

## **Impedance-based assay to evaluate potency of immunotherapy products**

**Juan Miguel Sánchez-Nieto**

**Analytical Development Scientist – Industrialisation team**



## About us

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Part of a **world-leading network** of technology and innovation centres



Provide access to unique technical **facilities** and **expertise** to help adopt, develop and exploit innovations



**Bridge the gap** between businesses and academic research



Established by Innovate UK as a **not-for profit**, independent centre

It is our vision for the UK to be a **global leader** in the development, delivery and commercialisation of cell and gene therapies.

Where **businesses can start, grow and confidently develop** advanced therapies, delivering them to patients rapidly and effectively.



## Development centre

- 1,200m<sup>2</sup> purpose built centre
- Analytical characterisation
- Process development
- Viral vector
- Stem cell differentiation
- **10<sup>th</sup> floor integration & collaboration centre**



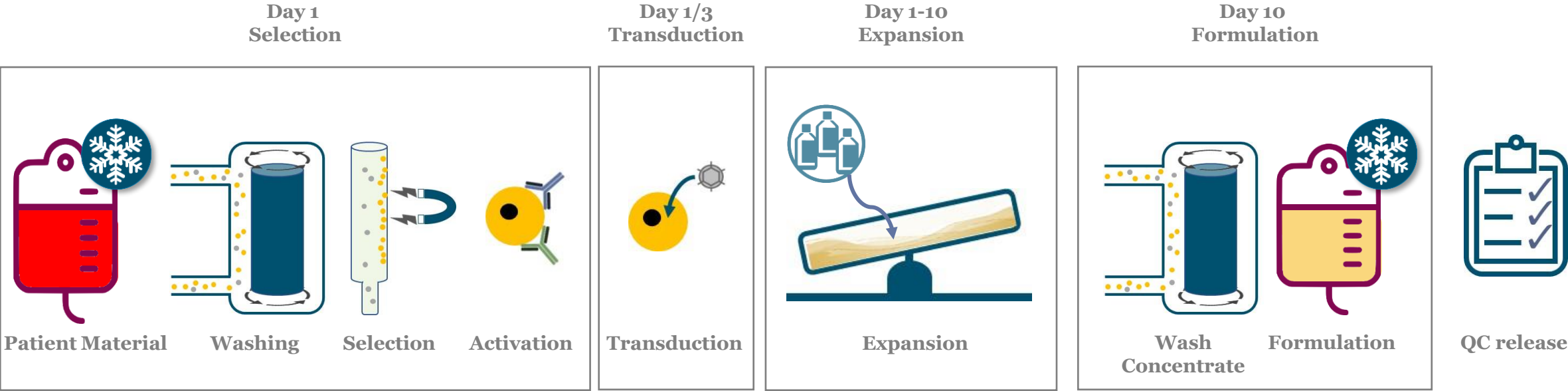
## Manufacturing centre

- 7,700m<sup>2</sup> manufacturing centre designed specifically for cell and gene therapies
- 12 segregated large clean room modules
- Secure supported collaboration model
- Centre of a cell and gene therapy cluster
- **Expanded QC capacity and capability**



# Potency assays for immunotherapies

# Challenge: reduce time between product formulation and patient administration



Identity
<ul style="list-style-type: none"><li>• Transduction efficiency</li><li>• Immunophenotype</li><li>• Appearance</li></ul>

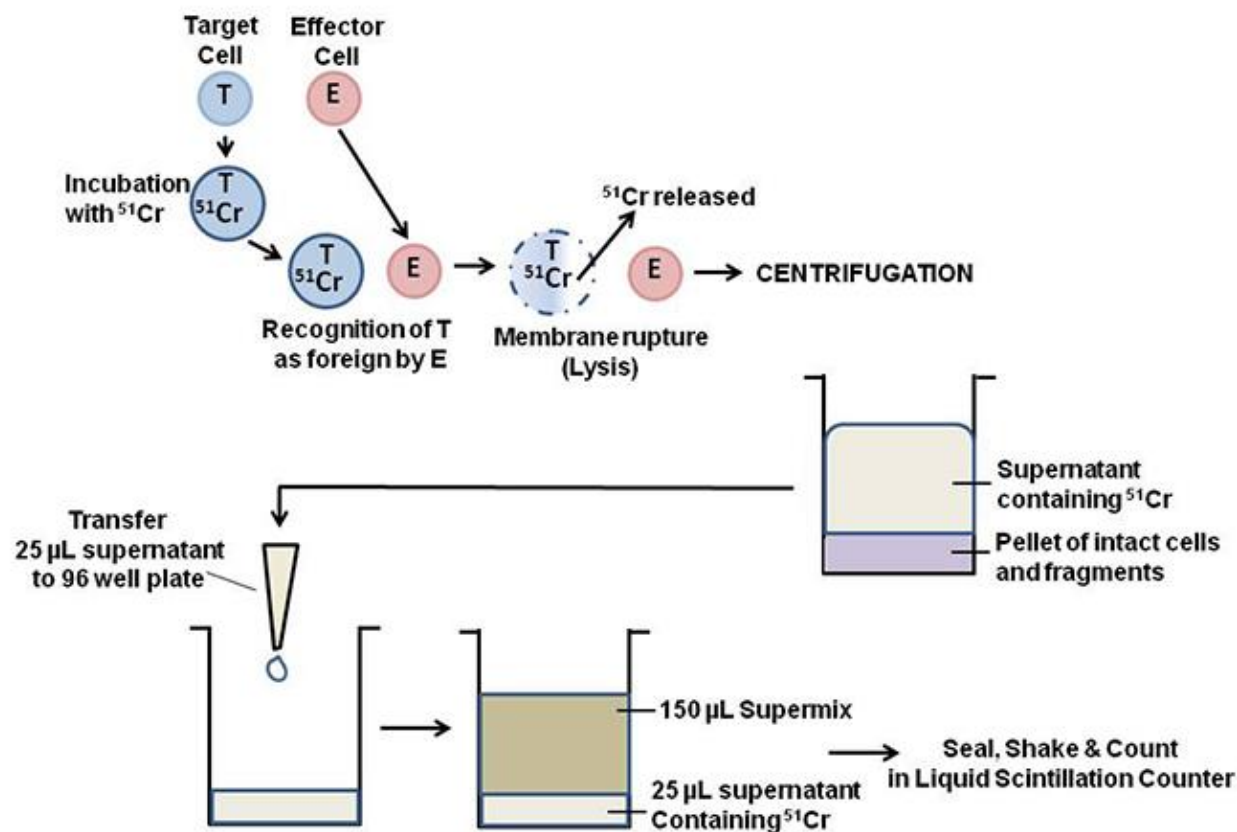
Impurities
<ul style="list-style-type: none"><li>• Percentage non-CD3<sup>+</sup> cells</li><li>• Large T antigen protein/DNA</li></ul>

Safety
<ul style="list-style-type: none"><li>• Genome viral copy number</li><li>• Sterility (EP 2.6.1)</li><li>• Mycoplasma (EP 2.6.7)</li><li>• Endotoxin (EP 2.6.14)</li><li>• Replication competent viruses</li></ul>

Potency
<ul style="list-style-type: none"><li>• Viable cell count</li><li>• CAR/TCR expression</li><li>• Cell killing activity</li><li>• Cytokine stimulation</li></ul>

- **Chromium release**

- Gold standard
- Limitations:
  - Time – leakage
  - Safety – use of radioactive material
  - Cell requirements – high effector to target ratios | physiological relevance



Assay	Measure	Readout
CytoTox 96 <sup>®</sup>	LDH	Absorbance
CellTiter-Glo <sup>®</sup>	ATP	Luminescence
Calcein-AM	Dye release	Fluorescence
DELFI <sup>®</sup> A EuTDA	BATDA release	Fluorescence
Flow cytometry	Cytokine/cell death	Fluorescence
Luminex <sup>®</sup>	Cytokine	Fluorescence

## Solution: impedance – based potency assay

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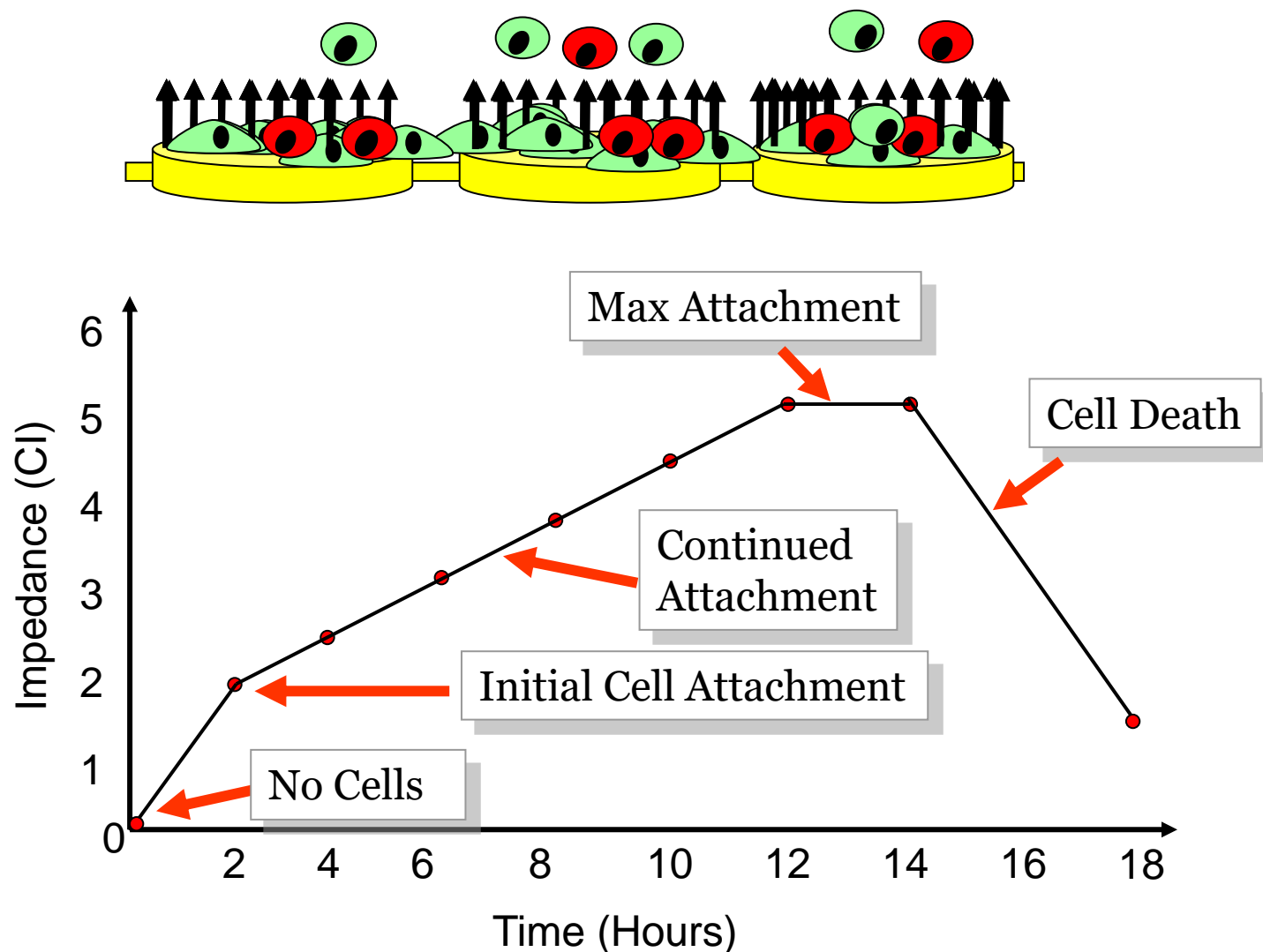
- Real Time Cell Analysis system:
  - Non-invasive system – electrical impedance
  - Label free
  - High throughput – 6x 96-well plates
  - Flexible
  - Limitation:
    - Optimisation required for each target cell line





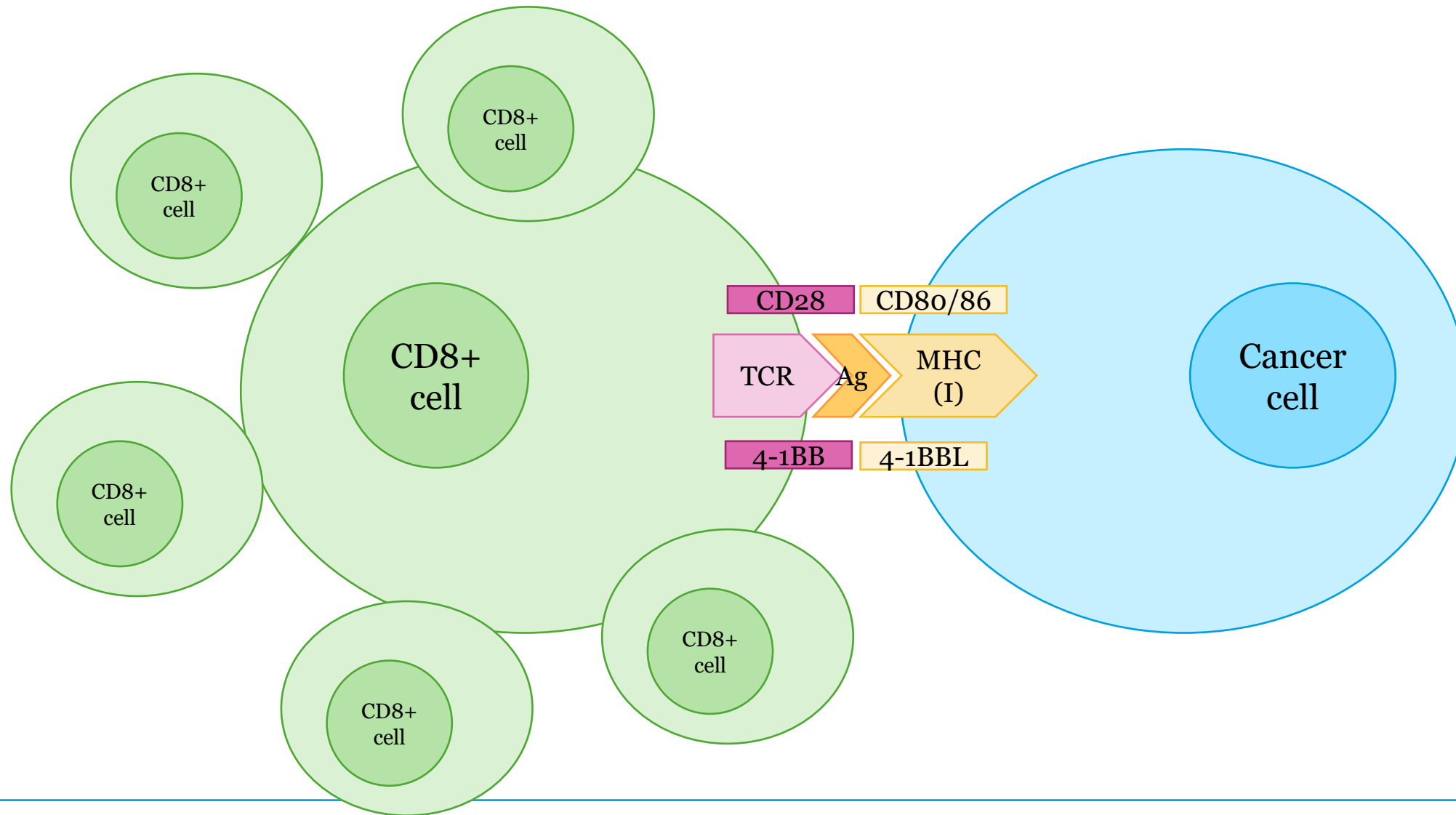
- Strategies followed for different types of immunotherapies:
  - T-cell receptor based therapies (TCR)
    - Non-adherent target cells
    - Adherent target cells
  - Chimeric Antigen Receptor (CAR)
    - Adherent target cells

