# Quantification of the antigen density activation threshold for targeted immunotherapeutics

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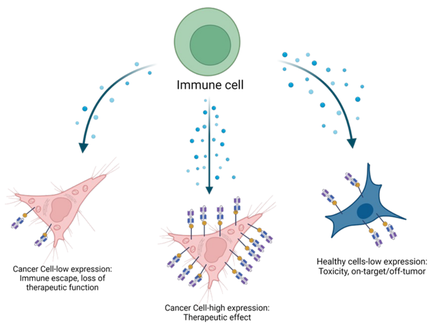
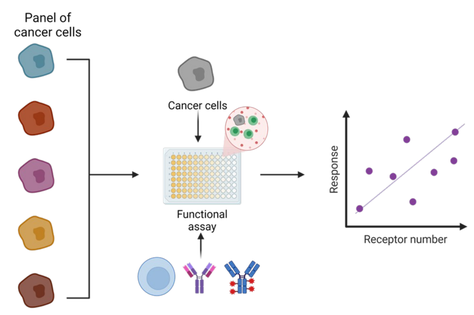
## Introduction

The number of antibody-targeted I-O therapeutics in development is growing Y-o-Y, yet there persists a lack of *in vitro* systems to interrogate the efficacy and safety of these modalities. We have developed IndEx-2, an *in vitro* cell-based platform which allows the expression of one, or two, target antigens over a range of biologically relevant levels, allowing the determination of the precise antigen density activation thresholds of targeted candidate immunotherapies.

## The importance of target antigen density

Bispecific antibody (BsAb)-based oncology therapeutics are promising therapeutic modalities, but their multi-specific nature introduces layers of complexity when trying to demonstrate efficacy and safety. There is a lack of appropriate *in vitro* systems to assess the impact of cell-surface protein levels of the respective targets on efficacy and safety. This is vitally important for T-cell engagers and CAR-T cells, which are hampered by 'on-target/off-tumor' toxicity towards healthy tissues (J. Immunotoxicol. 17, 67–85). This side-effect is often not observed until the clinical trial stage, at which point significant financial investment has been made.

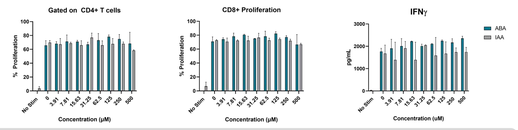



## CIP-based systems for titratable expression

Chemically Induced Proximity (CIP) systems use membrane-permeable, small molecule inducers to control dimerization between proteins of interest that are fused to the inducer-binding proteins. These systems can be adapted to switch on transcription of a target antigen of interest (TAOI) by means of bringing a DNA binding domain into proximity of a transactivator and placing the binding motif for the DNA binding domain upstream of the antigen of interest.

