Quantification of the antigen density activation threshold for targeted immunotherapeutics

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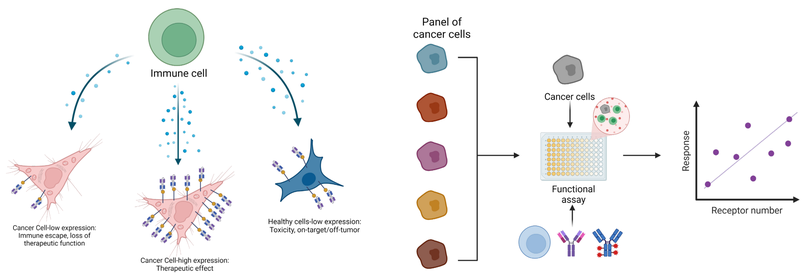
INTRODUCTION

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.

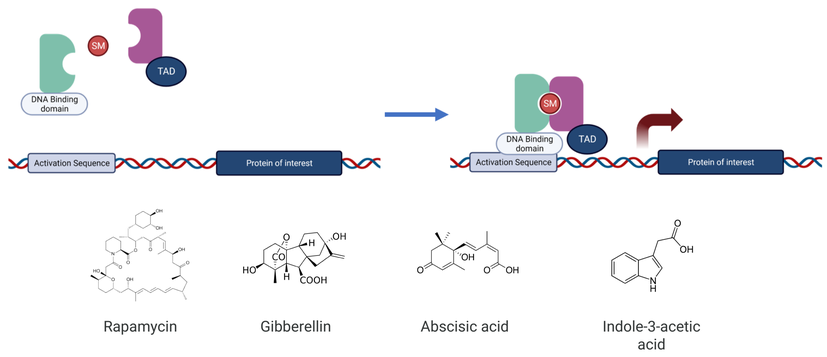
THE IMPORTANCE OF TARGET ANTIGEN DENSITY

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 the relative levels 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.

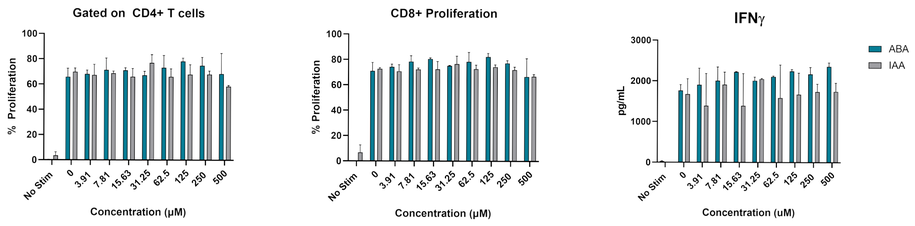


CIP-BASED SYSTEMS FOR TITRATABLE EXPRESSION

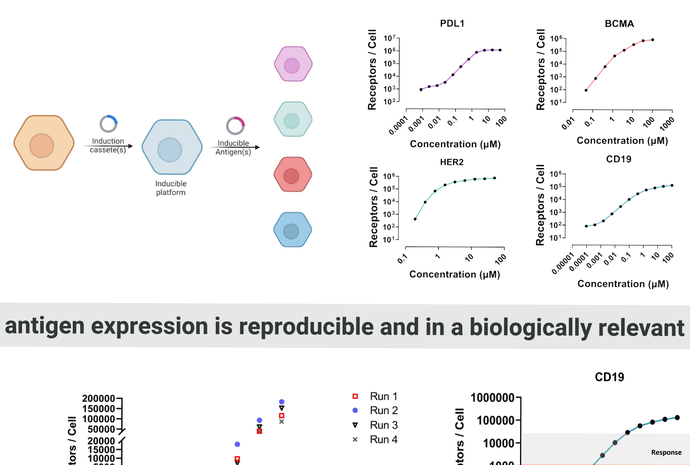
Chemically Induced Proximity (CIP) systems use membrane-permeable, small molecules inducers to control dimerization between proteins of interest that are fused to the inducer-binding proteins. These systems can be adapted to switch on transcription of a target antigen of interest (TAOI) by means of bringing 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.



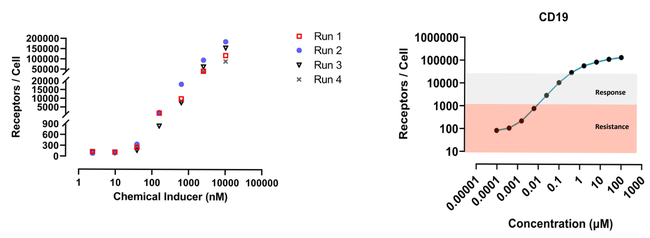
The small molecules do not have immunomodulatory activity



Titratable expression of therapeutically relevant targets



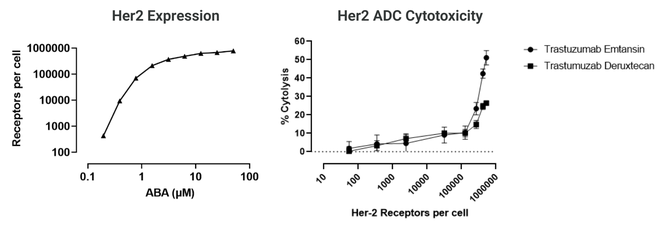
Target antigen expression is reproducible and in a biologically relevant range