# How does the impedance-based potency assay work?



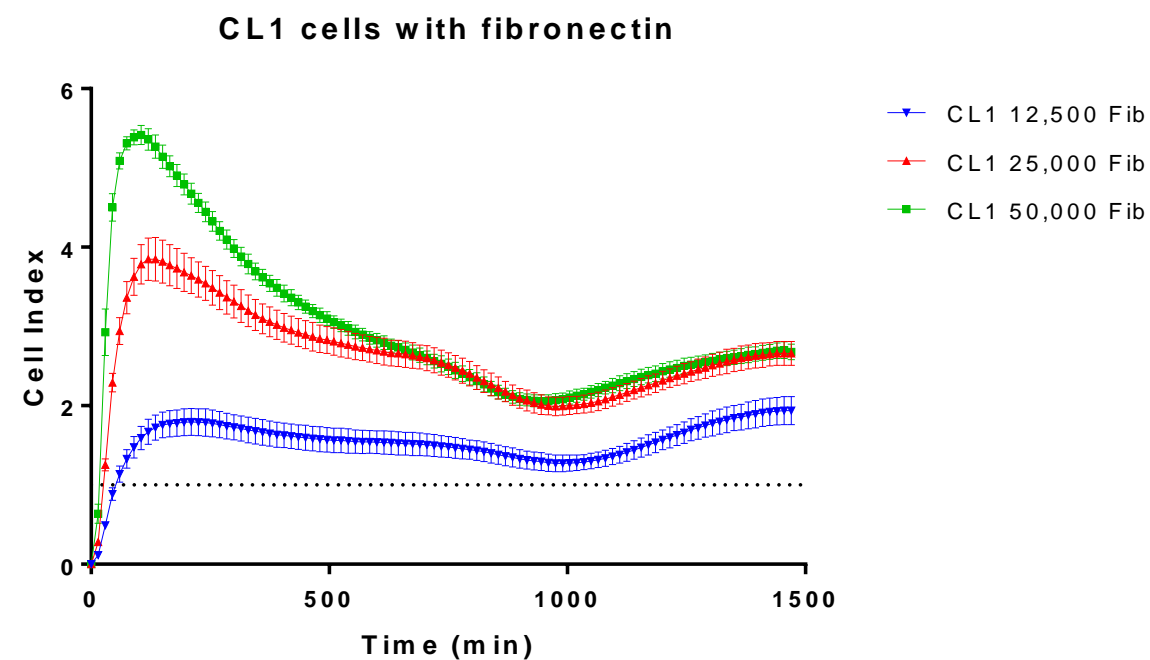
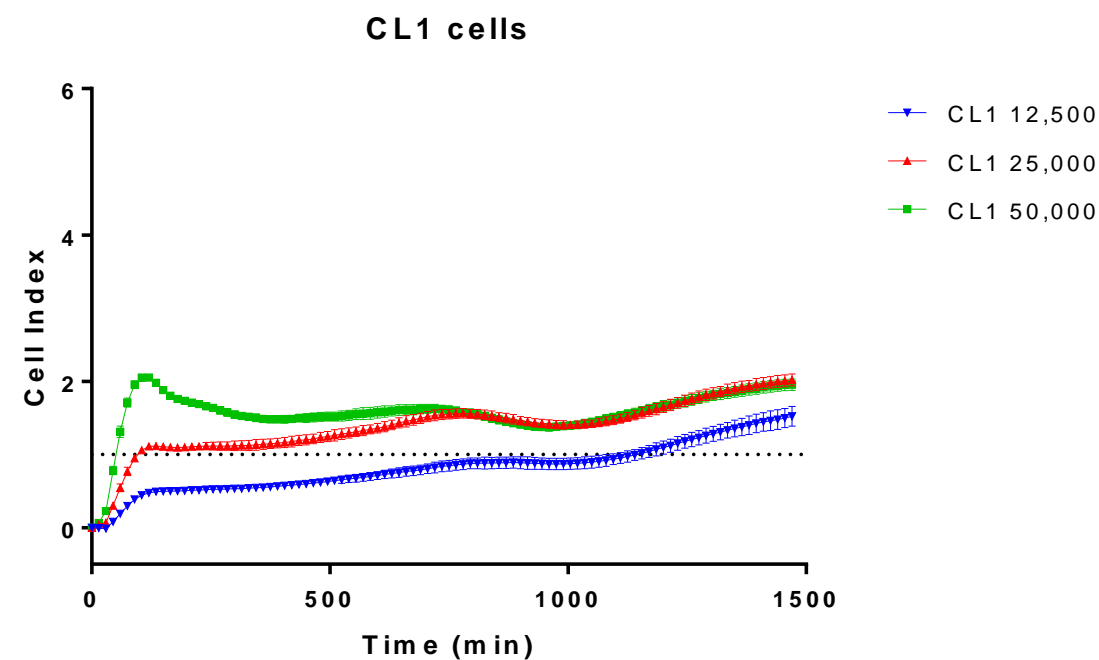
# TCR based products

# TCR mediated killing

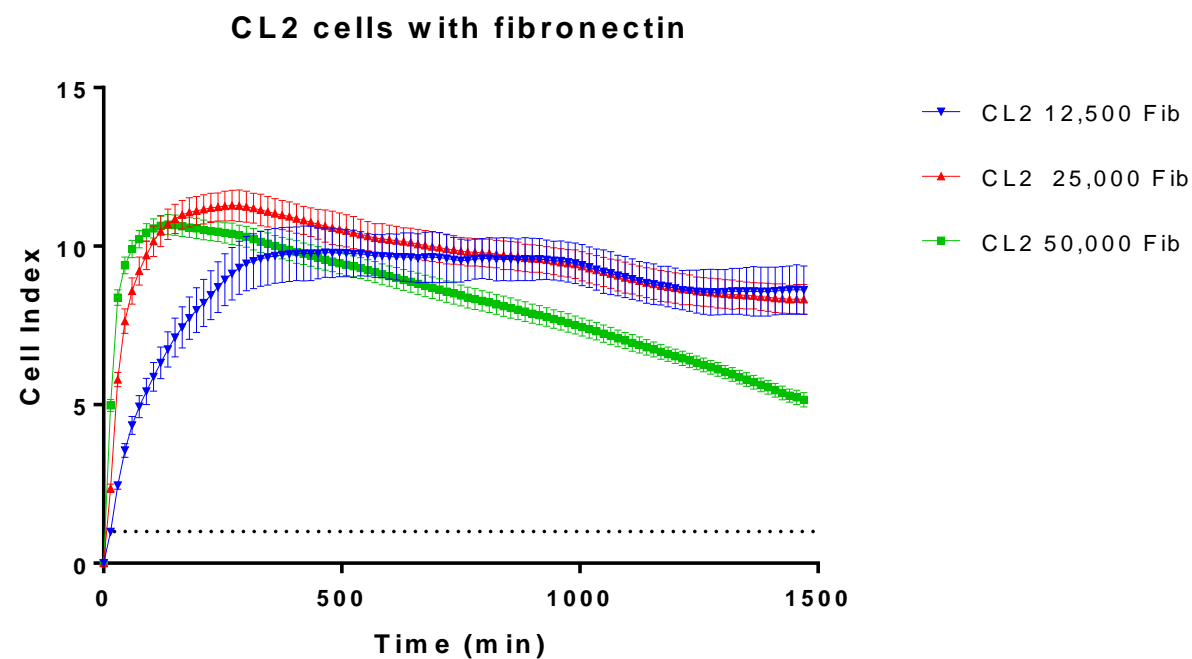
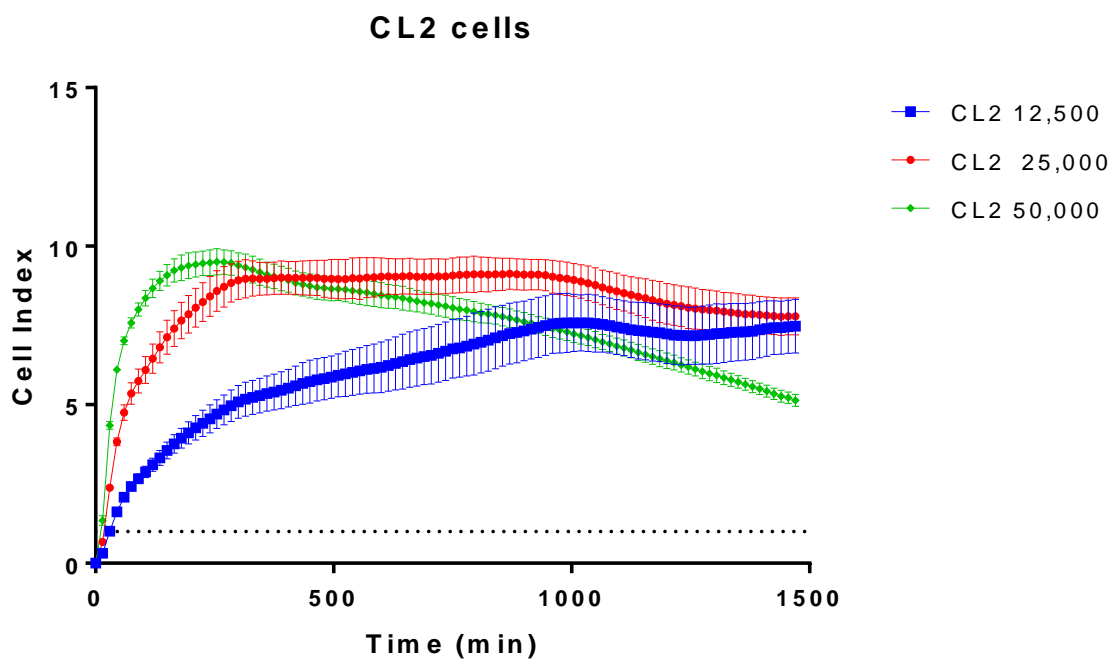




- Selection of a suitable target cell line
  - Cell lines to test:
    - CL1 and CL3 – melanoma cancer cell lines
    - CL2 – ovarian cancer cell line
    - Control cell line
  - Evaluation of optimal seeding density
  - Expression levels of HLA-A2
- Assay qualification
  - Instrument's linearity
  - Correlation between cytolytic potential and cell index
    - Killing time 50
    - CI at a specific timepoint
  - Intermediate precision/repeatability