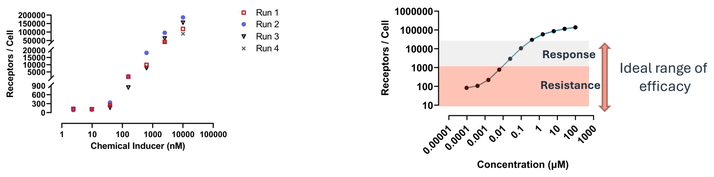

### The small molecules do not have immunomodulatory activity




### Titratable expression of therapeutically relevant targets




### Target antigen expression is reproducible and in a biologically relevant range



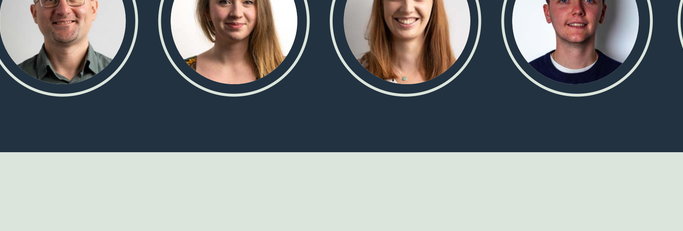




## Titratable antigen expression in assays

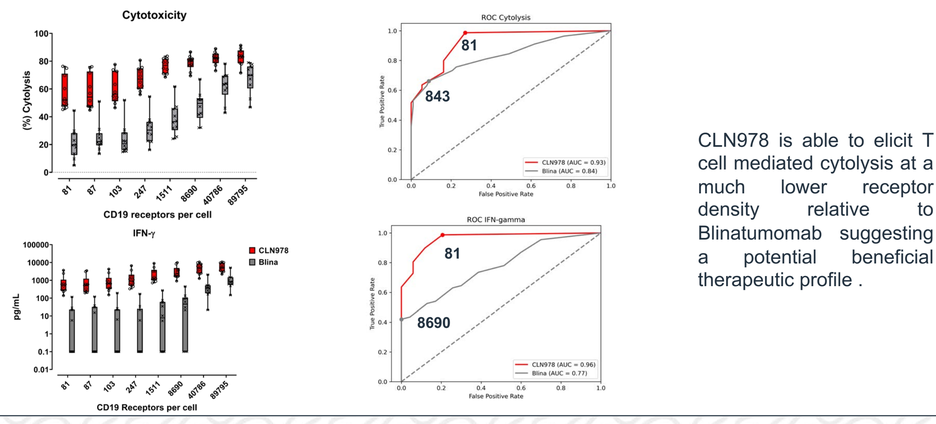
The inducible expression system can be used to assess the impact of target antigen density on the efficacy and safety of molecules such as T-cell engagers (TCEs), antibody drug conjugates (ADCs) and Chimeric Antigen Receptor (CAR)-T cell therapies.

### ADCs – comparing activity




A difference in the cytotoxicity of the anti-Her2 ADCs is apparent with Trastuzumab Emtansin achieving 50% killing at the highest level of expression of Her2.

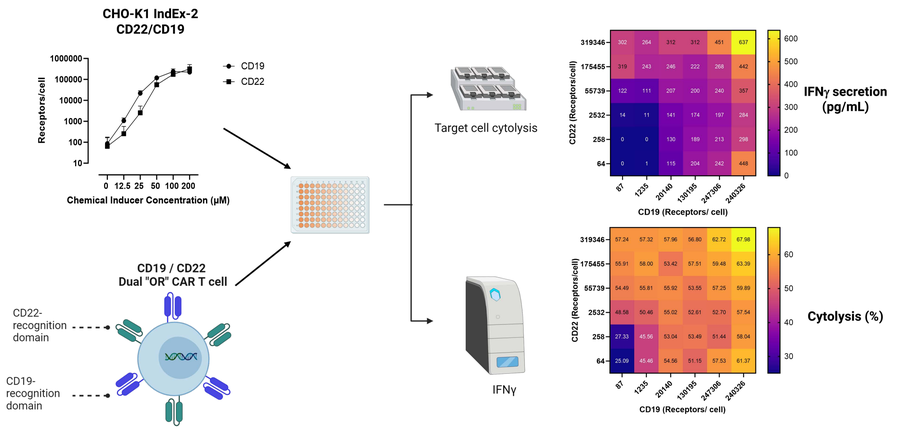
### Bispecific T cell engagers – assessment of minimal receptor number for activity




CLN978 is able to elicit T cell mediated cytolysis at a much lower receptor density relative to Blinatumomab suggesting a potential beneficial therapeutic profile .

## Controlling two target antigens

A dual targeting CAR specific for both CD19 and CD22 was employed. This CAR was designed to combat antigen escape and thus requires engagement of either CD19 or CD22. The dual inducible cell line was employed as the target cell in a cytolysis assay, with CD19 and CD22 expression under the control of two independent CIP systems. The CD19/CD22 'OR' CAR-T cells were employed as effector cells.




All images were generated using Biorender.

## Summary

We have demonstrated the applicability of the inducible cell line system in multiple assay formats, covering ADC, bispecific T cell engager and CAR-T modalities. The system allows for the elucidation of target antigen density required for eliciting a biologic effect by the therapeutic candidate. This data is important for the pre-clinical evaluation of the impact of target antigen density on the efficacy and safety of therapeutics.

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