SINGLE CELL ANALYSIS OF VIRAL TRANSDUCTION AS A NOVEL TOOLBOX FOR AN IMPROVED CHARACTERISATION OF CELL THERAPY PRODUCTS

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Ex-vivo gene modified cell therapies are increasingly being developed for the treatment of monogenic diseases and various forms of cancer. In the last few years three gene modified cell therapy products have been granted regulatory approval and there are currently over 600 clinical trials being conducted using this type of cell therapy. However, the risks associated with therapies based on genetically modified cells are not fully understood and safety concerns could hamper their ongoing development.
Retroviral or lentiviral vectors are often used in the generation of genetically modified cells, and these vectors integrate into the host genome for a long lasting and efficacious therapeutic effect. Due to their random integration, viruses may disrupt key genes in the host cells leading to therapy-induced secondary diseases. Hence, ensuring consistency during the transduction process through controlled delivery and integration of the viral vectors is critical to controlling the product quality.
Current methods are limited to the analysis of viral copy number on populations of cells. Whilst this gives an estimate of the viral cell copy number across the population, it does not account for cell-to-cell variations in the number of viruses integrating into the genome and the risk that rapidly proliferating clonal populations could compromise the efficacy and the safety of the product. Though this method is a regulatory requirement, the limitation of population-based assessment has prevented its use as an effective in-process control and development tool. New techniques to accurately determine the copy number per cell will be key to improving product characterisation and the regulatory criteria for product safety.

To address this need we have designed a single cell capture, processing and analysis approach to measure the viral copy number heterogeneity of a human T-cell immunotherapy model. Our unique and novel approach uses cutting-edge droplet digital PCR to evaluate the cell-to-cell variability within the population by accurately measuring the number of viral integrations per single cell. This method provides a robust and reliable toolbox for the analysis of integration events in single cells and can be fully customisable for the analysis of any viral or non-viral transgene.

The incorporation of this method within a process development pathway means that key factors that impact transduction and promote homogeneity in viral integration, including viral input, can be effectively optimised, improving a critical step in the immunotherapy manufacture workflow.
In addition, the use of this method as a replacement of the current population-based safety test, ensures that cell therapy products meet a high safety standard with a known number of cells containing a desired number of viral copies.
This assay is currently being incorporated into the manufacturing process of a cell therapy company and has the potential to play a key role in supporting the development of safe and efficacious cell therapy products for the treatment of a range of diseases.

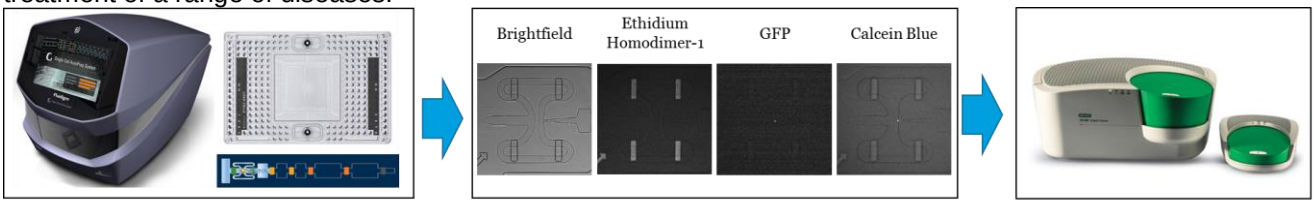

Figure 1: Single cell viral copy number workflow.