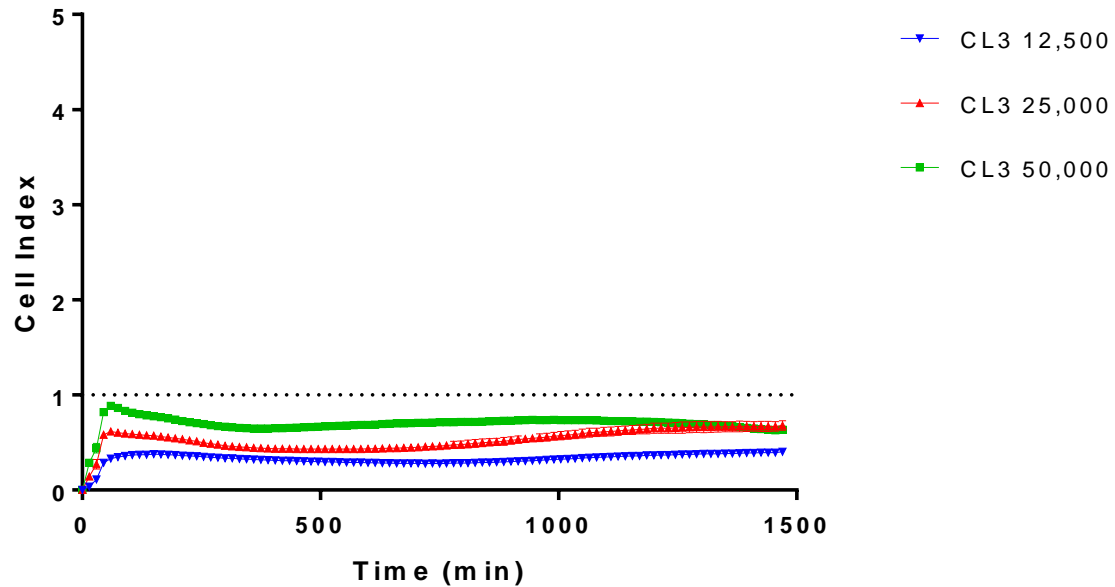


# Evaluation of optimal seeding density – effect of extracellular matrix

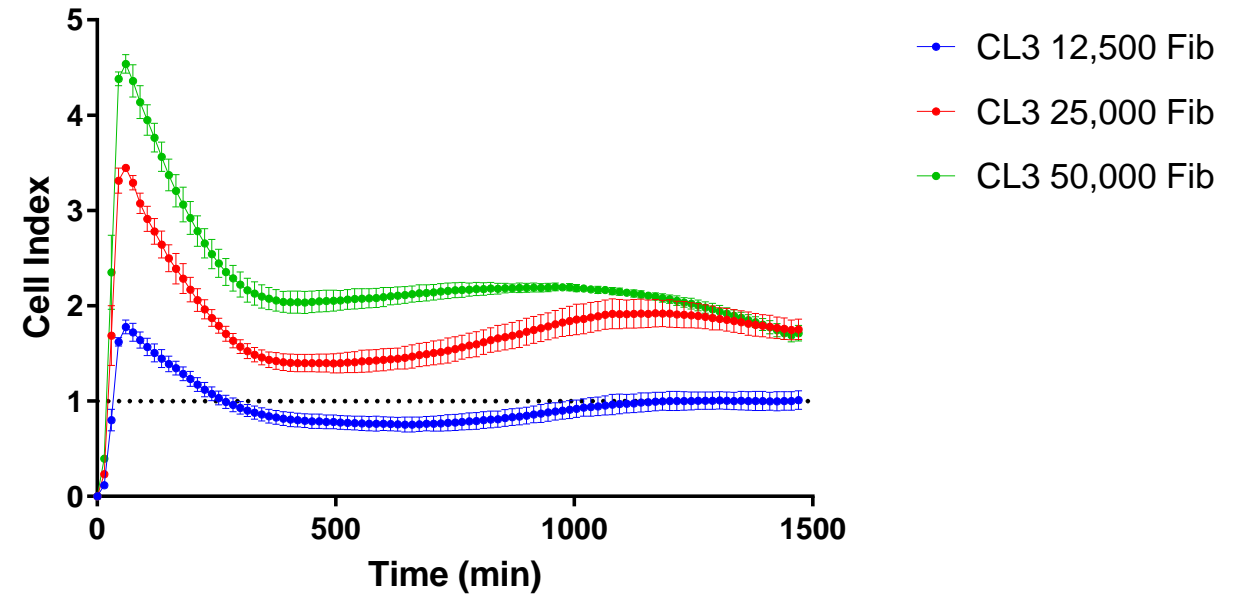


# Evaluation of optimal seeding density – effect of extracellular matrix

CL3 cells



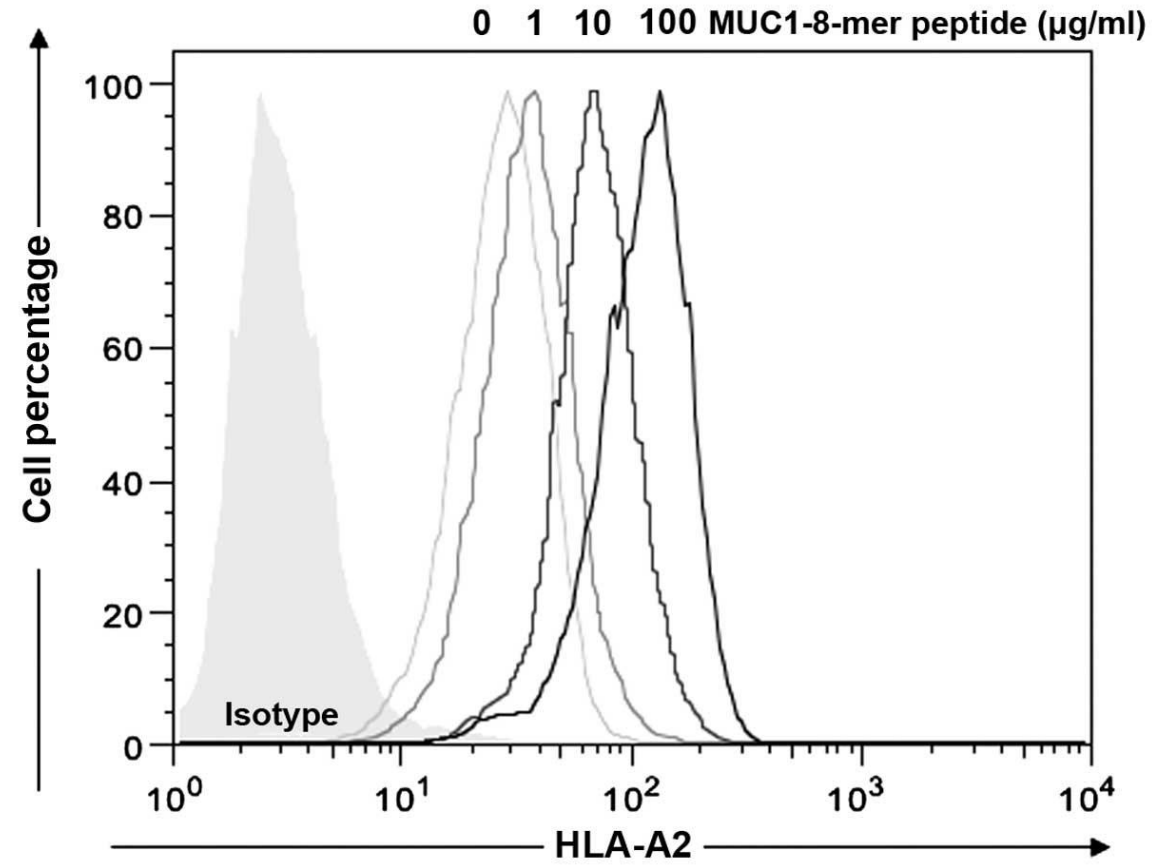
CL3 cells with fibronectin





# Selection of the cell line to perform the killing assay

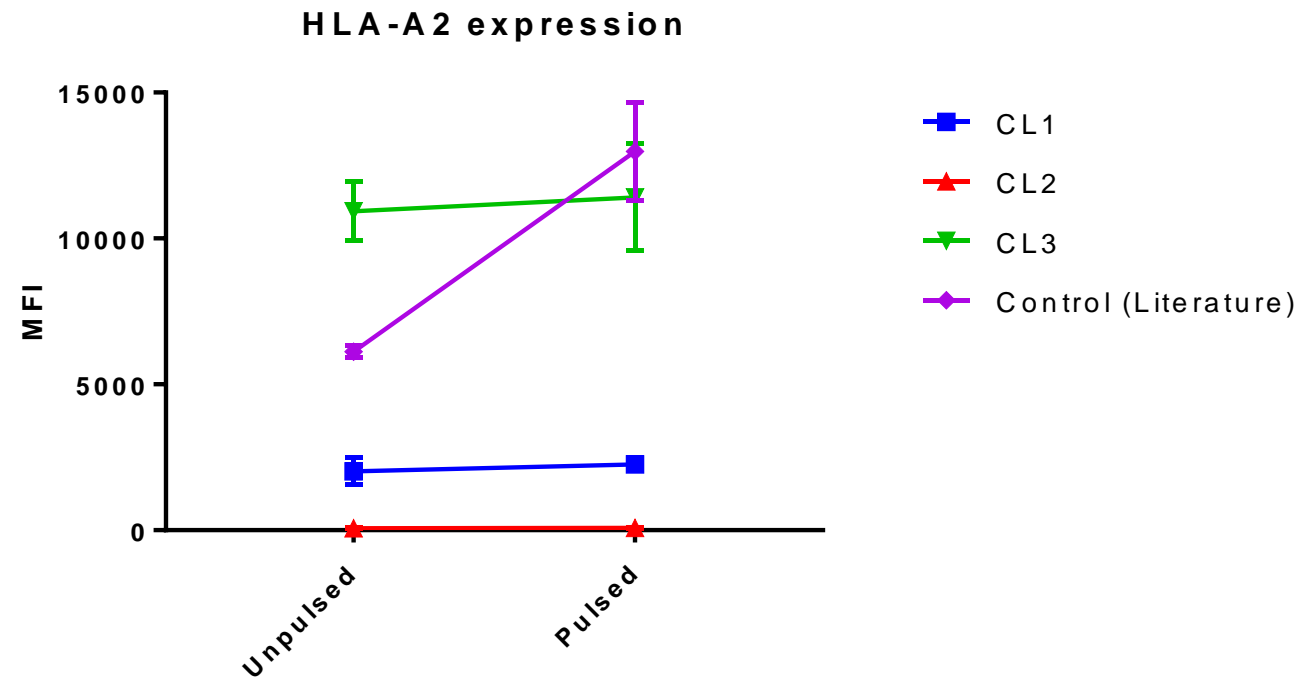
- Expression levels of MHC-1



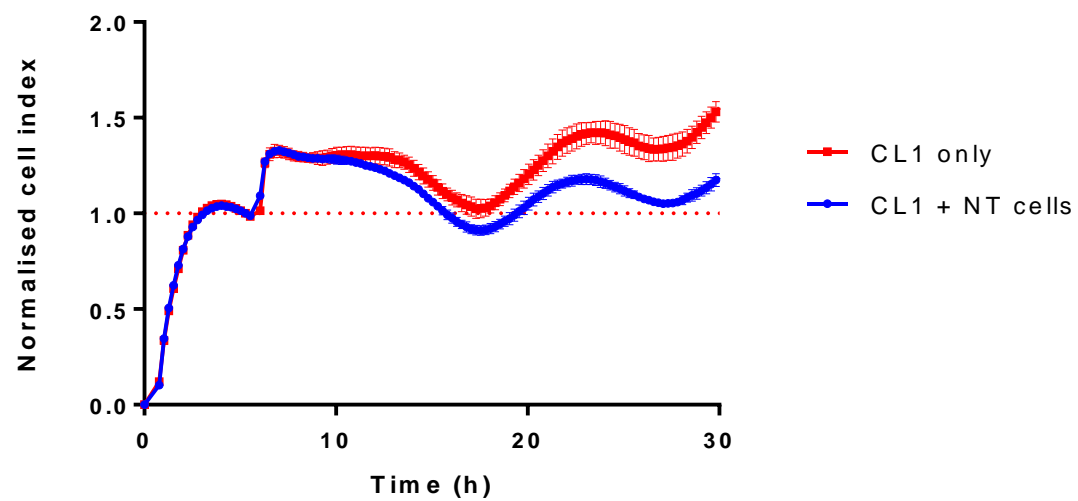
Atzin-Méndez, J. A. et al. 2015

# Selection of the cell line to perform the killing assay

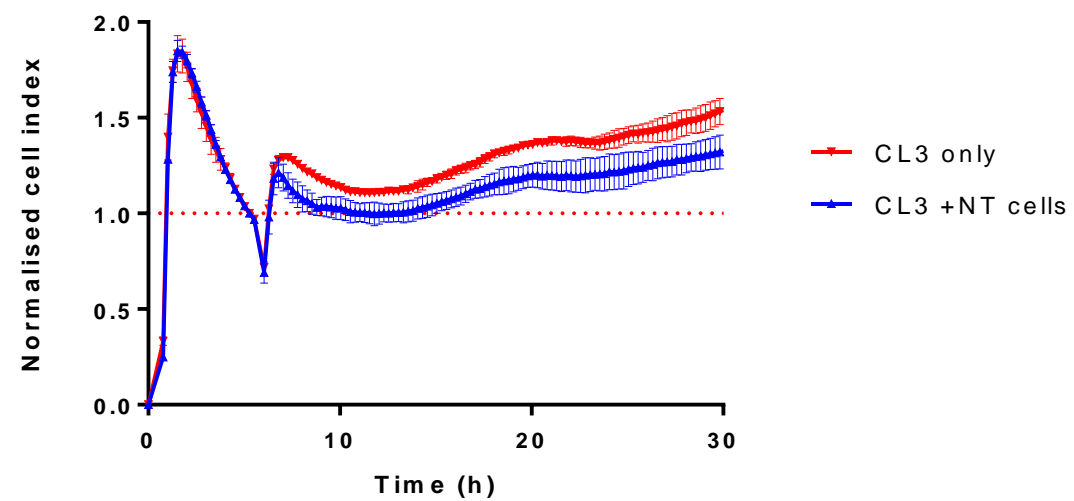
- Expression levels of MHC-I



## CL1



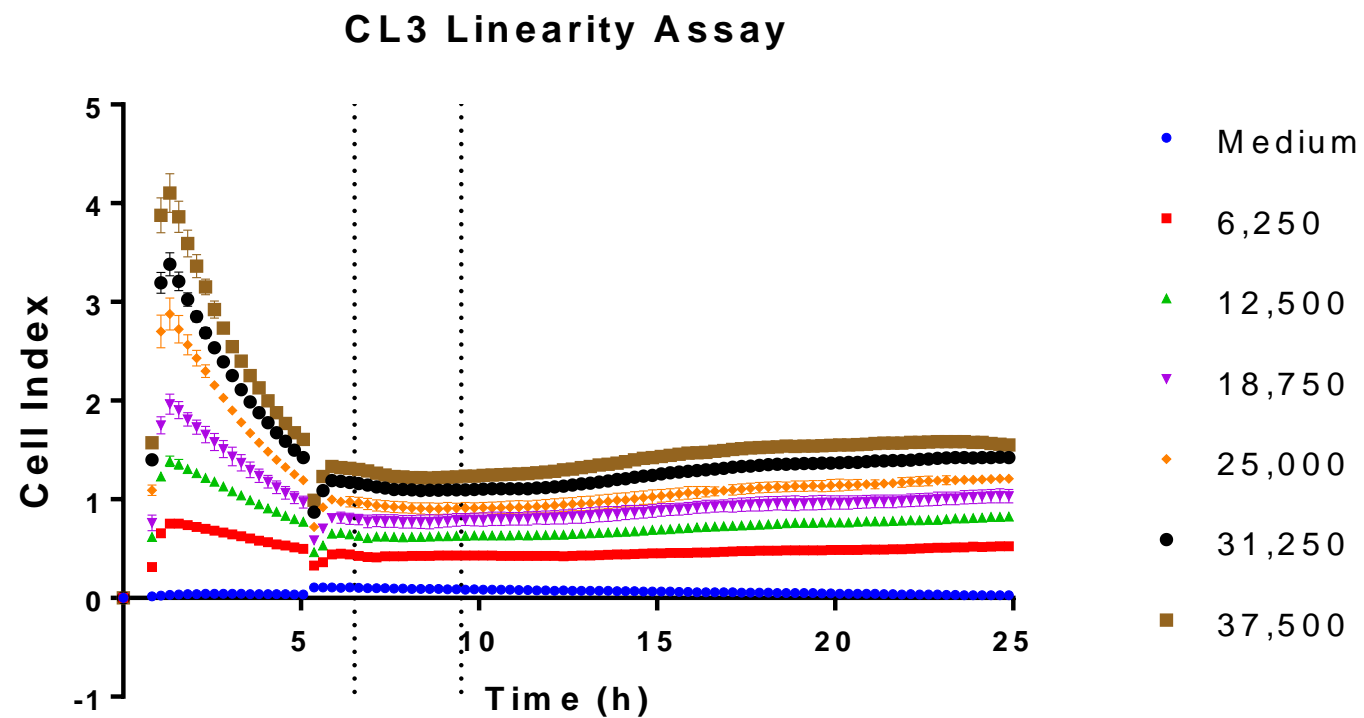
## CL3



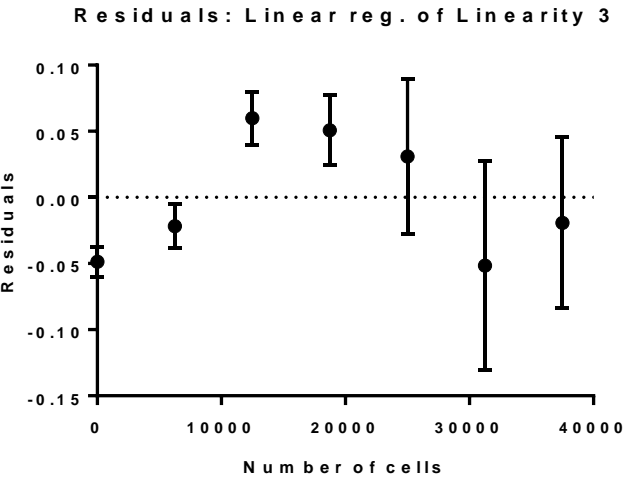
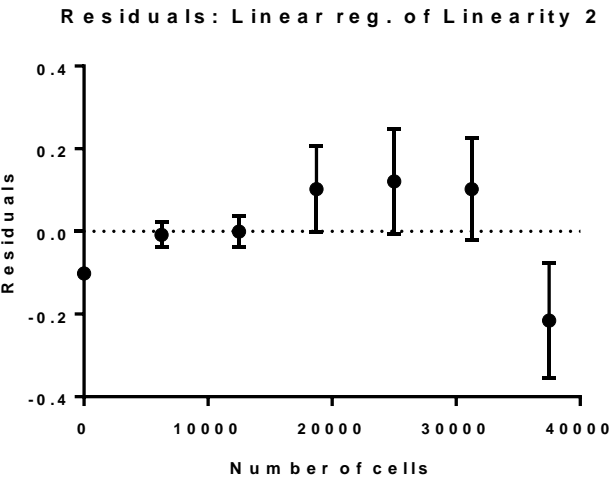
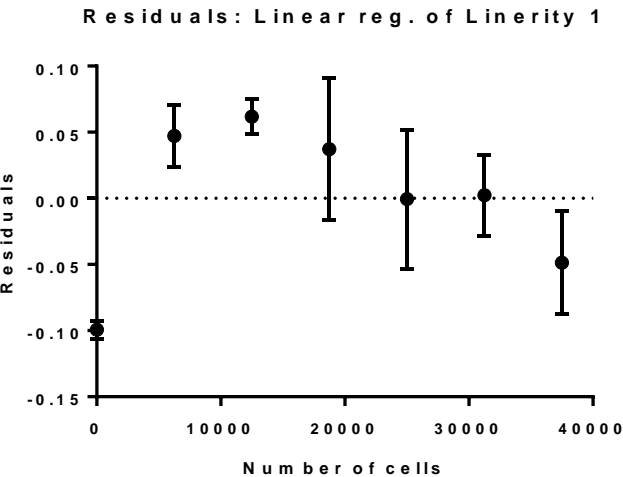
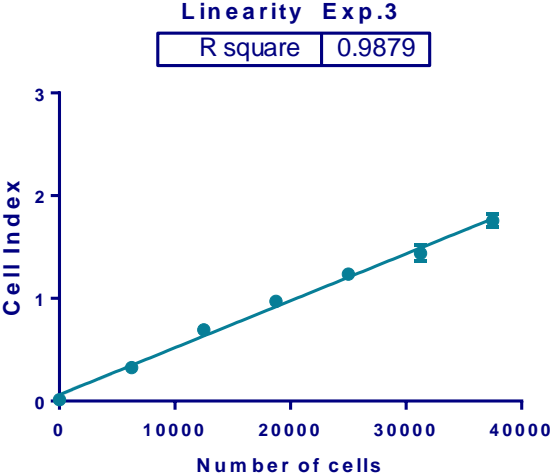
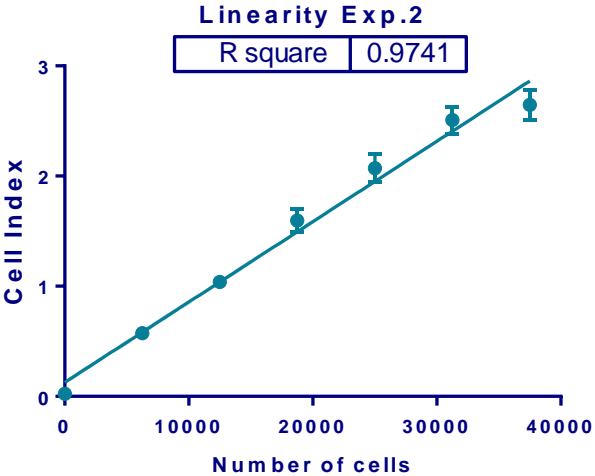
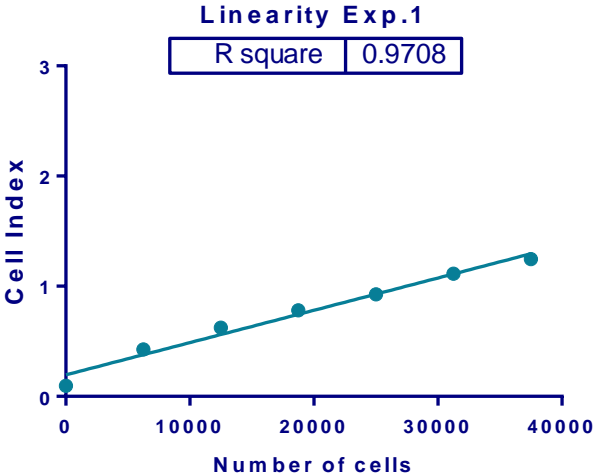
### CL<sub>3</sub>

