DEVELOPMENT AND OPTIMIZATION OF ANIMAL ORIGIN-FREE, SERUM-FREE MEDIA FOR HUMAN TREG MANUFACTURING

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Key Words: Regulatory T cells (Tregs), Media development, Serum-free, Dynabeads™, Treg immunotherapies

Regulatory T cells (Treg) constitute a small subset of immunosuppressive CD4+ T cells. Studies have shown that imbalanced or aberrant Treg function can result in autoimmune disorders. The importance of Tregs in dampening immune responses has been described in multiple studies and Treg immunotherapies are being explored to develop personalized therapies for various autoimmune diseases. Scalable commercial development of Treg therapies suffers similar challenges as other T cell immunotherapies: biosafety and supply chain concerns of human serum and limitations regarding bioprocess development due to serum variability. Additionally, there a challenges regarding Treg isolation for both magnetically isolated (blockade of CD25+ epitope may affect function and low purity) and flow cytometry sorted Tregs (low numbers and viability). In addition, the starting population and purity (measured by FOXP3 expression) can be low, resulting in small cell numbers post expansion which can impact dose escalation studies. To address these challenges, we are developing a serum-free, animal origin component – free, defined medium and Treg optimized Dynabeads™. Our strategy was to exploit metabolic differences between Tregs and conventional Tcells as well as optimizing the level of activation ligands to develop a defined Treg manufacturing system. Using design of experiment (DOE) approaches we explored factors described in the literature to be associated with Treg development. DOE studies were followed by testing in combination with Treg Dynabeads™ in development. Feasibility was evaluated with positively selected Tregs (CD4+CD25+CD127lo, n=5). Tregs cultured in our system achieve higher FOXP3+ frequencies (>60% FOXP3+) outperforming control containing 10% human serum (~30% FOXP3+). In summary, our results suggest that serum can be eliminated from Treg workflows to generate highly suppressive enriched FOXP3+ Treg immunotherapy product. We believe that our defined serum-free medium and Dynabeads™ Treg system will enable the development of better immunotherapies for autoimmunity.