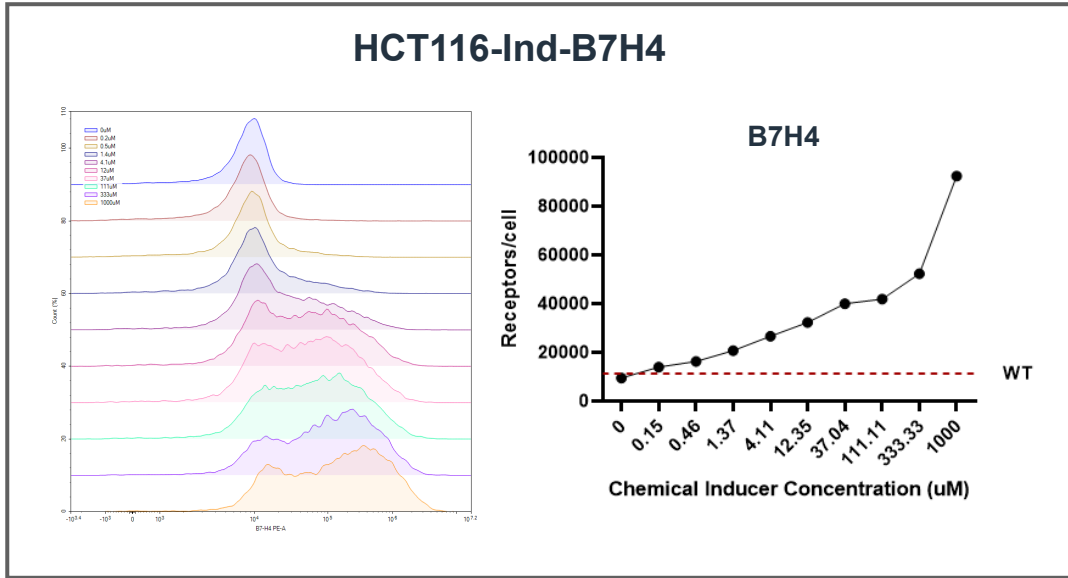
- A cell line system that allows fine titratable expression of antigens
- An assay system that allows the estimation of antigen thresholds for initiation of effector functions
- Applicable for multiple types of therapeutics;
 - mAbs, TCEs, ADCs and CAR-T





Appendices

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IndEx-2 in cancer cell backgrounds



⊕ Pre-developed IndEx-2 cell lines

Cell Line	Target	Inducer	Derivation
IndEx-2; CHO-K1-IndCD19	CD19	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndCD22	CD22	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndBCMA	BCMA	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndPD-L1	PD-L1	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndFOLR1	FOLR1	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndEpCAM	EpCAM	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndCEACAM5	CEACAM5	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndB7H3	B7-H3	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; CHO-K1-IndPSMA	PSMA	ABA	CHO-K1, ovary, epithelial-like
IndEx-2; A549-IndFOLR1	FOLR1	ABA	A549, epithelial carcinoma; lung
IndEx-2; HCT116-IndB7H4	B7-H4	ABA	HCT116, epithelial carcinoma; colorectal



Standard assay list / capability overview

Antibody characterisation	Immune cell biology	Tool generation	Safety assays
Antigen binding (SPR / Flow Cytometry / ELISA)	TDCC; T cell directed cytotoxicity (Flow Cytometry / luminometer / xCELLigence / ELISA / Luminex)	Target cell line development; Protein or receptor over-expression or attenuation	Cytokine release assay (Luminex/ELISA)
Fc Receptor and C1q binding (SPR / Flow Cytometry /ELISA)	Phenotyping and lineage characterisation (Flow cytometry / ELISA / Luminex)	Reporter cell line development; conditional promoter-linked luciferase expression	On-target, off-tumour assessment (flow cytometry / xCELLigence /ELISA / Luminex)
ADCC (Cell-based; Flow Cytometry / Luminometer)	Mixed lymphocyte reactions; one-way and two-way (Flow Cytometry / ELISA / Luminex)	Inducible cell line development; finely titratable protein/receptor expression	
CDC (Flow Cytometry / Luminometer)	Antigen recall assays (Flow Cytometry / ELISA / Luminex)	Protein expression & purification; e.g. Fc receptors	
ADCP (Flow Cytometry)	T cell suppression assays (Flow Cytometry / ELISA / Luminex)	Primary cell engineering; e.g. chimeric antigen receptors, reporters	
Epitope binning (SPR)	T cell activation (Flow Cytometry /ELISA / Luminex)	Cell banking; immune cell banks, immortalised cell banks	
Reporter cell assays (Luminometer)	T cell exhaustion model (Flow Cytometry / ELISA / Luminex)	Potency assay method development; GMP-ready lot release assays (Cell or ELISA-based)	
Trogoctosis (Flow Cytometry)	Cell activation, phenotype and function; T cells, dendritic cells, macrophages, NK cells, $\gamma\delta$ T cells, PBMCs, B cells, regulatory T cells, monocytes, primary tumour material, TILs (Flow Cytometry /ELISA/Luminex)		

The logo icon consists of two stylized, curved lines in a light orange color. The left line starts at the top left and curves downwards and to the right. The right line starts at the top right and curves downwards and to the left. They meet at a central point, forming a shape reminiscent of a pair of parentheses or a stylized 'R' and 'B' combined.

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