- Lower cell density: CI ~1
- Highest expression levels of HLA-A2 between the tested cell lines
- Relatively constant CI over time
  - Less non-specific killing observed





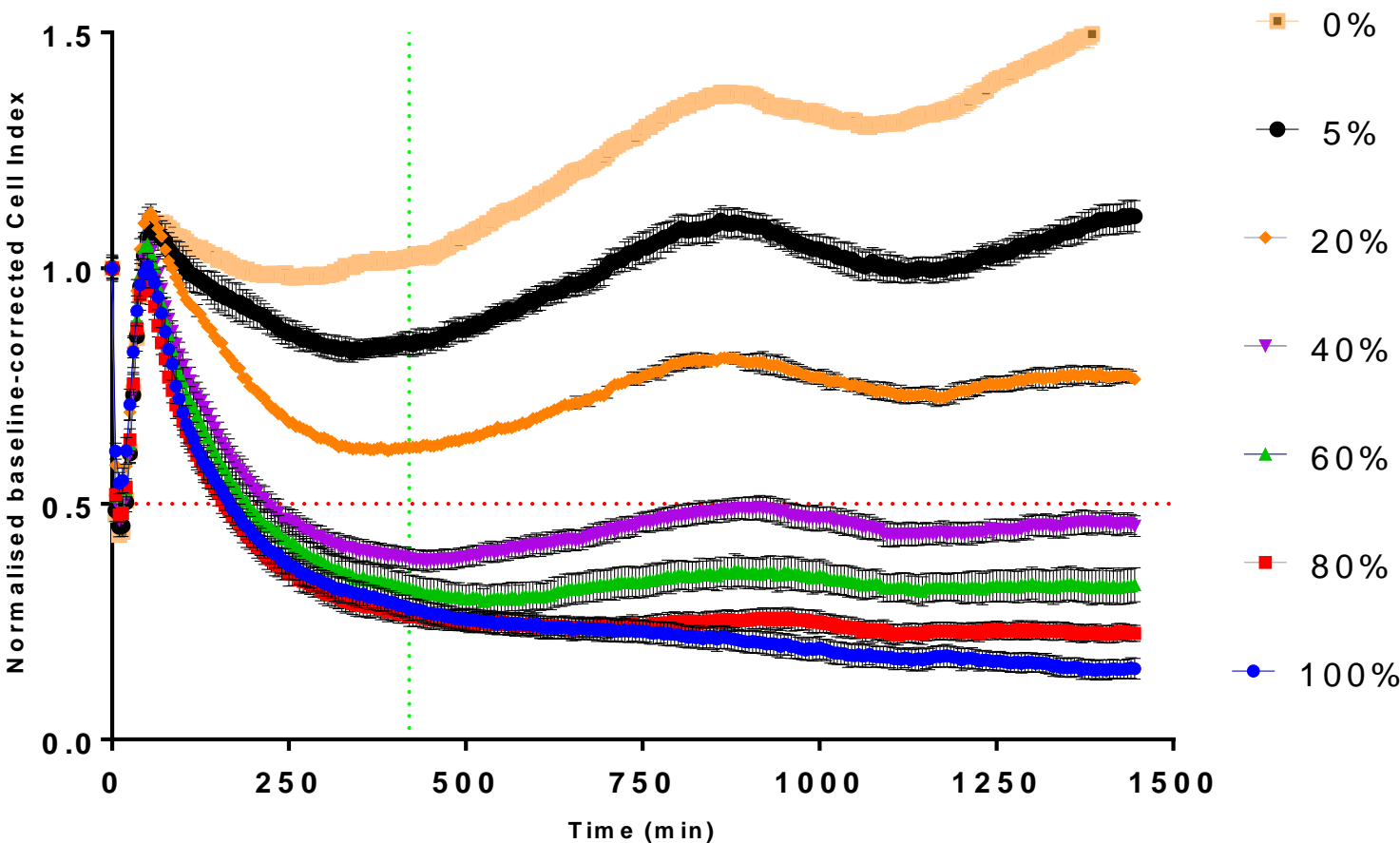
# Instrument's linearity



2:1 Effector to target ratio

% Transduced product	Number of Transduced cells	Number of non-transduced cells
100	25,000	0
80	20,000	5,000
60	15,000	10,000
40	10,000	15,000
20	5,000	20,000
5	1,250	23,750
0	0	25,000

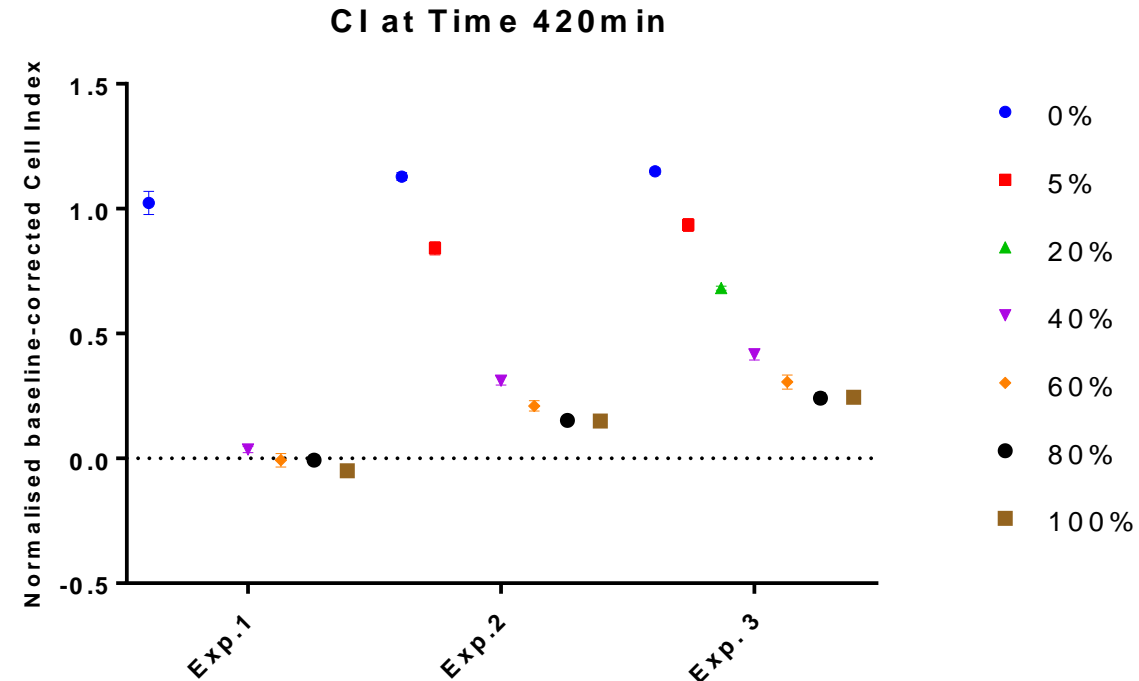
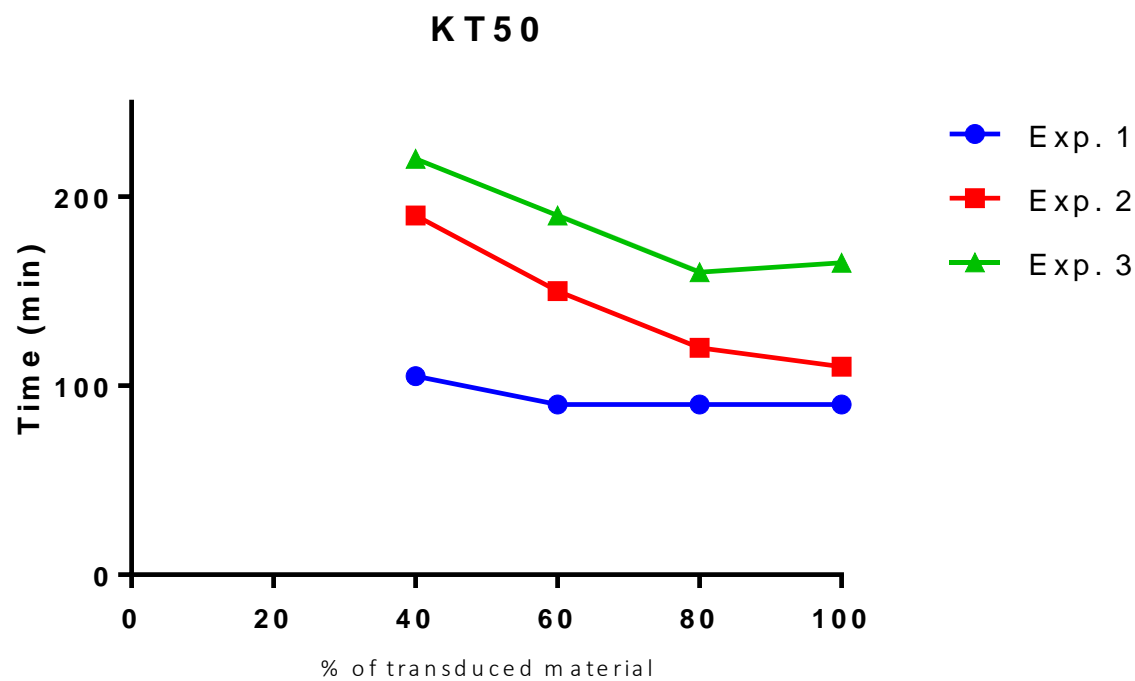
# Correlation between cytolysis and cell index



• KT50  
• Time 420min

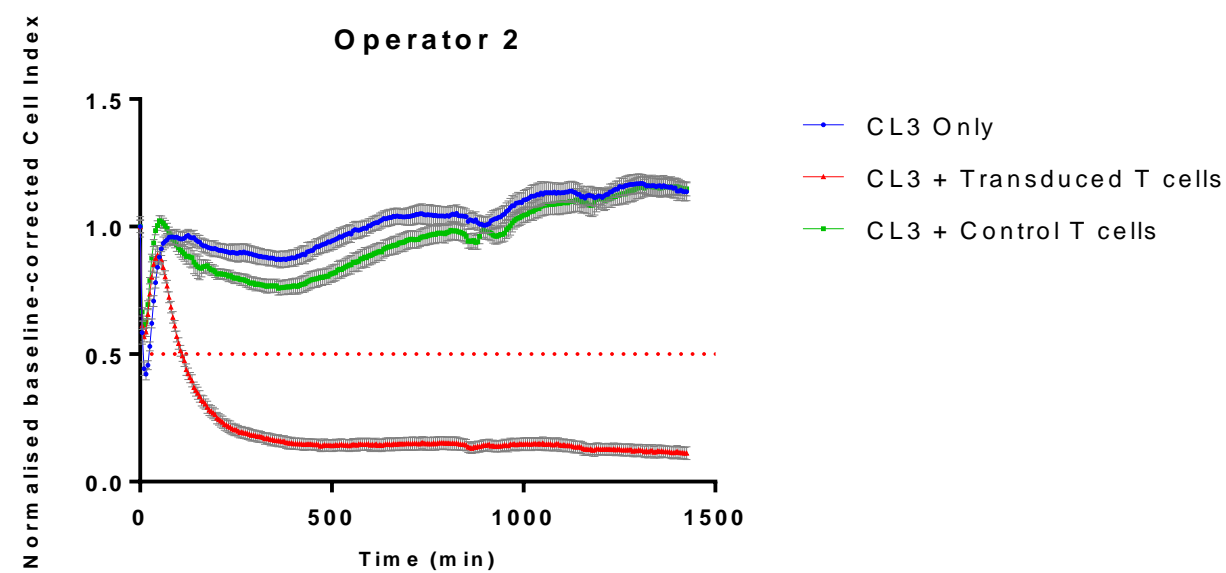
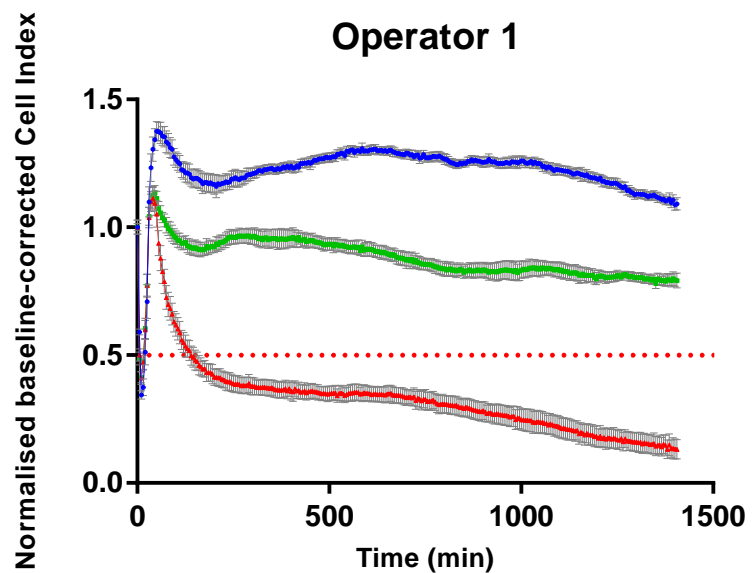


