AN AUTOMATED AND CLOSED SYSTEM FOR PATIENT SPECIFIC CAR-T CELL THERAPIES

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Key Words: automation, manufacturing, autologous, CAR-T, closed

Autologous cell therapies, particularly chimeric antigen receptor T-cell (CAR-T) immunotherapies, are becoming a promising treatment option for difficult diseases. Immunotherapies for blood cancers have dominated the pipeline, while treatments for solid tumors have started to become more successful. However, as the market continues to grow and more clinical trials begin globally, the challenge of manufacturing autologous cell therapies remains significant. A greater number of patients will lead to an increase in cost, labor, and the complexity of logistics for scaling out the commercial production of patient specific therapies. To enable clinical and commercial success, novel manufacturing platforms, such as closed and automated systems, will be required to produce cost effective and robust therapies. This abstract highlights a successful CAR-T process translation from a manual process to an automated patient scale system.

To accomplish a CAR-T process translation, we utilized a platform that automates cell seeding, activation, transduction, real time process monitoring, feeding, washing and concentration, and harvesting. In order to mimic a therapeutic CAR-T cell process, manual research scale processes were optimized, scaled up, and then programmed to run automatically without manual intervention. In these processes, 100 million peripheral blood mononuclear cells (PBMC) were first inoculated with CD3/CD28 activation beads. The following day, cells were transduced with HER-2 lentivirus vector. Cells were then expanded with a defined feeding strategy and IL-2 supplements until harvested when target yields were reached. After harvest, cells were analyzed for cell yield, viability, transduction efficiency, and an array of cell phenotype, potency and functionality via FACS and killing assays. Specifically, CAR-T cells were analyzed for the presence of naïve T cells, T stem cell memory, T central memory, T effector memory, and T effector cells.

We show here how we optimized, scaled up, and automated manual processes to reach clinical requirements. Automated runs using the above process with cells transduced by HER-2 virus yielded an average of 2×10^9 cells post harvest with a viability > 90%. Automated runs and associated controls were able to support the expansion of both CD4+ and CD8+ T cells with 73% CD4+ T cells and 20% CD8+ T cells. Harvested cells yielded approximately 80% NGFR+ cells with a higher detection of NGFR in the CD4+ fraction than in the CD8+ fraction for all samples. Both CD4+ and CD8+ subsets demonstrated T cell phenotype such as naïve T cells, T stem cell memory, T central memory, T effector memory, and T effector cells. Both subsets also only expressed between 15-20% of immunosuppressive regulatory T cells. Cell health was evaluated by the levels of exhaustion marker, PD-1, which was 19% in CD4+ T cells and < 1% in CD8+ T cells. Furthermore, there was a negligible amount of senescent T cells and anergic cells and < 10% expression of the apoptotic marker, Caspase-3. Subsequently, cells from multiple automated runs showed the specific killing of NGFR+ tumor line were correlated with high levels of effector cytokines: TNF-alpha (~34%) and IFN-gamma (20-25%) as compared to a manual control. In summary, automated CAR-T process in the Cocoon system yields a healthy populations of T cell subsets. This system is a viable solution to translate labor-intensive CAR-T process into a fully automated system, thus allowing scalability, high yield, reduction of manufacturing cost, and better process control to yield high quality CAR-T cells.