SCALING A XENO-FREE FED-BATCH MICROARRIER SUSPENSION BIOREACTOR SYSTEM FROM DEVELOPMENT TO PRODUCTION SCALE FOR MANUFACTURING XF hMSCs

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ABSTRACT

• There have been > 900 clinical trials investigating the use of human mesenchymal stem/stromal cells (hMSCs) for regenerative medicine, driving the need for an economical biomanufacturing paradigm.

• A scalable process and production technology that can generate billions to trillions of cells per manufacturing lot is needed to meet the clinical demand.

• Suspension bioreactors show great promise in reaching commercially-viable working volumes. However, scalability of cell production remains a challenge.

• This study developed a scalable xeno-free (XF) hMSC bioreactor process that maintains the final cell population doubling level (PDL) within the recommended range of 16-20 to ensure product quality.

• The ability to scale XF hBM-MSC expansion in a low shear, single-use, vertical-wheel suspension bioreactor was evaluated at small scale (0.1L), development scale (3L), pilot scale (15L), and production scale (50L), using high volume XF cell banks, an optimized XF fed-batch media system, and XF microcarriers in a scalable bioreactor system.

• Cell yields of >0.5M cells/ml were achieved in all bioreactor scales within 5 days of culture with no media exchange. Comparable nutrient and waste profiles, cell growth curves, and pH were observed at each scale.

• Cells from all bioreactor scales maintain the hMSC critical quality attributes of osteogenic, adipogenic, and chondrogenic differentiation potential and cell surface marker expression, as well as functional attributes of angiogenic cytokine secretion and inducible immunomodulatory potential (as measured by functional IDO activity), which are comparable to 2D control cells of similar PDL.

• Our expansion of XF hMSCs in a scalable bioreactor culture platform can provide significant time and cost savings for translational researchers and product developers in the fields of cell therapy, regenerative medicine, and tissue engineering.

FED-BATCH PROCESS OUTPERFORMS 1/2 MEDIA EXCHANGE & BATCH CULTURE, AND INCREASES MEDIA PRODUCTIVITY IN BIOREACTOR

• Fed-Batch process shows a distinct advantage on final cell yield and media productivity over 1/2 media exchange and Batch process. (*) indicates statistical significance between Fed-Batch and Batch systems at Day 5 and Day 6 (p<0.05). Comparison study was performed in scaled down 0.1L bioreactors.

• hMSC manufacturing platforms have evolved as the demand for cells increases.

• Suspension bioreactor production of hMSCs is a scalable manufacturing platform that can provide the necessary lot sizes of billion to trillions of cells while reducing the time, labor, and cost of goods (COGs) for regenerative medicine applications.

ESTABLISHED hMSC BIOREACTOR EXPANSION PROCESS

• hMSCs seeded into bioreactor on day 0 are fed with RoosterReplenish™-MSC-XF on day 3, and are ready for harvest on day 4 or 5 of culture.

• hMSC proliferation was monitored throughout culture as observed by the formation of cell-microcarrier agglomerates during expansion.

• Distribution of cells on microcarriers throughout the bioreactor culture was comparable across scales, demonstrating a scalable seeding strategy. Blue dashed lines indicate end of bioreactor culture.

• Feed-Batch processes achieved 4 times higher final cell yield and media productivity compared to 1/2 media exchange and Batch processes. (*) indicates statistical significance between Fed-Batch and Batch systems at Day 5 and Day 6 (p<0.05). Comparison study was performed in scaled down 0.1L bioreactors.

• Concentration of glucose, glutamine, lactate, and ammonia were comparable in all bioreactor scales (0.1L, 3L, 15L, 50L), and are maintained at desired levels throughout culture to support cell expansion. Glutamine is present in excess in media, and breaks down into ammonia with increased storage duration.

• XF hMSCs expanded in the bioreactor maintained their tri-lineage differentiation potential, expansion potential, and cell surface marker expression identity.

• Cell functional attributes are maintained as shown by comparable inducible interleukin 2,3-dioxogenase (IDO) activity when stimulated with interferon-gamma (IFNγ), and angiogenic cytokine secretion profile.

• A robust, scalable XF hBM-MSC culture process was developed in a vertical-wheel suspension bioreactor up to production scale (50L).

• Consistent yields of >0.5M cells/ml is achieved in all scales

• hMSC critical quality attributes and functionality are maintained

The demonstrated scalability of hBM-MSC culture allows significant time and cost savings as a standardized system, using streamlined processes, for translational researchers and hMSC-based product developers.

MAINTAINED CELL CRITICAL QUALITY ATTRIBUTES AND FUNCTIONALITY ACROSS BIOREACTOR SCALES

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• Cell functional attributes are maintained as shown by comparable inducible interleukin 2,3-dioxogenase (IDO) activity when stimulated with interferon-gamma (IFNγ), and angiogenic cytokine secretion profile.

SUMMARY