PROCESS DEVELOPMENT AND MANUFACTURE OF PRIMARY HUMAN T-CELLS IN SCALABLE, AUTOMATED STIRRED-TANK BIOREACTORS

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Key Words: T-cell manufacturing, stirred-tank bioreactor, T-cell phenotype, metabolite flux.

Engineered Chimeric Antigen Receptor (CAR) T-cell products have recently gained FDA and EMA approval and have demonstrated significant clinical efficacy against non-Hodgkin lymphoma and pediatric B-cell acute lymphoblastic leukemia. Despite the significant clinical and commercial progress these products represent, the high costs associated with patient-specific cell therapy manufacture needs to be addressed.

The work presented here focuses on the growth of human primary T-cells and CAR-T cells across a range of commercially available expansion platforms, including stirred tank bioreactors, which although routinely employed for the production of biologics, are not commonly used for the manufacture of T-cells. Initial experimental studies were carried out in an automated ambr® 250 single use bioreactor system which has demonstrated significant success for suspension and adherent mammalian cell culture applications. Building on previous work undertaken in the group which developed a new bioreactor vessel for microcarrier culture, both the new and existing bioreactor vessels were characterized with respect to cell yield, fold expansion, viability, metabolite profile, T-cell subpopulations and kLa. The comparison between the two vessels was performed based on power per unit volume, kLa and stirring speed, ranging from 100 to 200 rpm, using at least 3 different donors per condition.

T-Flask expansion of human primary T-cells was carried out as a static control and results were compared with the dynamic culture conditions (Figure 1). Results revealed a significantly higher fold expansion ($p<0.05$) in the ambr® 250 bioreactor at 200rpm (24.53 ± 1.50) compared to the static platform (16.38 ± 2.91). Moreover, the final product composition in terms of cell phenotype was not affected by the stirring regime. The dO_2 concentration, pH, and metabolite flux was measured throughout enabling for a better understanding of culture performance.

Further studies have compared the growth and quality of human primary T and CAR-T cells across a range of different expansion platforms including a rocking-motion (WAVE®) bioreactor, G-Rex® cell culture device, gas-permeable cell culture bags and the ambr15® microbioreactor. For each platform, the same culture conditions were used including cell source, medium formulation, and seeding density. The findings from this extensive comparability study will be presented, including an overview of the cell phenotype and quality. The findings will be used to identify the capability of each expansion platform for CAR-T process development and manufacture with the aim of developing a cost effective process for both autologous and allogeneic CAR-T cell therapies.

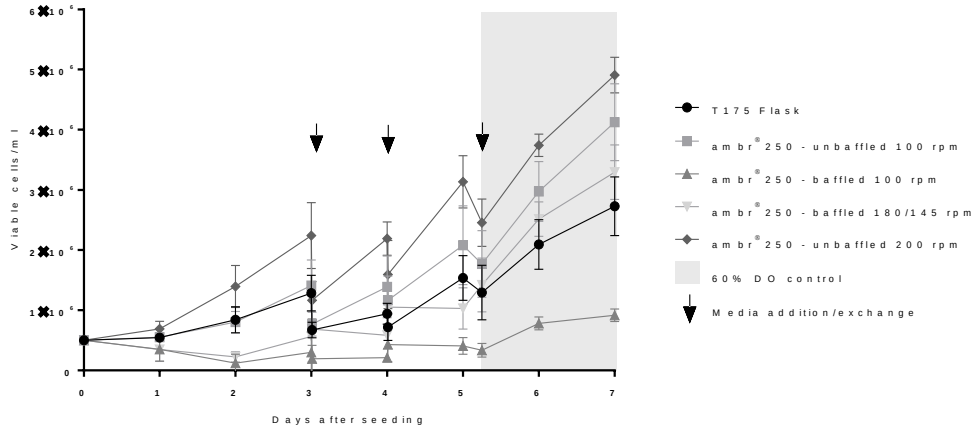

Figure 1 – Growth curves showing the viable cells density in two different vessels. The stirring speed range was varied between 100 and 200 rpm. Media addition was performed on day 3, 4 and 5, while a 60% DO control was introduced from day 5 until day 7. The cells were then harvested and stained for flow cytometry analysis.