Impedance-based assay to evaluate potency of immunotherapy products

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Analytical Development Scientist – Industrialisation team
About us

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.

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Our assets

**Development centre**
- 1,200m² purpose built centre
- Analytical characterisation
- Process development
- Viral vector
- Stem cell differentiation
- 10th floor integration & collaboration centre

**Manufacturing centre**
- 7,700m² 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

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Potency assays for immunotherapies
Challenge: reduce time between product formulation and patient administration

**Day 1**
- Selection

**Day 1/3**
- Transduction

**Day 1-10**
- Expansion

**Day 10**
- Formulation

**Identity**
- Transduction efficiency
- Immunophenotype
- Appearance

**Impurities**
- Percentage non-CD3⁺ cells
- Large T antigen protein/DNA

**Safety**
- Genome viral copy number
- Sterility (EP 2.6.1)
- Mycoplasma (EP 2.6.7)
- Endotoxin (EP 2.6.14)
- Replication competent viruses

**Potency**
- Viable cell count
- CAR/TCR expression
- Cell killing activity
- Cytokine stimulation

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Current methods to evaluate T-cell potency

- **Chromium release**
  - Gold standard
  - Limitations:
    - Time – leakage
    - Safety – use of radioactive material
    - Cell requirements – high effector to target ratios | physiological relevance
### Alternatives to Cr51 release assay

<table>
<thead>
<tr>
<th>Assay</th>
<th>Measure</th>
<th>Readout</th>
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<tbody>
<tr>
<td>CytoTox 96&lt;sup&gt;®&lt;/sup&gt;</td>
<td>LDH</td>
<td>Absorbance</td>
</tr>
<tr>
<td>CellTiter-Glo&lt;sup&gt;®&lt;/sup&gt;</td>
<td>ATP</td>
<td>Luminescence</td>
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<tr>
<td>Calcein-AM</td>
<td>Dye release</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>DELFIA&lt;sup&gt;®&lt;/sup&gt; EuTDA</td>
<td>BATDA release</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Flow cytometry</td>
<td>Cytokine/cell death</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>Luminex&lt;sup&gt;®&lt;/sup&gt;</td>
<td>Cytokine</td>
<td>Fluorescence</td>
</tr>
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Luminex<sup>®</sup> is a trade mark of R&D Systems. DELFIA<sup>®</sup> is a trade mark of PerkinElmer. CytoTox 96<sup>®</sup> and CellTiter-Glo<sup>®</sup> are trade marks of Promega.
Solution: impedance – based potency assay

• 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
Use of the xCELLigence® MP to assess T-cell cytotoxicity

• 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

xCELLigence® is a trade mark of ACEA Biosciences
How does the impedance-based potency assay work?
TCR based products
TCR mediated killing

CD8+ cell

CD8+ cell

CD8+ cell

CD8+ cell

CD8+ cell

Cancer cell

CD8

CD80/86

Ag

MHC (I)

CD28

4-1BB

4-1BBL

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Assay development

- 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

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Evaluation of optimal seeding density – effect of extracellular matrix

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Evaluation of optimal seeding density – effect of extracellular matrix

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Evaluation of optimal seeding density – effect of extracellular matrix

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Selection of the cell line to perform the killing assay

- Expression levels of MHC-1

Atzin-Méndez, J. A. et al. 2015

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Selection of the cell line to perform the killing assay

- Expression levels of MHC-I
CI profile

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Cell line selection

CL3

• 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

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Instrument’s linearity

**Linearity Exp.1**

- **R square**: 0.9708

**Linearity Exp.2**

- **R square**: 0.9741

**Linearity Exp.3**

- **R square**: 0.9879

**Residuals: Linear reg. of Linearity 1**

**Residuals: Linear reg. of Linearity 2**

**Residuals: Linear reg. of Linearity 3**

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Correlation between cytolysis and cell index

2:1 Effector to target ratio

<table>
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<tr>
<th>% Transduced product</th>
<th>Number of Transduced cells</th>
<th>Number of non-transduced cells</th>
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<tbody>
<tr>
<td>100</td>
<td>25,000</td>
<td>0</td>
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<tr>
<td>80</td>
<td>20,000</td>
<td>5,000</td>
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<tr>
<td>60</td>
<td>15,000</td>
<td>10,000</td>
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<tr>
<td>40</td>
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<tr>
<td>20</td>
<td>5,000</td>
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<tr>
<td>5</td>
<td>1,250</td>
<td>23,750</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>25,000</td>
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</table>
Correlation between cytolysis and cell index

- KT50
- Time 420min
**KT50: Correlation between cytolysis and cell index**

- **KT50**
  - Time (min)
  - % of transduced material

- **CI at Time 420 min**
  - Normalised baseline-corrected Cell Index

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<tr>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
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</thead>
<tbody>
<tr>
<td>NA</td>
<td>0.99</td>
<td>0.95</td>
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**Exponential R²**

<table>
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<tr>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
</tr>
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<tbody>
<tr>
<td>0.99</td>
<td>0.99</td>
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Specificity – CL3

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Specificity – CL2

Operator 1

- CL2 + Control T cells
- CL2 + Transduced T cells
- CL2 only

Operator 2

- CL2 + Control T cells NT
- CL2 + Transduced T cells
- CL2 only

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Intermediate precision and repeatability

Operators

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Non-adherent cell line
Optimisation of cell attachment – capture antibody

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Optimisation of cell attachment – cell density

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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**

- Effector only
- Non-pulsed
- Non Specific 1
- Non Specific 2
- Specific Peptide

**Viability %**

- 100%
- 80%
- 60%
- 40%
- 20%

**time (h)**

- 0
- 5
- 10

**8 hours**

- Live
- Early apoptotic
- Late apoptotic
- Dead

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Correlation with impedance and quantitative image analysis

Target cells | Effector Cells | Dead cells
Summary

- 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
CAR-T cells
Chimeric Antigen Receptor (CAR) T cell therapies

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Challenges of targeting solid tumours

- Access to the tumour tissue
- Lack of appropriate target antigens
- Immunosuppressive tumour microenvironment
Tumour angiogenesis: potential therapeutic approach

Small tumor  Sprouting capillary  Growing tumor

Angiogenic factors

Nutrients from blood  Metastatic spread
CLEC14A – tumour endothelial marker

Breast carcinoma

Liver carcinoma

Bladder carcinoma

Human endothelial marker (ULEX)  CLEC14A  Nuclei (DAPI)

Mura et al. Oncogene 31:293 (2012)
Reduction in tumour burden in RipTag2 mouse model
(F. Maione, E. Giraudo, Turin)
Large scale generation of mRNA CAR-T cells

Starting Material
Electroporator
Cell Culture
CD4/8 Isolation
Harvest

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Real time detection of product’s potency within 4h

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Persistence of potency over time

**Cl at 20h**

Normalized cell index vs. time for transfected, transduced, and target cells only.

**IFN-gamma (pg/mL) vs. Granzyme B (pg/mL)**

Bar chart showing IFN-gamma and Granzyme B levels in untransfected and transfected conditions.

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Summary

- 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.

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### Acknowledgements

<table>
<thead>
<tr>
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