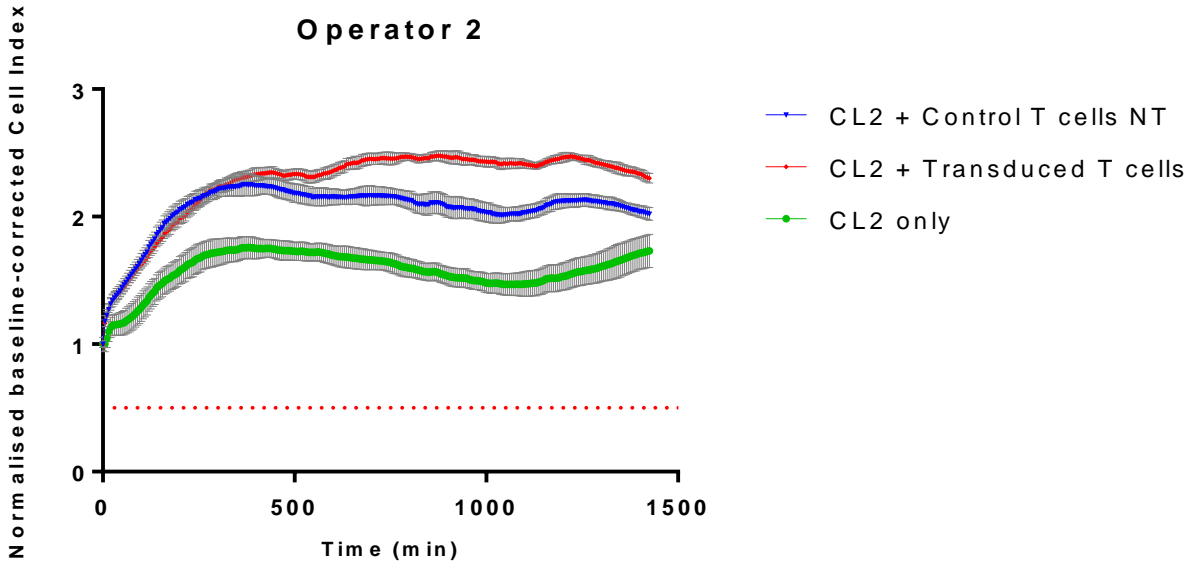
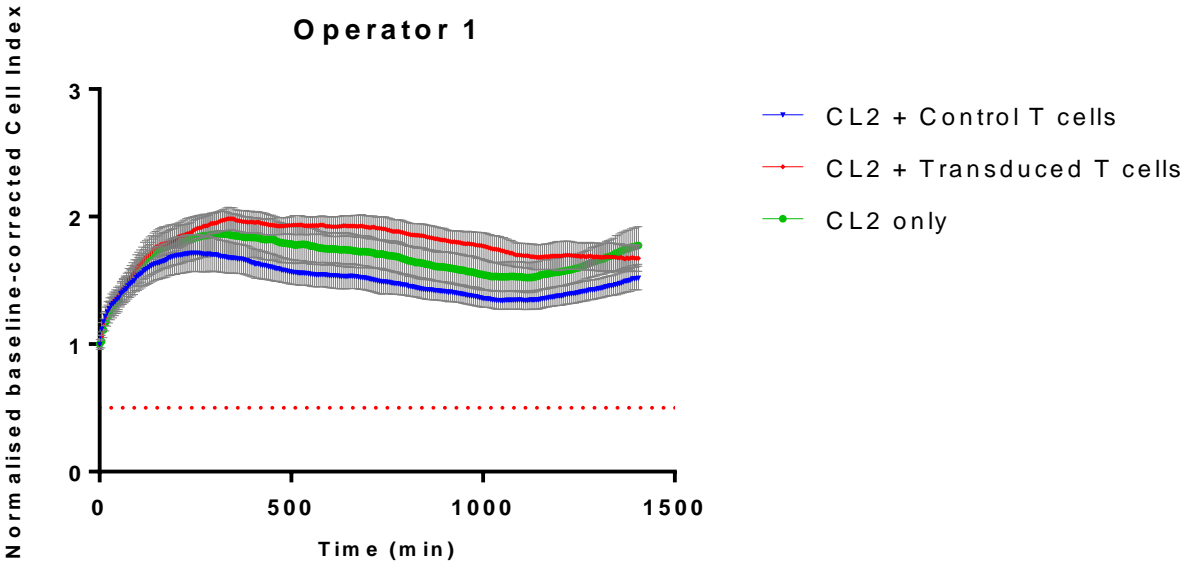
# KT50: Correlation between cytotoxicity and cell index

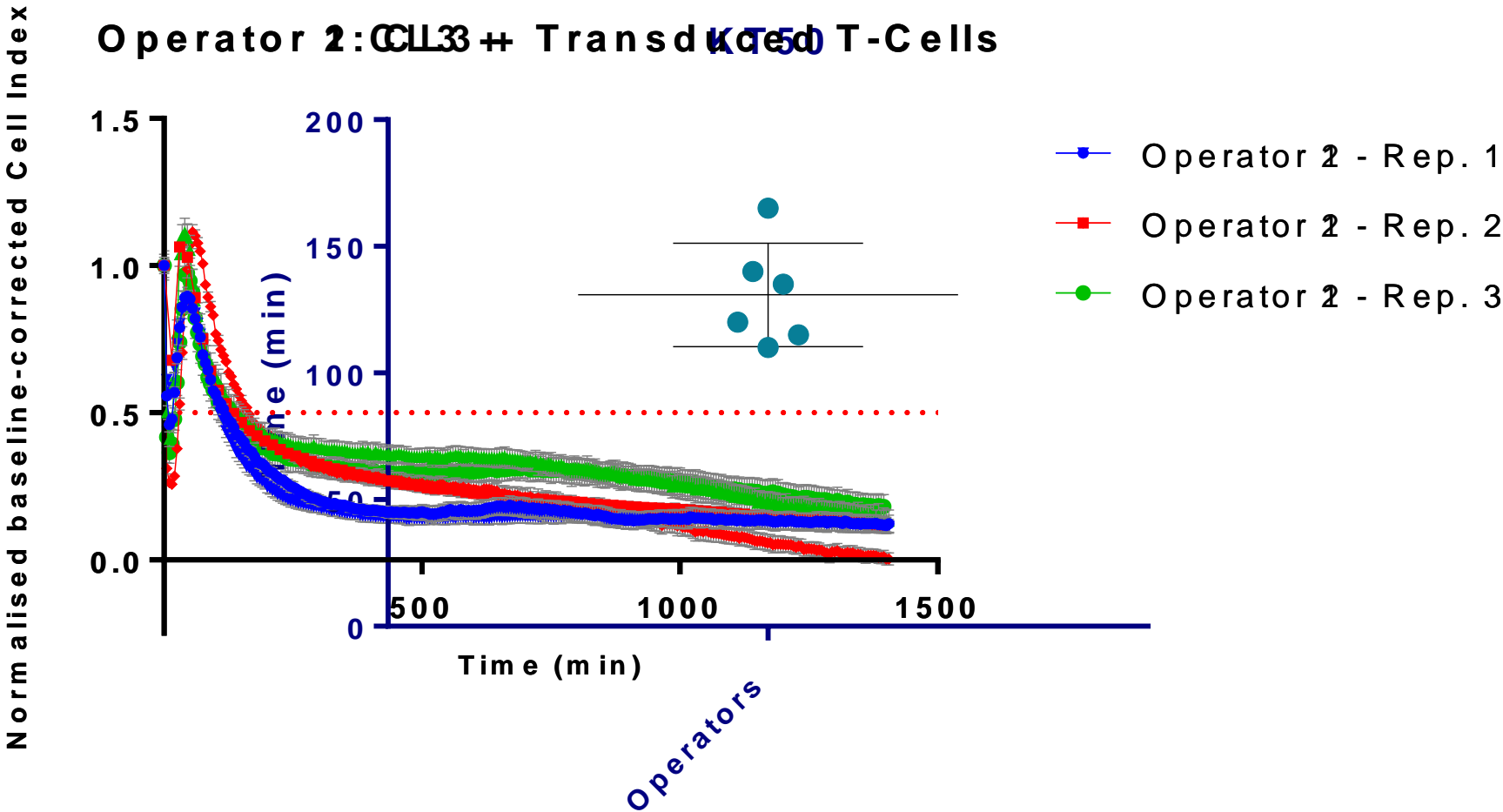


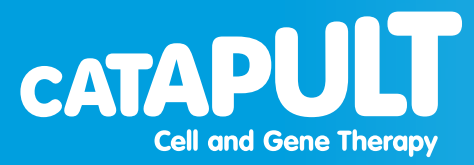
Exponential R²		
Exp. 1	Exp. 2	Exp. 3
NA	0.99	0.95

Exponential R²		
Exp. 1	Exp. 2	Exp. 3
0.99	0.99	0.99







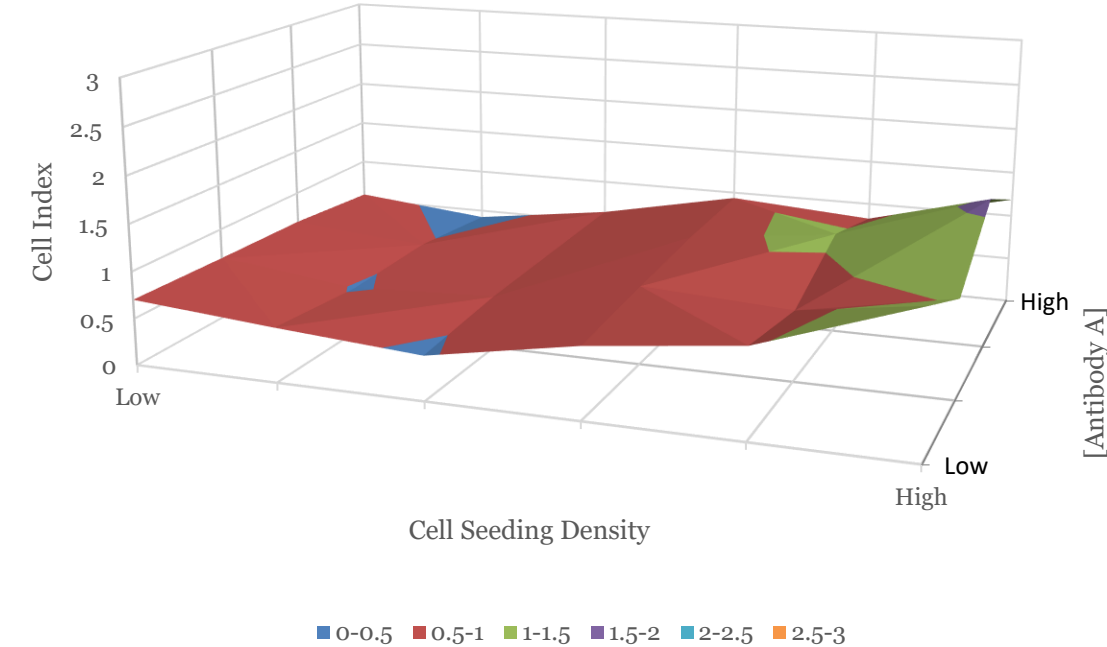


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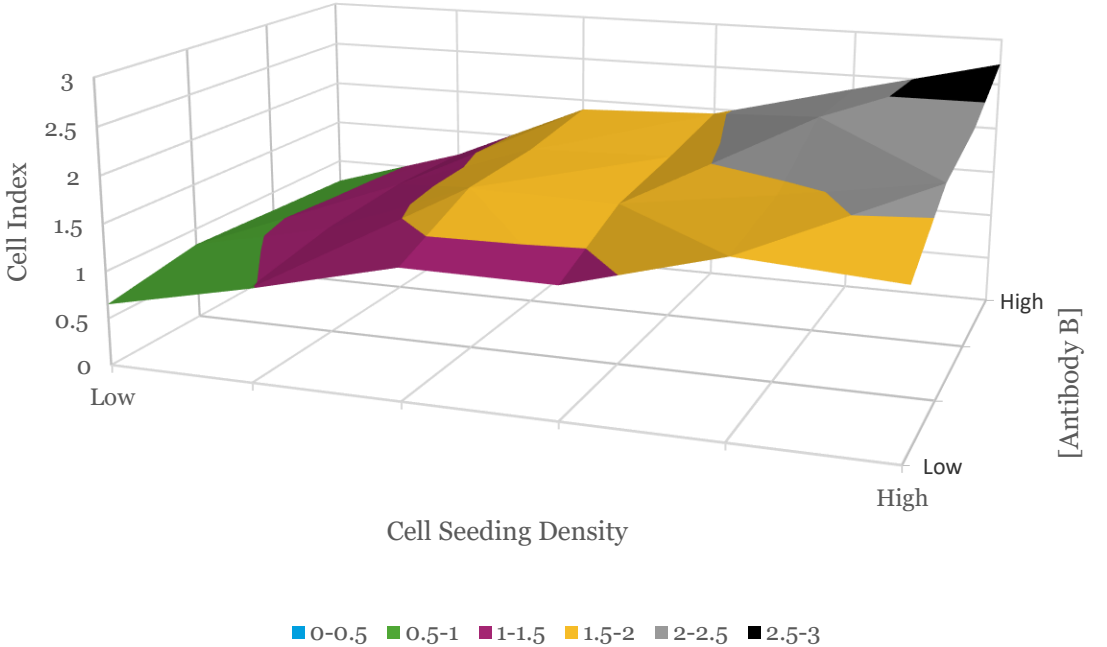
## Non-adherent cell line



