in the CD13+ myeloid compartment (88.3 ± 4.5%) compared to other lineages, including CD20+ lymphoid (9.1 ± 3.9%), CD235a+ erythroid (0.2 ± 0.1%) and CD41+ megakaryocytic (0.6 ± 0.2%) lineages. Interestingly, human myeloid engraftment was superior in recipient mice engrafted with human CD18+ cells (81.5 ± 4.3%) compared to animals transplanted with non-transduced (CD18-) LAD-1 cells (65.3 ± 11.3%). Integration site analysis of engrafted human cells is ongoing. Thus, FVV-mediated transduction of human LAD-1 CD34+ cells leads to clinically significant levels of CD18 expression, supporting the use of this CD18-expressing FVV in a human clinical trial.

Vector and Cell Engineering/Manufacturing

286. Genome Editing of Inducible Cell Lines for Scalable Production of Improved Lentiviral Vectors for Human Gene Therapy

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Lentiviral vectors (LVs) represent efficient and versatile vehicles for gene therapy. Current manufacturing of clinical-grade LVs mostly relies on transient transfection of plasmids expressing the multiple vector components. This method is labor and cost intensive and becomes challenging when facing the need of scale-up and standardization. The development of stable LV producer cell lines will greatly facilitate overcoming these hurdles. We have generated an inducible LV packaging cell line, carrying the genes encoding for third-generation vector components stably integrated in the genome under the control of tetracycline-regulated promoters. These LV packaging cells are stable in culture even after single-cell cloning and can be scaled up to large volumes. In order to minimize the immunogenicity of LVs for *in vivo* administration, we set out to remove the highly polymorphic class-I major histocompatibility complexes (MHC-I) expressed on LV packaging cells and incorporated in the LV envelope. We performed genetic disruption of the β-2 microglobulin (B2M) gene, a required component for the assembly and trafficking of all MHC-I to the plasma membrane in LV producer cells, exploiting the RNA-guided Cas9 nuclease. The resulting B2M-negative cells were devoid of surface-exposed MHC-I and produced MHC-free LVs. These LVs retain their infectivity on all tested cells *in vitro* and efficiently transduced the mouse liver upon intravenous administration. Strikingly, the MHC-free LVs showed significantly reduced immunogenicity in a T-cell activation assay performed on human primary T cells co-cultured with syngeneic monocytes exposed to LV, from several (n=7) healthy donors. To reproducibly generate LV-producer cell lines from these cells, we insert the LV genome of interest in the *AAVS1* locus, chosen for robust expression, exploiting engineered nucleases and homology-directed repair. By this strategy, we have obtained several independent producer cell lines for LVs that express marker or therapeutic genes and are devoid of plasmid DNA contamination. LVs produced by these cells reproducibly show titer and infectivity within the lower bound range of standard optimized transient transfection, and effectively transduce relevant target cells, such as hematopoietic stem/progenitor cells and T cells *ex vivo* and the mouse liver *in vivo*. Overall, we provide evidence that rationally designed targeted genome engineering can be used to improve the yield, quality, safety and sustainability of LV production for clinical use.

287. Production of KTE-C19 (Anti-CD19 CAR T Cells) for ZUMA-1: A Phase 1/2 Multi-Center Study Evaluating Safety and Efficacy in Subjects with Refractory Aggressive Non-Hodgkin Lymphoma (NHL)

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Introduction: An ongoing anti-CD19 CAR T cell study with KTE-C19 in subjects with refractory, aggressive NHL achieved objective responses in 5/7 subjects, including 4 complete remissions (Locke, ASH 2015). To support this trial (ZUMA-1, NCT02348216) and other multicenter trials in lymphoma, we developed a robust and efficient strategy to generate autologous anti-CD19 CAR-engineered T cell products. The newly developed process aims to minimize the time between subject leukapheresis and product administration, and generate a KTE-C19 lot for every enrolled subject. **Methods**: Upon confirmation of eligibility, leukapheresis was performed to process 12-15 L of blood targeting collection of 5-10 x 10^9 peripheral blood mononuclear cells (PBMC). After collection at the investigational site, subject apheresis material was shipped to the central manufacturing site, where it was processed to enrich for the T cell-containing PBMC fraction on a closed system Ficoll™ gradient. In a closed bag system, T cells in the PBMC fraction were then activated using an anti-CD3 monoclonal antibody and cultured in serum-free medium containing 300 IU/ml of IL-2. Magnetic beads were not used for either cell selection or activation. Activated T cells were transduced with a gamma retroviral vector that contains the anti-CD19 CAR gene and further expanded for 4 to 6 days to achieve the target cell dose of 2 x 10^6 CAR-positive T cells/kg (minimum of 1 x 10^6). Final KTE-C19 product was washed, cryopreserved and tested for identity, potency, and adventitious agents. In-process samples were collected for analysis by flow cytometry for CAR expression and other characteristics. After meeting acceptance criteria, the KTE-C19 product was shipped back to the clinical sites using a validated cryo-shipper. **Results**: 7 subjects were dosed in the phase 1 portion of ZUMA-1. KTE-C19 was successfully manufactured in all cases despite a broad range of baseline leukocyte counts (median 5.4x10^3 cells/μl, 2.1-11.1) and low numbers of baseline lymphocytes (median 0.9x10^3 lymphocytes/μl, 0.1-1.4) prior to apheresis. Apheresed cell populations used to generate clinical lots had a broad range of phenotypic characteristics including lymphocyte / monocyte ratio (median 1.1, 0.04-3.5), CD8/CD4 ratio (median 4.2, 0.3-7.7), and naïve plus central memory (Tcm) / more differentiated T cells (median 0.3, 0.04-1.3). Fold-expansion of T cells was consistent among the 7 product lots (average 6-fold) from transduction to harvest. All KTE-C19 lots contained predominantly CD3+ T cells (median 96%; 90-99%), with CD8+ T cells (median 57%, 27-82%) and CD4+ T cells (median 43%, 18-73%). Both CD4+ and CD8+ T cells expressed CAR at similar levels. The product lots contained predominantly effector memory (median 42%, range 30-56%) and Tcm cells (median 34%, 15-58%), with the remainder being naïve (median 14%, 6-19%) and effector T cells (median 5%, 1-22%). All KTE-C19 lots met release specifications and were available for clinical administration within ~2 weeks of apheresis. **Conclusions**: We developed a robust KTE-C19 manufacturing process that successfully generated biologically and clinically active product irrespective of the characteristics of the starting cell populations processed to date. Product lots met release specifications and were available for subject treatment within the target timeframe. This centralized manufacturing process is well suited to support multicenter clinical trials.