Development of a Novel Potency Assay to Quantify Immune Cell-Mediated Cancer Cell Killing

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Introduction
Adaptive T-cell therapy for the treatment of haematological cancers and solid tumours is one of the fastest growing areas in the cell and gene therapy field. These immunotherapies use genetic modifications of T-cells to engineer their specificity or enhance their function.

Potency is a critical quality attribute (CQA) for any cell therapy product and, while often difficult to measure, its assessment is required to determine quality, consistency, and stability. Here we demonstrate a novel rapid potency assay developed for a gene-modified T-cell receptor (TCR) immunotherapy product.

This assay utilises impedance based spectroscopy (sCELLigence RTCA, ACEA Biosciences) to give a rapid real-time, label-free measurement of cell killing activity. A suspension target cell line was loaded with a tumour specific peptide before being anchored to the impedance plate. Following attachment, engineered T-cells are added and the killing activity can be monitored by the decreasing impedance measurement.

Results

Assay Development
Tumour-specific peptide loaded antigen presenting cells (APC) were anchored to an electrode array using capture antibodies. When an electrical current is passed across the array the attached cells create resistance (impedance) which can be converted to Cell Index (CI). Capture antibody concentration and target cell seeding density were optimised to obtain the highest CI/impedance (Figure 1).

Figure 1 Optimization of target cell anchoring to the electrode array.

Transduced effector T-cells were added in increasing ratios from 0:1 (Effector:Target) to 5:1, the killing activity, as seen by the decrease of CI was measured over 24 hours (Figure 2). This data can be normalised as seen in Figure 3 to the target cell viability to determine the EC50 and maximum cell killing.

Figure 2 Impedance plots showing variable Effector:Target ratios over 24 hours.

Conclusion
- Suspension target cells were successfully adhered to the surface of the impedance plate electrodes using capture antibodies
- Use of an established cell line as target cells enhances assay consistency and reliability
- Engineered T-cells, which were directed against the tumour antigen, were able to specifically target tumour peptide-loaded cells
- This assay is label-free and does not require pre-loading with radioactive isotopes or other detection labels
- Assay readout correlates to both flow cytometry and quantitative imaging
- Real-time data showing the kinetics of killing rather than single end-point measurement

Though these results demonstrate the use of this assay in gene modified TCR immunotherapy product, it is equally applicable for other T-cell therapies. This novel potency assay provides a rapid, label-free, kinetic assessment of engineered T-cell cytolytic activity.

Figure 3 Normalized cell viability for different Effector:Target ratios.

Assay Cross-Validation
To cross-validate the targeting and killing effect, quantitative live cell imaging and flow cytometry analysis were performed (Figure 4 a and b).

Live Cell Imaging – Effector cells were stained with Hoechst dye (blue) and target cells were stained with Calcein AM (green). Propidium iodide was added to identify dead cells (red).

Flow Cytometry – Effector cells were pre-labelled with CFSE to differentiate them from target cells. After 8 hours, the cell population was stained with 7-AAD (dead cells) and Annexin-V (apoptotic cells).

Figure 4 a) Live cell imaging (left) showing time course specific targeting of effector T-cells (blue) to target cancer cells (green) followed by cytotoxic killing (red). Right plots show comparison between impedance and quantitative imaging. b) Flow cytometry analysis performed at 8 hours following effector T-cell addition.