344 COMPARATIVE ASSESSMENT OF PRODUCT QUALITY ATTRIBUTES ASSOCIATED WITH PRODUCTION OF CHIMERIC ANTIGEN RECEPTOR T CELLS EXPRESSING SCFV CD-19 AND SCFV IL-13RA2 BY TWO MANUFACTURING PLATFORMS

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Background The recent advances in adoptive immunotherapy of cancer have led to the FDA approval of chimeric antigen receptor modified T (CAR-T) cells targeting CD19 antigen on advanced hematological malignancies, which has resulted in dramatic responses in large numbers of patients. Because of several challenges and complexities in manufacturing for characterizing CAR-T cell products, it is important to optimize their manufacturing and critical quality attributes (CQAs). Herein, we performed a comparative assessment of scFv-CD-19-CAR-T and IL-13Ra2-CAR-T cells and manufactured using conventional and G-REX platforms.

Methods We manufactured lentiviral vectors (LVV) by employing common molecular biology techniques using HEK 293T as a producer cell line, transfected with scFVIL-13Ra2 or CD19 transgene plasmids along with three helper plasmids. The LVVs were used for transducing PBMCs from normal human blood donors after their activation and were expanded in T cell growth medium supplemented with cytokines including IL-2 or IL-2 + IL-15, IL-7 + IL-15 for specified durations. A battery of activation and exhaustion phenotype markers was studied at the end of expansion along with their potency to lyse the target cells and secrete Interferon-gamma in a co-culture assay.

Results Our results showed that the T cell activation was efficient by commercially available reagents such as anti-CD3/CD28 coated magnetic beads or nanoparticles and transduction either by retronection or Vectofusin. The CAR-T cell yields were at least two logs higher in the G-REX platform. Analysis of phenotype markers for T cells (CD3, CD4, CD8), T cell activation (CD25, CD45, CD69)/exhaustion markers (PD-1, LAG-3, TIM-3) and Central memory T cells, {CD45RA low, CD62L (CCR7 high} revealed better health and yield of CAR-T cell products manufactured in G-REX platform.

Conclusions We conclude that G-REX platform was superior to conventional methods for manufacturing both types of CAR-T cells. The data also suggest that better activation and transduction by specific reagents and expansion in combination with selected cytokines are key issues associated in manufacturing healthy and potent CAR-T cell products.

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