TITRATABLE ANTIGEN EXPRESSION IN ASSAYS

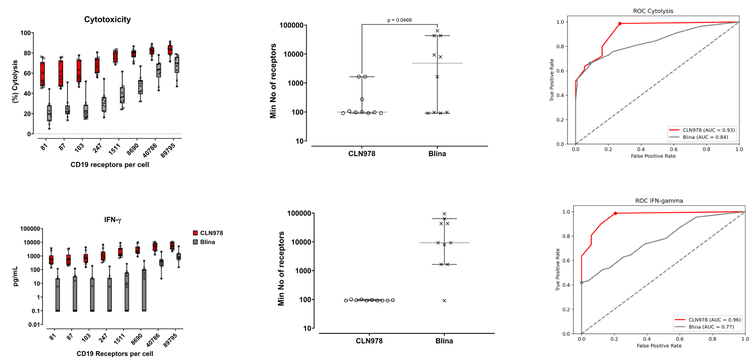
The inducible expression system can be used to assess the impact of target antigen density on the efficacy and safety of molecules such as T-cell engagers (TCEs), antibody drug conjugates (ADCs) and Chimeric Antigen Receptor (CAR)-T cell therapies.

ADCs – comparing activity



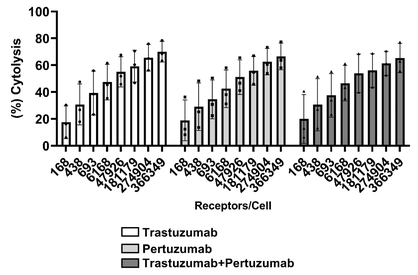
A difference in the cytotoxicity of the anti-Her2 ADCs is apparent with Trastuzumab Emtansin achieving 50% killing at the highest level of expression of Her2.

Bispecific T Cell Engagers – assessment of minimal receptor number for activity



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

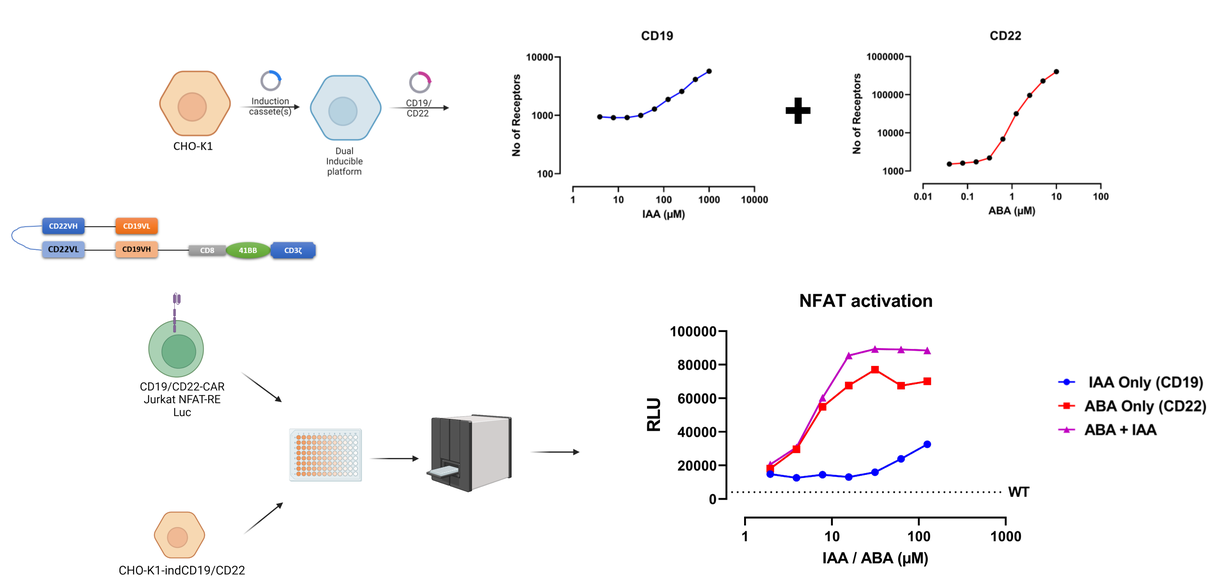
ADCC monoclonal antibodies – is combination therapy better?



There was no observable difference in the ability of the different treatment to induce ADCC in response to the different levels of HER2 receptor expression.

CONTROLLING TWO TARGET ANTIGENS

We employed a dual targeting CAR specific for both CD19 and CD22. This CAR was designed to combat antigen escape in leukaemias and thus requires engagement of either CD19 or CD22. We used the dual inducible IAA CD19 / ABA CD22 CHO-K1 cells as targets in a reporter assay with Jurkat NFAT luciferase reporter cells expressing the CD19/CD22 dual targeting CAR as the effector cells.



Dual engagement of CD19 and CD22 enhances functional capacity of T cells

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 cell therapeutic 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.

SCAN THE CODE

Let's discuss further how we can accelerate your drug development to the clinic.