PROCESS DEVELOPMENT AND SCALE-UP FOR GENE CIRCUIT ENGINEERED CAR-NK CELL MANUFACTURING

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Allogeneic Natural Killer (NK) cell therapy has shown promise in recent years for treating cancer in patients without inducing graft versus host disease and with potential for off-the-shelf administration. Senti Bio is using gene circuits to introduce logic-gating and regulated expression of payloads into next-generation CAR-NK cell therapies to broaden the therapeutic indications and improved efficacy in liquid and solid tumors. Key process development objectives for gene circuits include the ability to efficiently and stably transduce multi-gene constructs into primary NK cells while retaining cell expandability and anti-cancer function. Here, we describe a scalable GMP-ready manufacturing process for generating clinically relevant numbers of CAR-NK cells, and we demonstrate its potential applicability to our product pipeline.

To achieve a batch size target of >10^11 NK cells, we aimed to develop a process to start with ~25*10^6 isolated NK cells, achieve >40% CAR+ transduction, and obtain >5,600-fold expansion over 21 days. Enrichment of adult apheresis material from 12 healthy donors via CD3 depletion and CD56 selection yielded an average of ~3*10^8 NK cells, which were cryopreserved for later use. Upon thaw, NK cells were activated using proprietary irradiated gene-modified feeder cells and expanded in a closed system 1L G-Rex chamber. Seven days later, NK cells were transduced with retroviral vectors using closed system procedures, resulting in up to 80% CAR+ population. Gene circuits were tested across multiple retroviral vector delivery systems, and successful constructs were developed into producer cell lines (HEK293) using various single cell cloning techniques with the goal of generating stable, high titer vector producer clones. Primary NK cell transduction efficiency was optimized by testing a range of MOI, comparing different vector addition and spinoculation vessels, and the effect of GMP-compatible transduction enhancers. Transduced NK cells were expanded further in multiple closed system G-Rex culture vessels for a total process time (initial NK thaw to CAR-NK harvest) of approximately 21 days. Different expansion methods were assessed including different irradiated modified cell lines and feeder-free NK expansion technologies achieving ~10,000-fold expansion in the 1L vessels. At cell harvest, the cell suspension was volume-reduced, harvested and formulated into cryopreservation medium using an automated cell processing system, yielding ~4*10^9 cells per liter of culture. Formulated cells were filled in vials and stored in liquid nitrogen vapor phase. Functional assessment was performed via both *in vitro* and *in vivo* studies, demonstrating significant CAR-specific cancer cell killing compared to non-transduced NK cells. We also evaluated multiple donors for transduction efficiency, growth characteristics, cancer cell killing specificity, scalability, immunomodulatory function, single cell transcriptomics and distribution and kinetics *in vivo* to determine desirable attributes for manufacturing. This CAR-NK manufacturing process is expected to be suitable for translation to GMP clinical manufacturing in support of Senti Bio's internal allogeneic CAR-NK cell pipeline.