

DEVELOPMENT OF A CLOSED CAR-T MANUFACTURING PROCESS

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The field of immunotherapy has emerged as a promising new type of treatment for cancer with the approval of the first two CAR-T therapies. The clinical success of T-cell based immunotherapies necessitates a robust manufacturing process for these products to be consistently produced at commercial scale. Our CAR-T workflow combines unit operation specific solutions for thaw of an apheresis unit, wash, CD3 selection, T-cell activation, lentiviral transduction, incubator- and reactor-based expansion culture, harvest, formulation, cryopreservation and thaw of CAR-T product. We have evaluated the impact of both serum-containing and xeno-free culture media, commercially available T-cell selection and activation reagents, closed small-scale culture vessel options, alternative solutions to enhance transduction, and the specific timing of process steps to develop a modular platform process that is robust and flexible for the varied needs of CAR-T developers. Frozen apheresis units are processed using the SmartWash protocol on the Sepax™ 2 and T-cells are isolated with EasySep™ Release CD3 Positive Selection Kit. The cells are then activated with ImmunoCult CD3/CD28/CD2 T-cell activator before being transduced 24 hours later using the Sepax™ 2. Expansion of T-cells are carried out in two stages: incubator-based culture before going into the Xuri™ Cell Expansion System W25 with a perfusion feeding regime. Cultured cells are then harvested and washed in Plasmalyte-A with human serum albumin and formulated with CryoStor® CS10 using the FlexCell protocol on the Sefia™ Cell Processing System. The final cell products are cryopreserved using the VIA Freeze controlled-rate freezer. We have also accessed a point-of-care thawing strategy using the VIA Thaw. Our CAR-T process achieves greater than 1.0E10 expanded T-cells with >80% eGFP transduction efficiency across an 8-day manufacturing process.