OPTIMIZATION OF HUMAN T CELL EXPANSION *EX VIVO* USING SERUM-FREE MEDIUM AND THE GAS-PERMEABLE RAPID EXPANSION CELL CULTURE DEVICES (G-Rex)

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Adoptive immunotherapy with *ex vivo*-modified T cells shows immense promise as an emerging strategy for patients with advanced malignancies. Although promising, most current methods for expansion of gene-modified T cells *ex vivo* are complicated and labor intensive, limiting the broad application of adoptive immunotherapy in the future. Traditional T cell expansion protocols are marked by flaws in consistency, safety, the frequency of human intervention required, and the length of the manufacturing process. These expansion protocols have traditionally involved the use human serum, which is characterized by batch inconsistency, and can potentially expose the patient to adverse viral contamination. Moreover, the duration of the expansion phase has typically been 10-12 days and involves extensive hands-on operation, while an ideal process would be significantly shorter and require minimal cellular disturbance. For the purpose of safety and consistency, serum-free T cell expansion media have been developed, and to minimize complexity of the manufacturing process, many researchers in the field are turning to the Gas Permeable Rapid Expansion (G-Rex) culture platform, which has shown superior cell output and a reduction in the number of required technician manipulations compared to conventional approaches. However, to date, there is a dearth of knowledge regarding the use of serum-free media in G-Rex systems. To this end, we tested several commercially available serum-free media to see how effectively they could expand human T cells in the G-Rex culture system. The results demonstrated that none of the serum-free media consistently performed as well as conventional methods that employ serum-containing culture media. However, when we further supplemented the best performer from this cohort of serum-free media with 4mM GlutaMAX™ and 2% of a chemically defined serum replacement, this medium supported ample T cell expansion, with yields similar to or better than media containing human serum. Furthermore, the resultant cell population displayed a higher frequency of the desirable central memory phenotype than the cells grown in serum-containing media and was indistinguishable from the serum-grown population with regards to both CD8/CD4 ratio and functionality. We conclude that combining serum-free media with the G-Rex culture platform can be effective for human T cell expansion and that applying this cell culture strategy to the production of T cell therapies could potentially address some of the concerns associated with traditional protocols by ensuring safety and consistency, shortening the expansion phase, and reducing the excessive amount of technical intervention required.