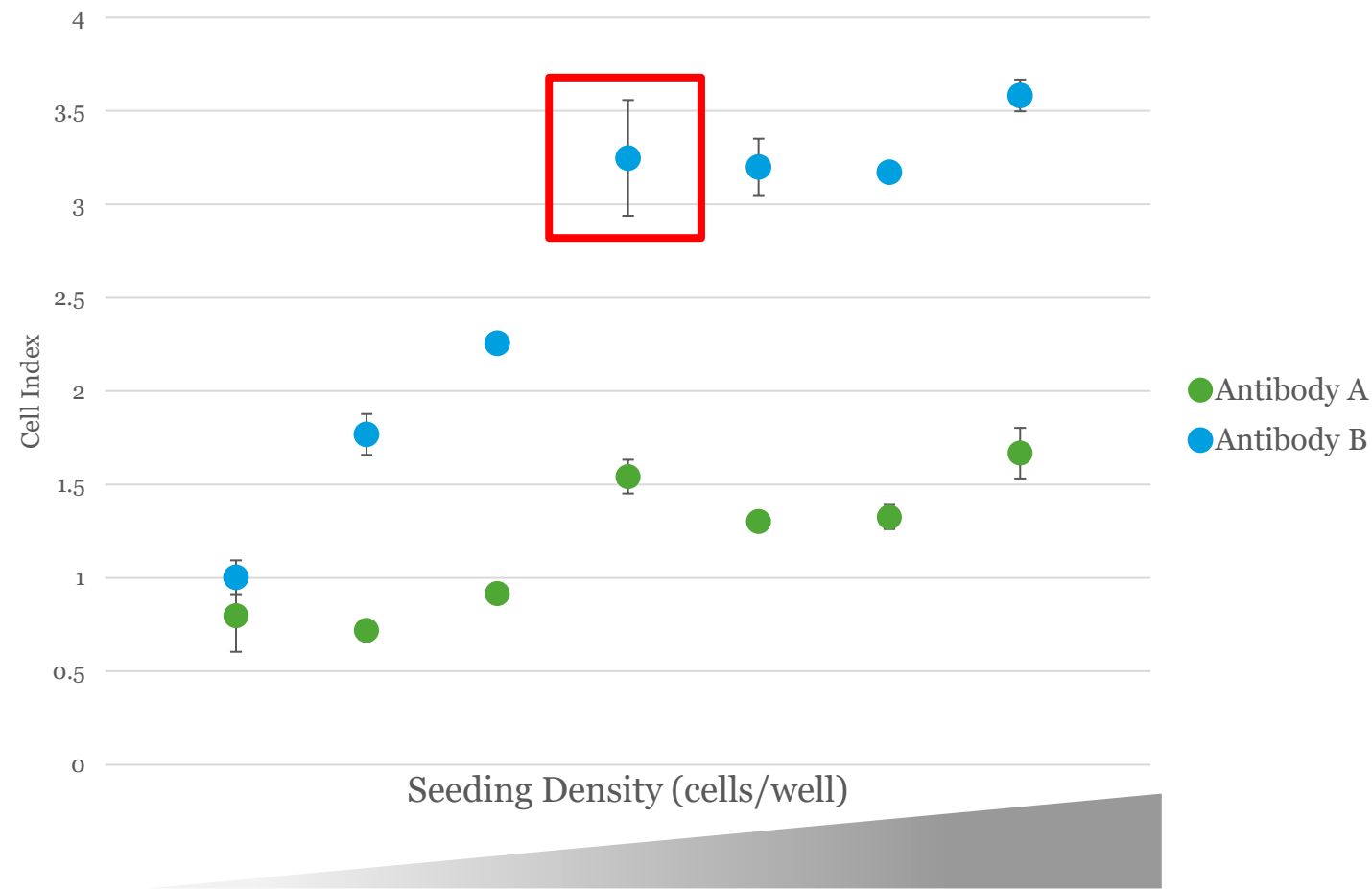
Antibody A



Antibody B



# Optimisation of cell attachment – cell density

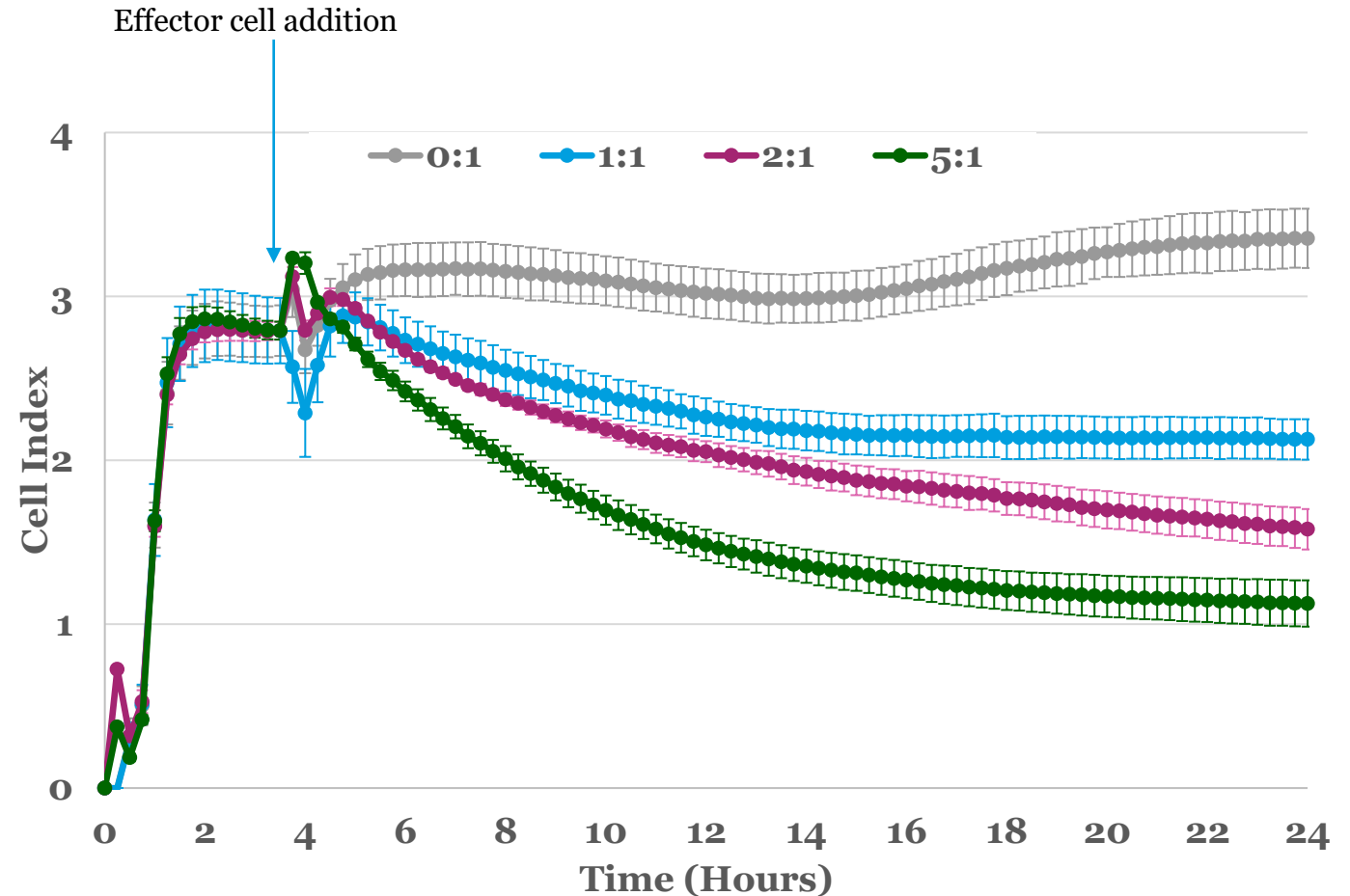




# Optimisation of Effector:Target cell ratios for a TCR therapy

## Assay outline:

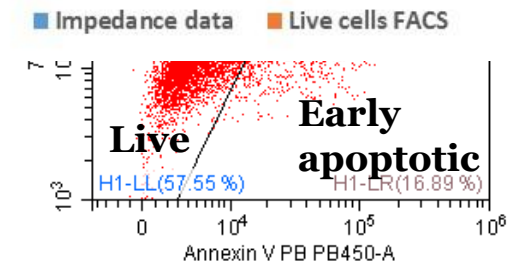
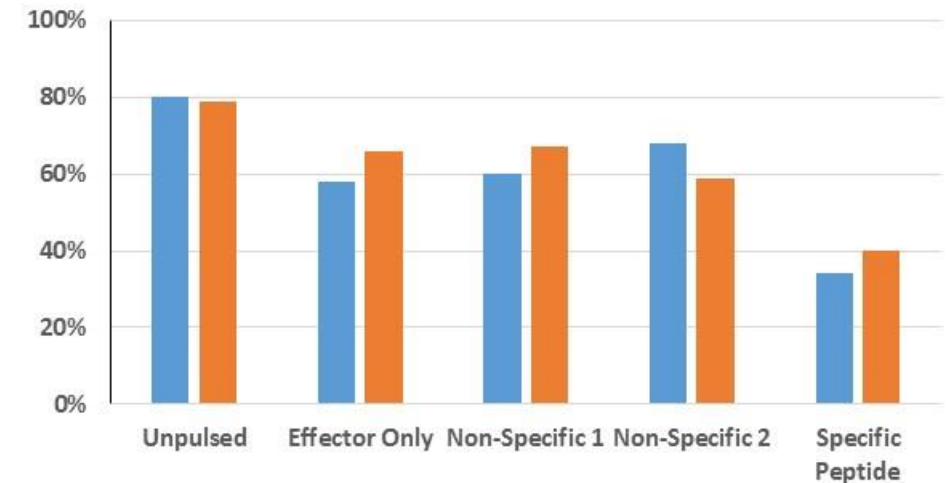
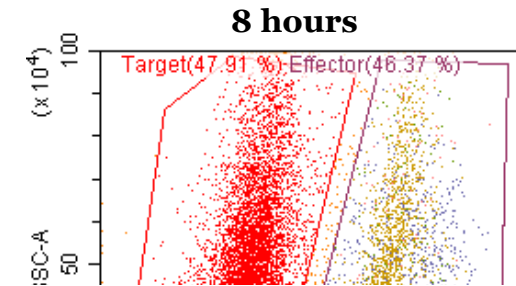
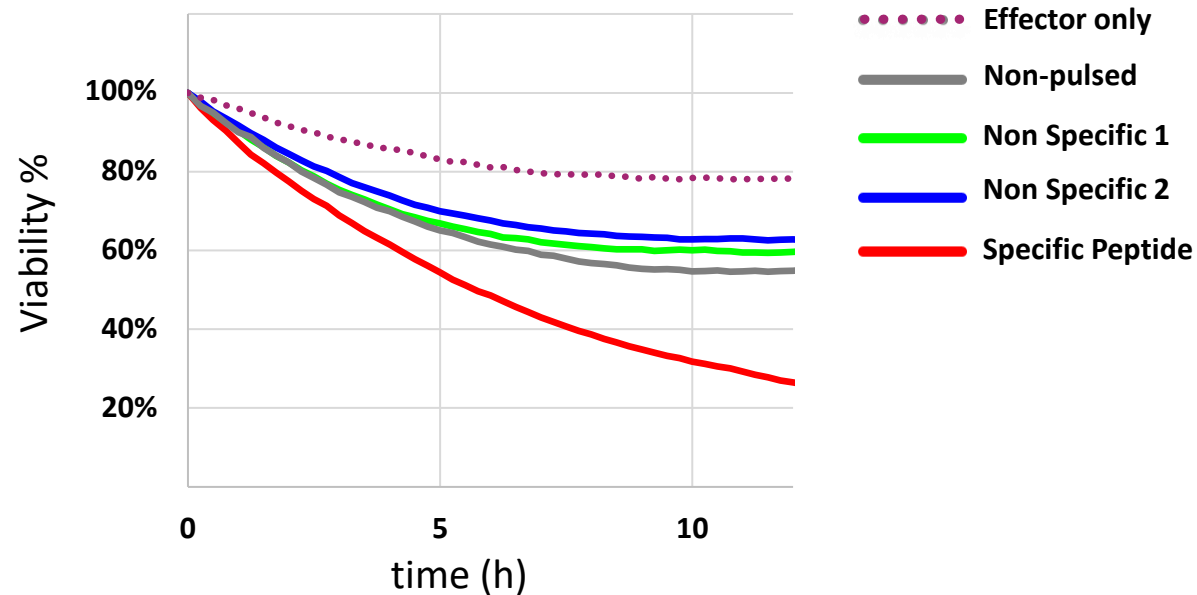
1. Target cells are pulsed for 2 hours with peptide prior to plating
2. Target cells are plated and allowed to attach for 4 hours – impedance readings are initiated
3. Cells are washed prior to killing assay
4. Transduced T cells are added
5. Killing response is measured every 15 minutes for up to 24 hours



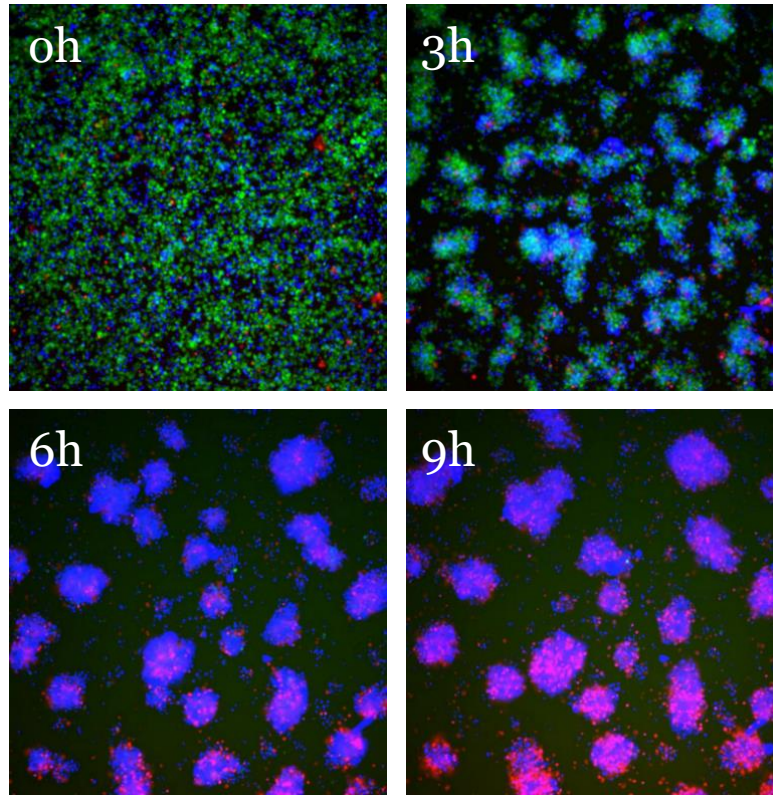
# Comparability between impedance and flow cytometry – TCR therapy



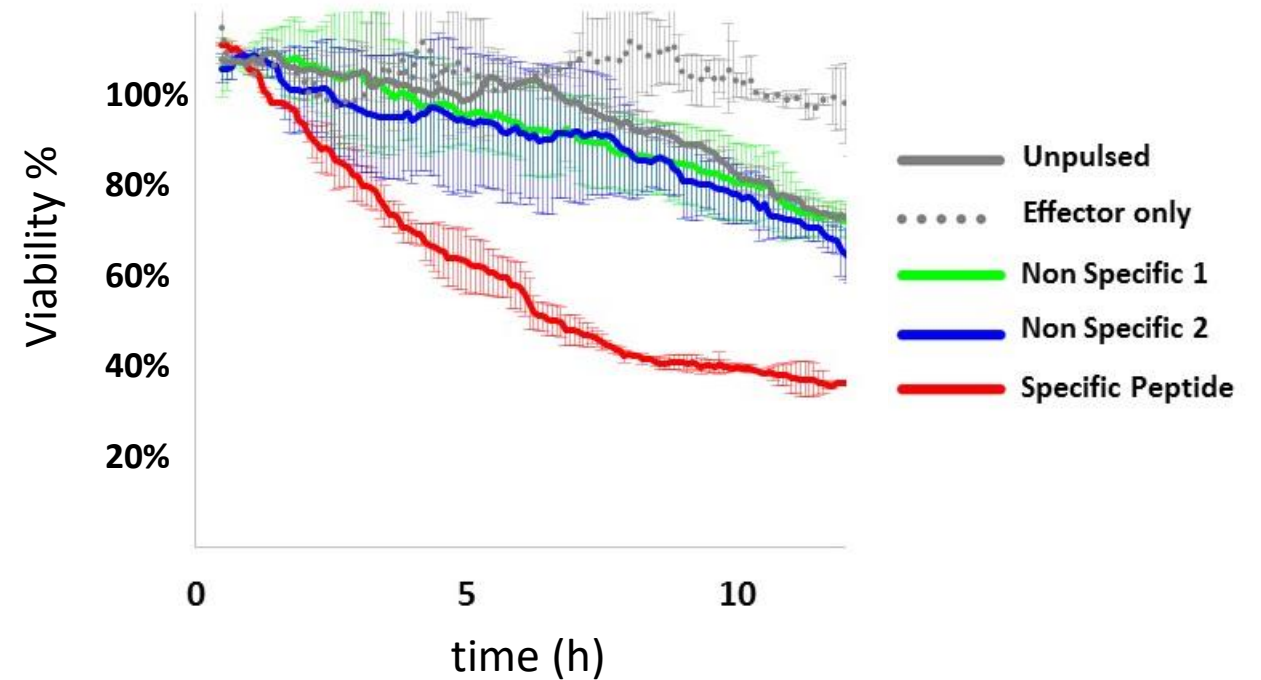
5:1 effector/target



# Correlation with impedance and quantitative image analysis



Target cells | Effector Cells | Dead cells



- TCR immunotherapy potency can be reliably measured using impedance spectroscopy
- We have shown two different methods to monitor T-cell cytotoxicity
  - Adherent cell lines
  - Non adherent cell lines
- Assay readout correlates with flow cytometry and image analysis
- The impedance assay is label free and provides kinetic data of cell killing

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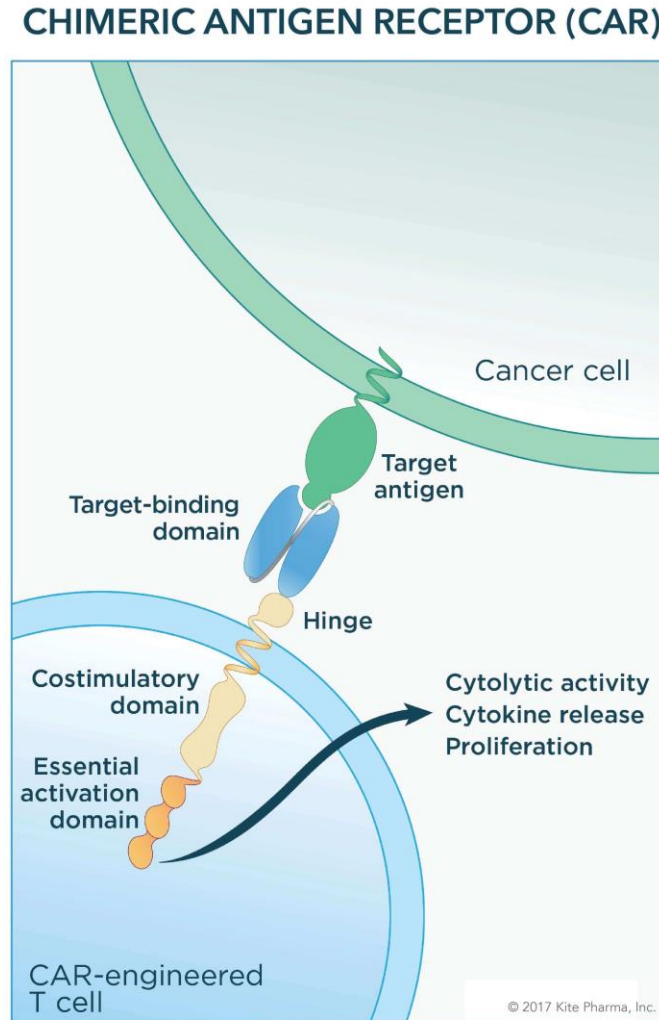
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# CAR-T cells



For Research Use Only. Not for use in diagnostic procedures.

