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AUTOMATION OF FLOW CYTOMETRY ANALYSIS IN CELL THERAPY PRODUCTION

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Keywords: flow cytometry.

Background & Aim: Flow cytometry is routinely used in cell therapy production to assess incoming material, for in- process testing, and for QC testing of cell product prior to release. An important topic in the quality of flow cytometry results is the process of data analysis. Even with a defined standard operating procedure for analysis, there is a risk of subjective operator-to-operator variation, the difficulty of training new operators, and significant time required to analyze results. An objective and automated approach to implement a user-defined analysis procedure would be beneficial. Here we present such an approach and compare it to expert operator results for incoming apheresis material and final cell product.
Methods, Results & Conclusion: The approach is termed adaptive gating and developed as a beta plugin for BD FlowJo™ software. Much as an expert flow cytometrist, this approach adapts analysis gates to a new sample based on prior knowledge from similar samples. The algorithm consists of three main steps. First, images are created of the reference (training) plot and the ungated (target) data plot. Next, image registration is performed to find a function which transforms the training plot to look like the target plot. Finally, this function is applied to the gate vertices, such that they are adjusted to the target data. The function is actually a collection of functions forming a grid over the entire plot space such that local adjustments occur to better account for biological variation. To test the adaptive gating approach, we analyzed 15 FCS files from apheresis samples (3 different lots, each prepared, stained, acquired, and analyzed in replicates of 5) and 15 FCS files from final product samples (3 different lots, each prepared, stained, acquired, and analyzed in replicates of 5). Manual gating of all files was performed by two operators and the results were averaged and compared with the automated, adaptive gating results. For the apheresis samples, %T cells and %B cells were compared. For the cell product, %CAR of viable T cells was compared. The percentage change between manual and automated results was within 2% for the apheresis samples and within 15% for the cell product, which met pre-defined acceptance criteria of 20%. This adaptive gating approach can be evaluated in additional cell therapy production contexts and considered as a useful tool to make flow cytometry analysis more automated and objective.

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A SUPPLY CHAIN CRISIS STORY: CULTURE BAG SHORTAGE ENFORCED VALIDATION OF AN ALTERNATIVE EXPANSION SYSTEM FOR CAR T CELLS

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Keywords: Supply, Chain, Challenge.

Background & Aim: The recent supply chain crisis highlights a need to establish alternative manufacturing (MFG) protocols ensuring continuity of existing and new cell therapy (CT) clinical trials. Our academic CT program, and likely others, experienced purchasing delays and restrictions caused by diversion of critical supplies to meet COVID-19- related research demands and/or reduced vendor capacity due to resource constraints, including attrition of skilled workforce. Mitigation strategies aimed at creating process redundancies overcome production challenges resulting from a scarcity of goods. Here, we validated an alternative ex vivo culture system to clinically MFG lentiviral vector (LV) modified CAR T cells due to limited availability of cell expansion culture bags for the Wave bioreactor, a critical unit of operation that we have used to successfully MFG thousands of gene-modified T cell products for 30+ clinical trials.
Methods, Results & Conclusion: The disposable G-REX culture vessels were compatible and seamlessly integrated with our closed system platform. Mesothelin CAR T cells were manufactured in parallel via the G-REX or conventional Wave bioreactor using consented patient starting material. Critical quality attributes of the final T cell products, including viability, transduction efficiency, phenotype and function were assessed. Transduction efficiencies assessed by flow cytometry and/or molecular qPCR were lower in products generated in the G-REX compared to the wave using the same multiplicity of infection. However, at least 50-fold expansion was achieved, with cell viabilities greater than 90% and with comparable cellular phenotypes. The Meso CAR T cells generated by either process were capable of eliciting CAR-mediated cytotoxicity and effector cytokine production. Strikingly, 2-4 billion T cells were harvested from a starting seed number of just 50 million T cells in the 1L G-REX, which may be sufficient to meet most protocol- specified cell therapy doses, suggesting that a full apheresis collection may not be needed. Notably, this process required just 1/3 of the starting material, 1/5 of the media and decreased manual effort through culture duration compared to the Wave. Additionally, the reduced reliance on specialized capital equipment combined with a small footprint enables simultaneous MFG of several immunotherapy products. These advantages propose consideration in replacement of current expansion platform as well as validating an alternative process for MFG CAR T cells.

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A NOVEL HIGHLY SCALABLE, MICROFLUIDIC APPROACH TO ELECTROPORATION

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Keywords: Electroporation, Microfluidic, Scalable.

Background & Aim: The need for non-viral methods of cell modification is widely recognised. A key driver for this includes the desire to avoid the high cost of vector production. In addition, the supply chain challenges that arise in securing both manufacturing capacity and ensuring vector capacity matches the demand result not only in high costs but also uncertainty in production. In some geographies, the viral vector is regulated as a drug product increasing the approvals challenges. The desire to overcome these requires new technologies to be developed. Electroporation has the potential to resolve these challenges. It also has the potential to deliver a wider range of pay loads, and larger payloads than can be delivered by viral vectors. However, it suffers from being a bulk process and has trade-offs around key performance metrics such as cell viability and transfection efficiency. This results in electroporation having scalability and performance issues which have so far hampered its widespread adoption in high volume cell therapy manufacture.
Methods, Results & Conclusion: By utilising a high throughput, single cell, microfluidic approach, it is possible to achieve both scalability and efficiency. This approach has the ability to ensure all cells experience the same conditions allowing for precise optimisation of condi-