The use of adoptive 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, with oncology targets accounting for approximately 40% of all cell therapy clinical trials currently being performed. A significant number of these immunotherapies use genetic modifications of T-cells using viral vectors to engineer their specificity or enhance their function. Examples of these products include gene modified T-cells expressing Chimeric Antigen Receptors (CAR) which direct specificity against cancer cell surface markers, or engineered T-cell receptors (TCR) which can target intracellular proteins through the presentation of their fragments on the cell surface by HLA molecules. Various strategies are applied for the manufacture of gene modified immunotherapies but the use of patient cells as a starting material and the use of viruses to deliver the CAR or TCR construct can lead to variability in terms of transduction efficiency, CAR/TCR expression and product potency. Characterisation is therefore critical both during manufacture to maintain consistency, and post manufacture to ensure sufficient function. Strategies for the characterisation of gene modified immunotherapies are continually evolving. However, a number of the methods commonly used for measurement of viral transduction and potency are complex, semi-quantitative, require complex pre-labelling of cells or are based on the detection of surrogate markers.

In this paper we demonstrate two novel approaches for the characterisation of a gene modified TCR immunotherapy product targeting the Wilms-tumour 1 (WT1) protein. WT1 expression has been demonstrated to be elevated in haematological malignancies such as acute myeloid leukaemia (AML) chronic myeloid leukaemia (CML) and myelodysplastic syndrome (MDS). The first approach uses single cell analysis to directly measure viral copy number integration into the genome and the expression of the WT1-TCR mRNA following transduction. This assay offers advantages over currently used techniques. From a safety perspective it provides high level characterisation of viral integration which can be used to optimise the manufacture process to control the number of integration events within the genome. It also offers a method to optimise the amount of virus used during manufacture which could have a significant positive impact on the cost of goods for product manufacture. Single cell mRNA analysis offers a direct functional measurement of TCR expression following viral transduction which overcomes the limitations of available antibodies specific for the antigen recognised by the TCR. The second approach demonstrated in this paper is for a novel potency assay which uses impedance spectroscopy to give a label free, real time measurement of cell killing by the WT1-TCR gene modified T-cells. This has many advantages over commonly used alternative methods such as the chromium-51 killing assay or surrogate assays looking at the stimulation of cytokine release. Firstly it is label free and does not require pre-loading of the target cells with a radioactive isotopes or other detection labels which can interfere with the assay readout. Secondly it can be performed with established cell lines which can act as antigen presenting cells reducing the assay variability associated with the use of primary cells. Thirdly, the impedance assay provides real time data showing the kinetics of the killing response rather than just a single end-point measurement.

These new assays are a valuable addition to the repertoire of techniques which can be applied to characterise immunotherapy products and while this paper demonstrates their use with a gene modified TCR products they are equally as applicable for CAR T-cell therapies and for measuring lentiviral based immunotherapy products.