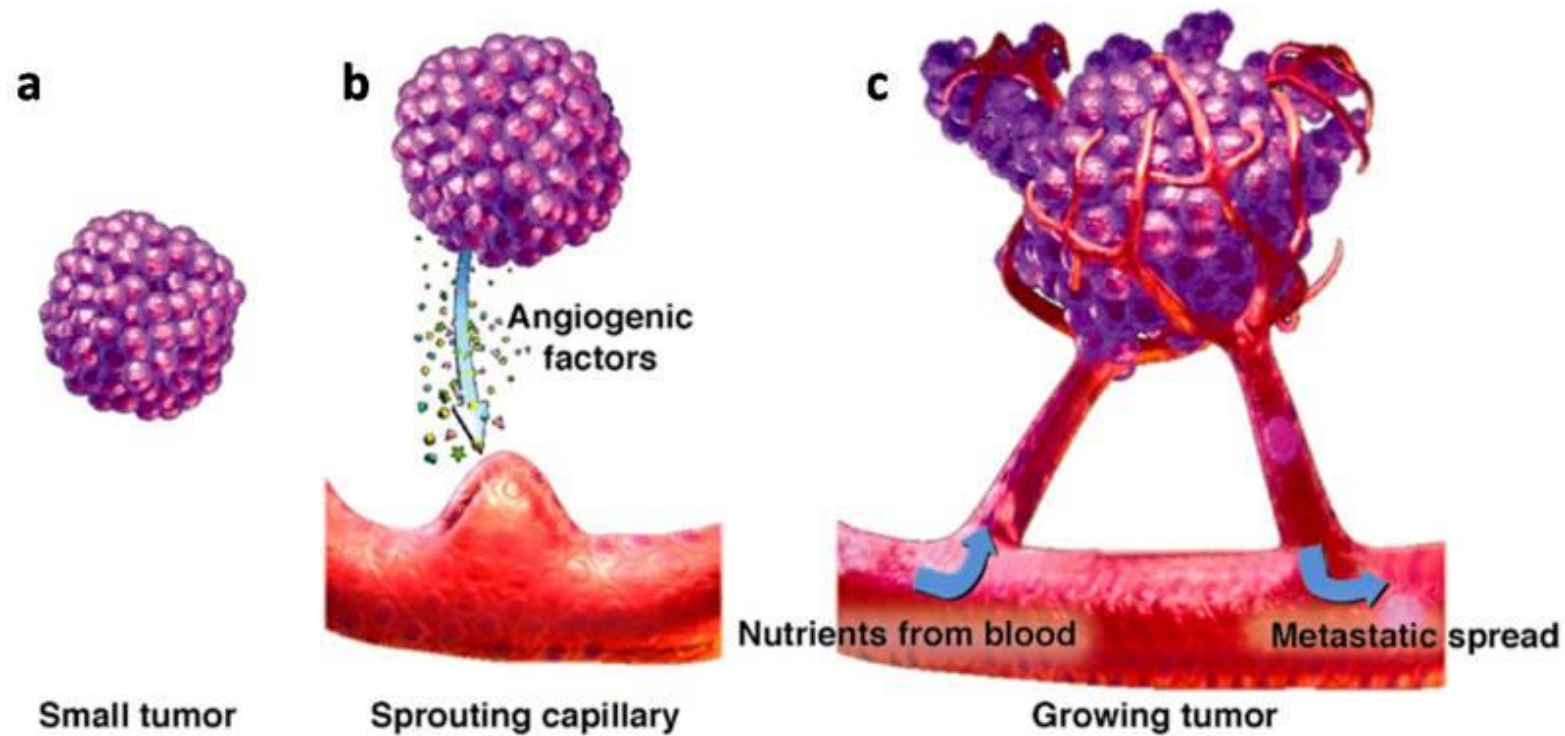
# Chimeric Antigen Receptor (CAR) T cell therapies



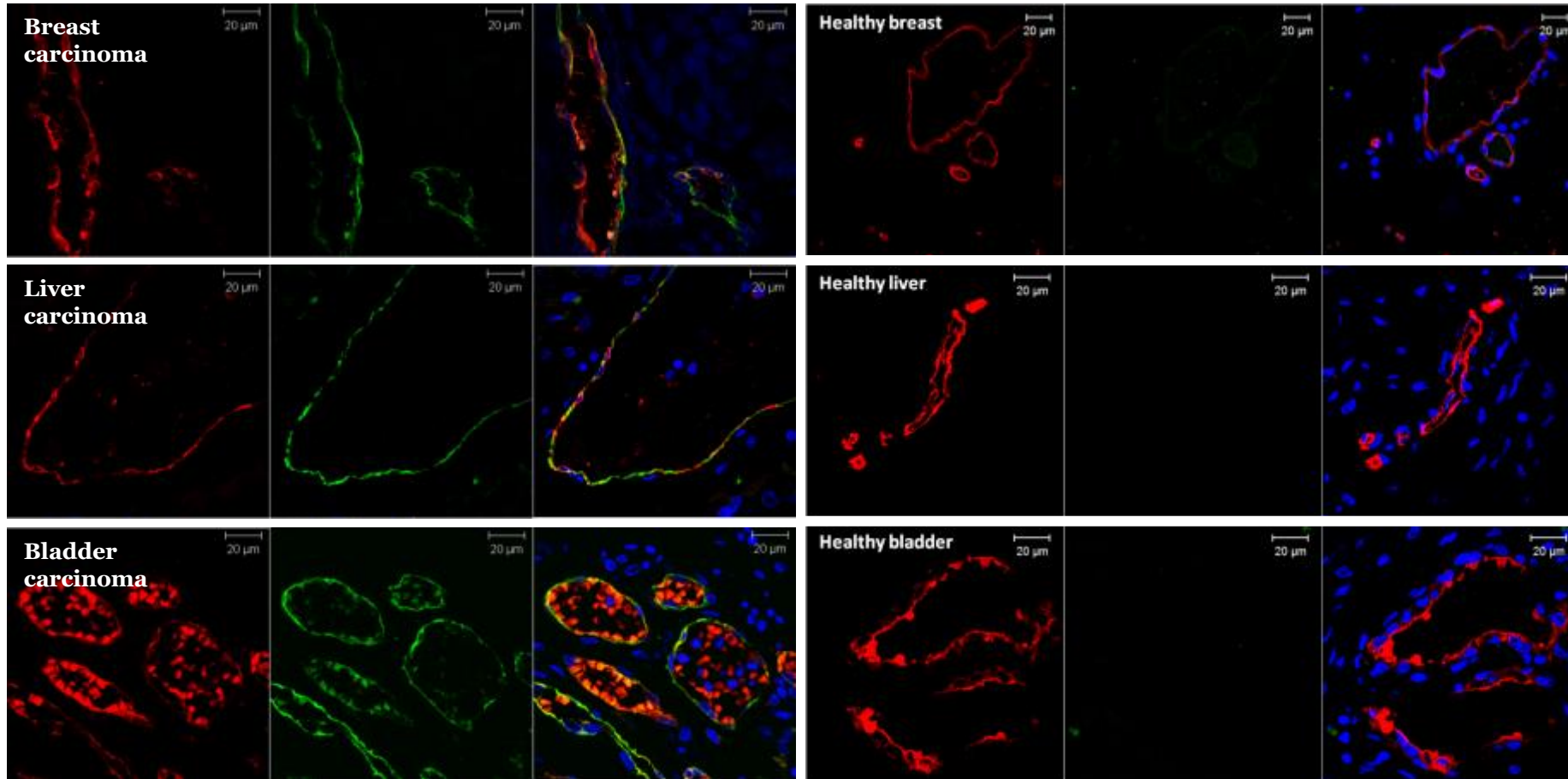
- Access to the tumour tissue
- Lack of appropriate target antigens
- Immunosuppressive tumour microenvironment



# Tumour angiogenesis: potential therapeutic approach



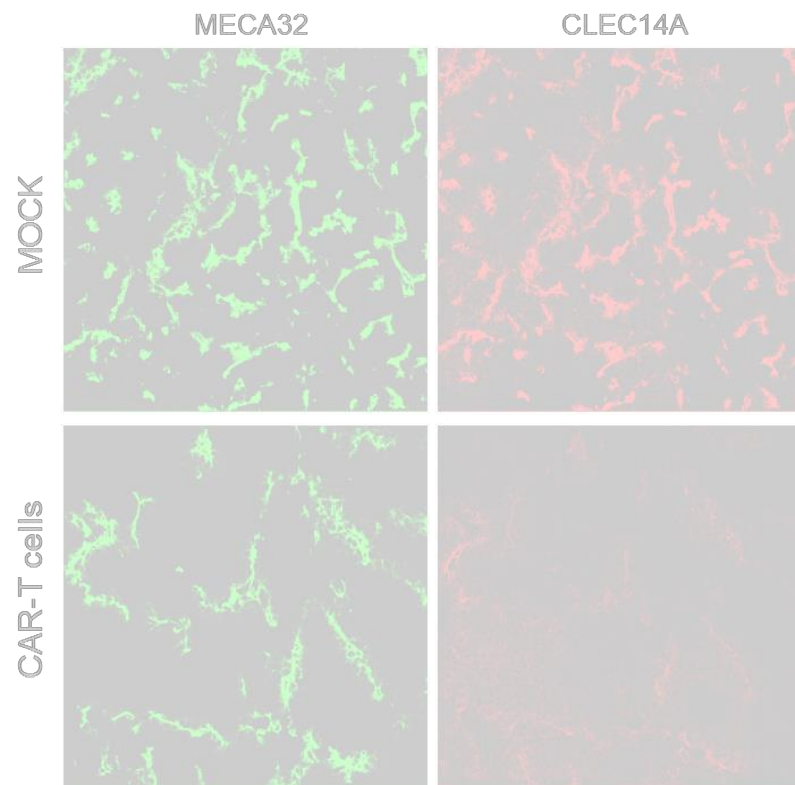
# CLEC14A – tumour endothelial marker



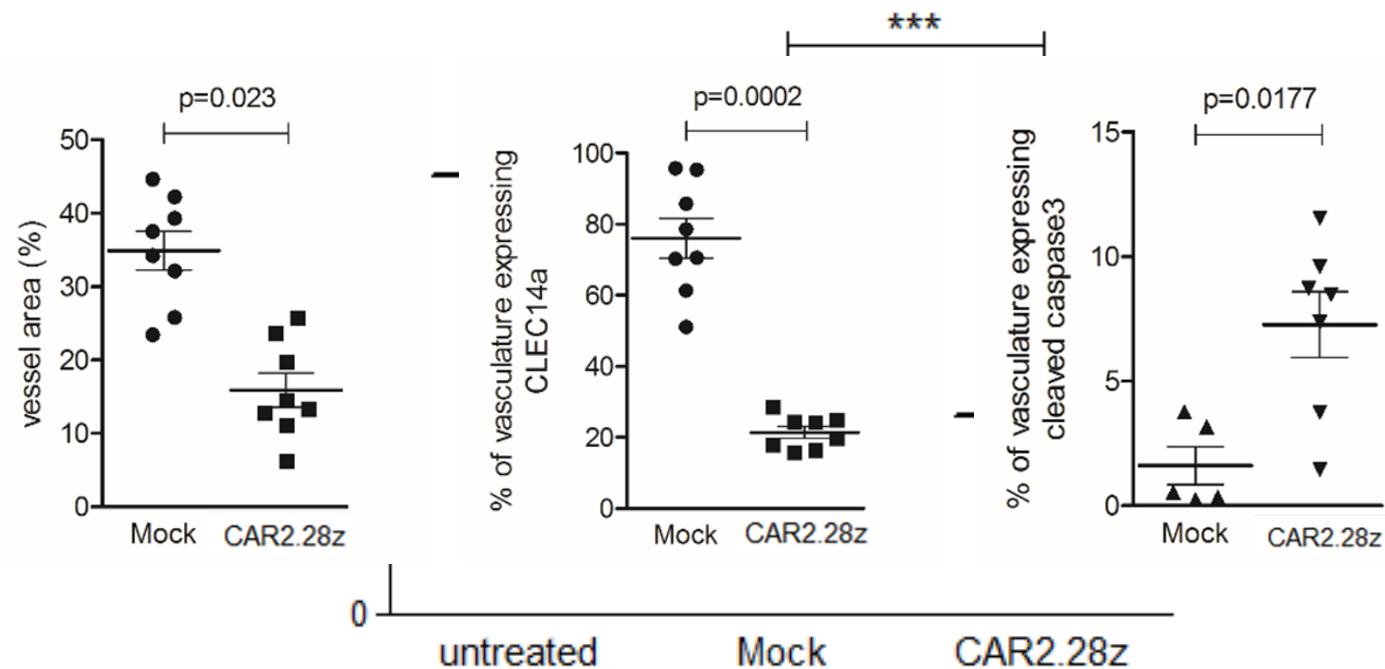
■ Human endothelial marker (ULEX)    ■ CLEC14A    ■ Nuclei (DAPI)

# Reduction in tumour burden in RipTag2 mouse model

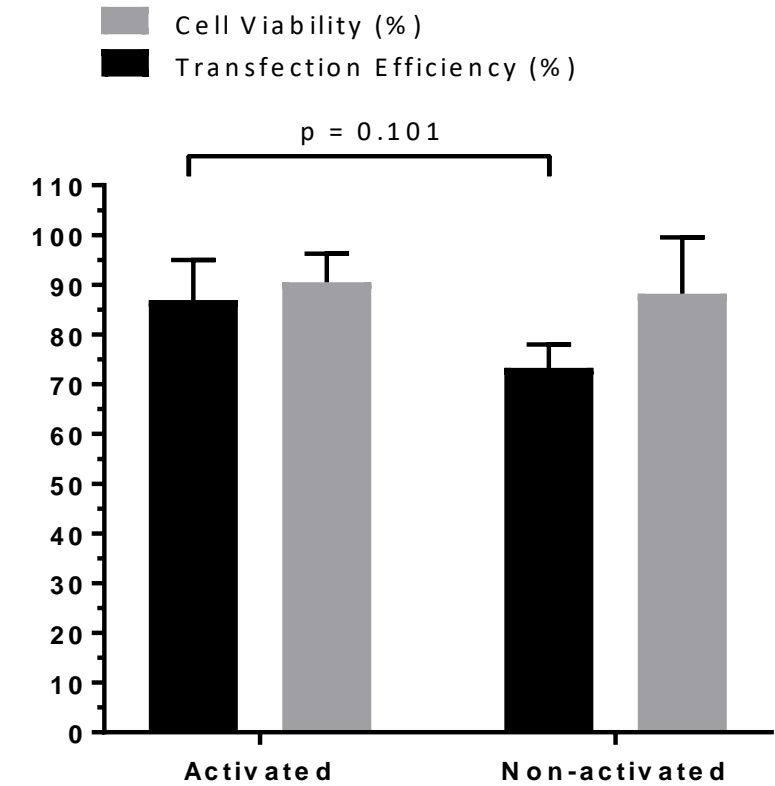
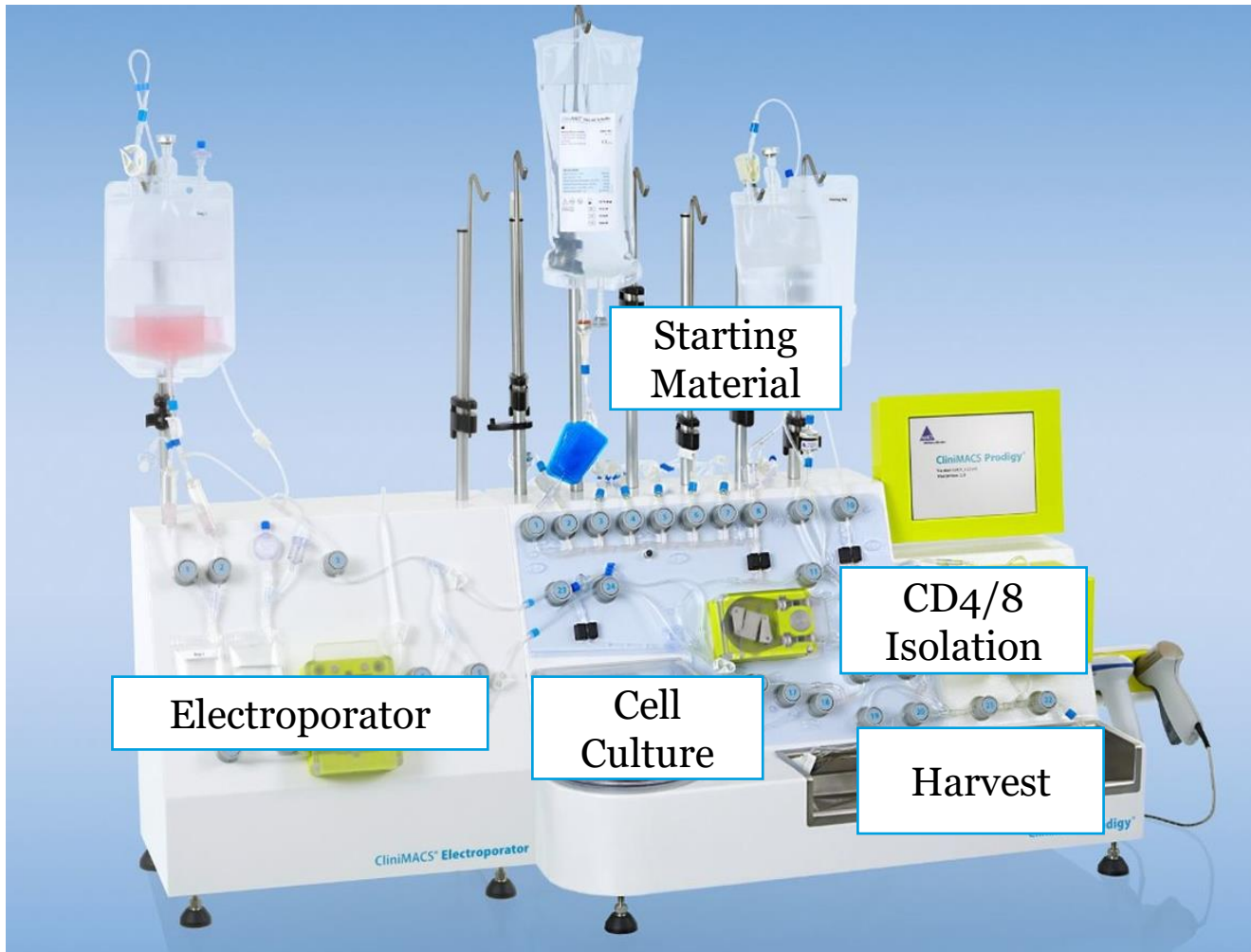
(F. Maione, E. Giraudo, Turin)



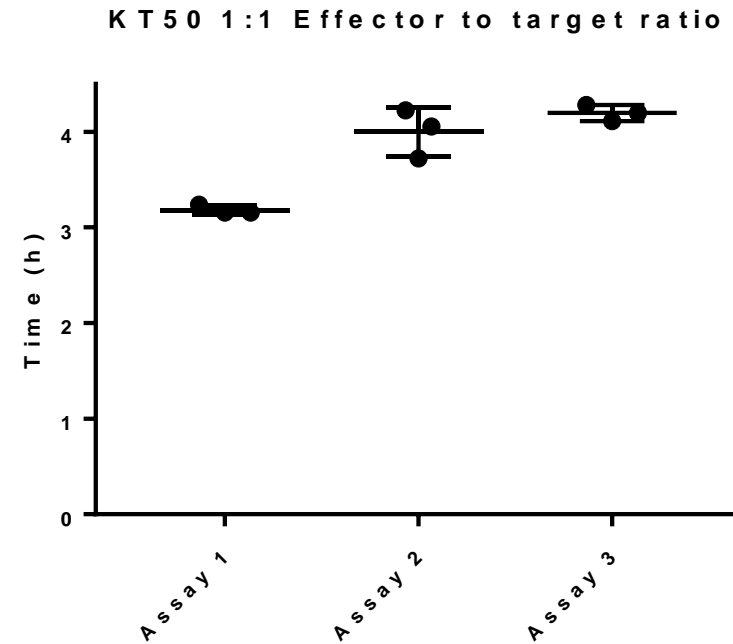
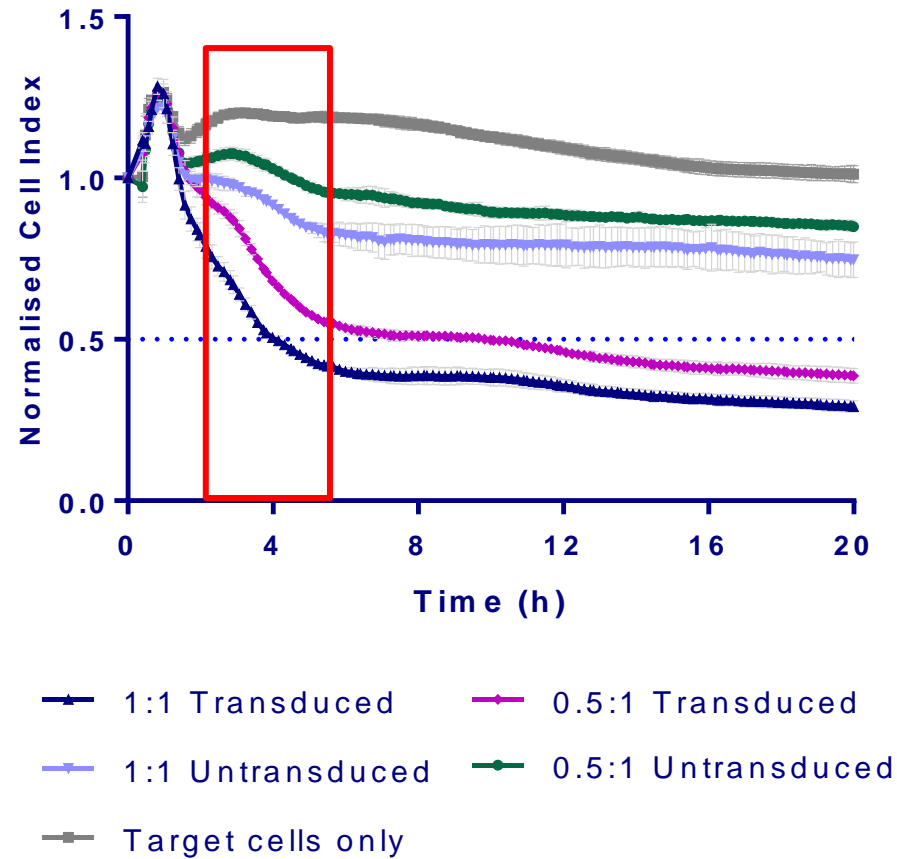
MECA32 (endothelial marker)  
CLEC14A



# Large scale generation of mRNA CAR-T cells

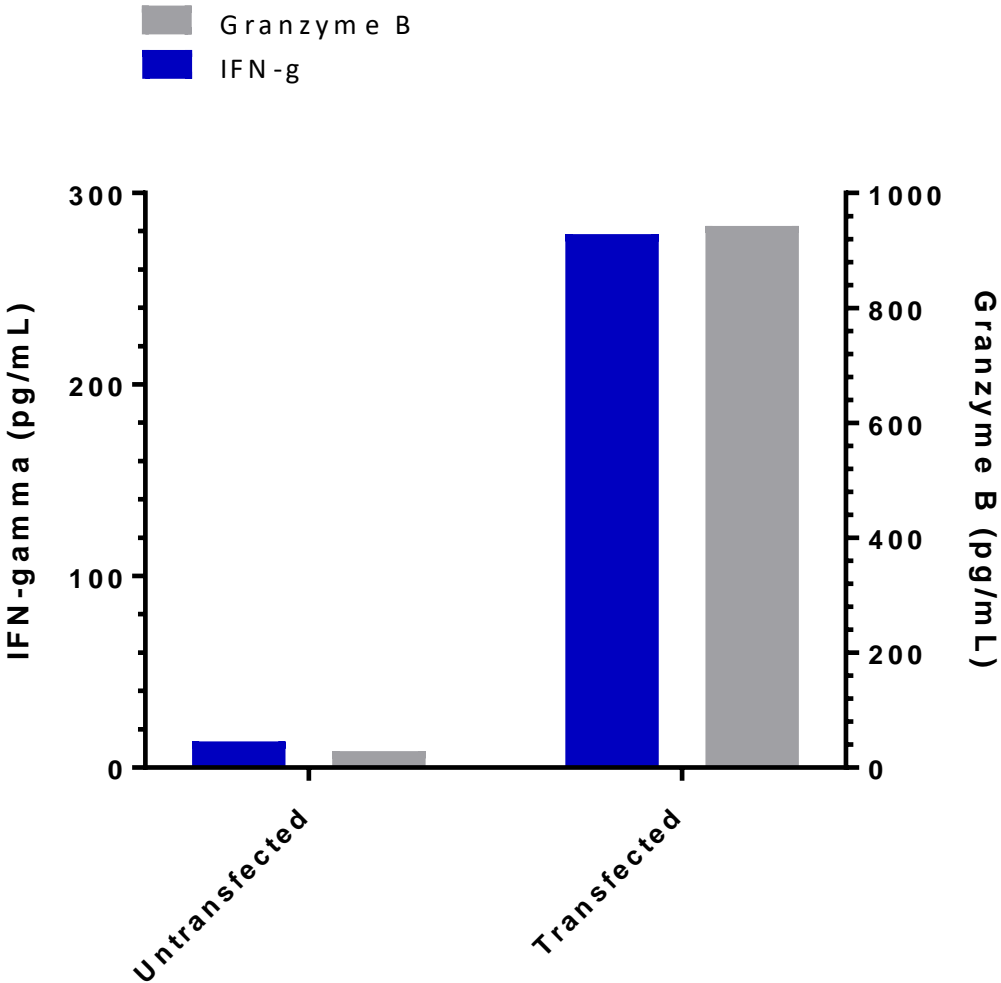
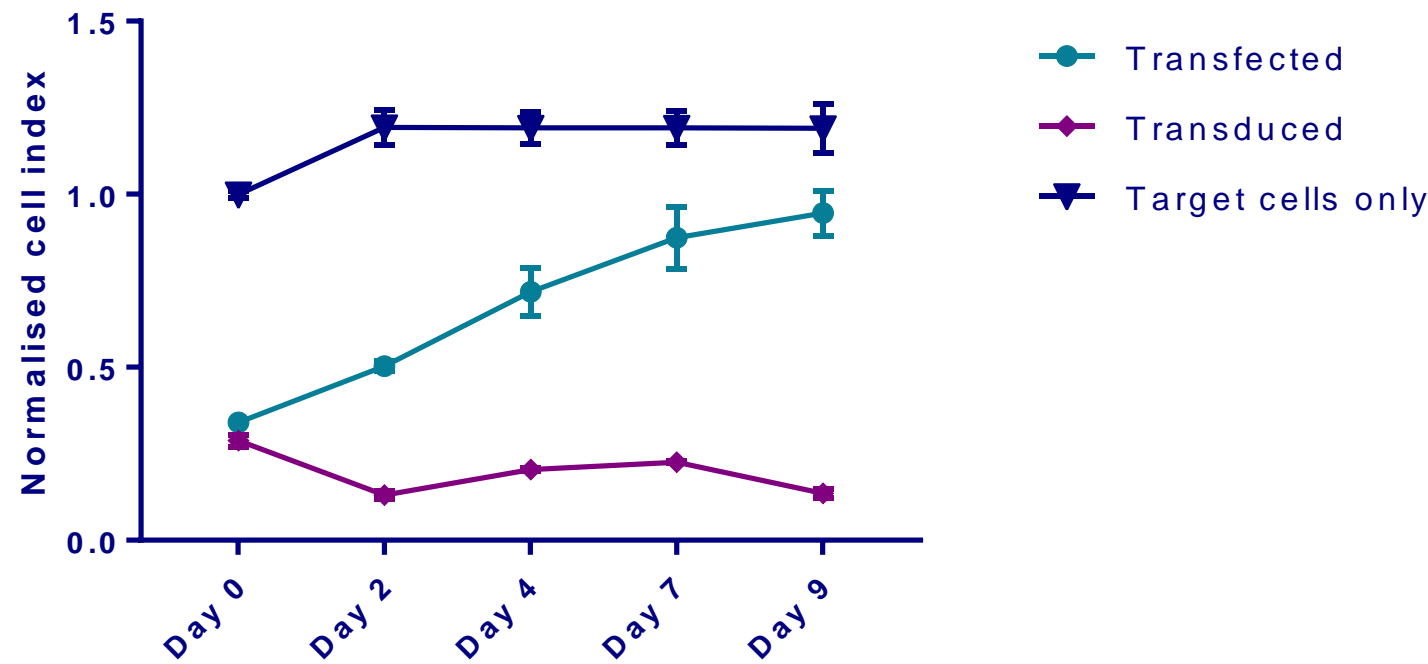


# Real time detection of product's potency within 4h



# Persistence of potency over time

CI at 20h



- TCR and CAR-T immunotherapy potency can be reliably measured using impedance spectroscopy
- We have shown specificity of the assay independently of the therapy used
- The impedance assay is label free and provides **kinetic data** of cell killing
  - During assay
  - Over different periods of time
- This assay provides a **fast** and **high-throughput** alternative to current methodologies



# Acknowledgements

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**Thank you**

# CATAPULT

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12th Floor Tower Wing  
Guy's Hospital  
Great Maze Pond  
London SE1 9RT

[info@ct.catapult.org.uk](mailto:info@ct.catapult.org.uk)  
[ct.catapult.org.uk](http://ct.catapult.org.uk)  
Twitter: @CGTCatapult

