THE DEVELOPMENT OF A 14-DAY NON-VIRAL ENGINEERED CAR T-CELL PROCESS

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Immunotherapy utilizing chimeric antigen receptor (CAR) T cells is a promising strategy for the treatment of several types of cancer. Many preclinical and clinical studies engineer CAR T cells through a viral vector, presenting the potential for genotoxicity or insertional mutagenesis. We propose a 14-day non-viral process where we introduce the gene of interest via electroporation; integration can be achieved with the Sleeping Beauty transposon system. Minicircle (MC) DNA constructs containing the CAR, a surface marker (EGFRt), and a double mutant of dihydrofolate reductase (DHFRdm) are electroporated into previously frozen, unstimulated CD4/CD8 T cells with an RNA construct coding for the Sleeping Beauty transposase. After electroporation, cells are bead-stimulated with CD3/CD28 without the use of feeder cells throughout the process. CAR+ cells expressing DHFRdm are rendered insensitive to an FDA-approved small molecule drug, methotrexate (MTX), which allows for chemical selection of the cells of interest while avoiding a magnetic bead sort. The entire process is completed in 2 weeks with a media formulation that contains a serum-free replacement.

First, we standardized electroporation conditions and observed that lower DNA concentrations resulted in lower basal levels of integration within T cells based on flow cytometry. Conversely, increased cell concentrations did not necessarily result in higher proportions of transgene positive cells. We also found that altering the concentration of MTX for chemical selection can select for cells that have a higher level of expression of the gene of interest based on MFI. Using this 14-day process and chemical selection, we were able to reach purities of >90% CD19CAR+ T cells where the majority of cells at the end of culture were of a minimally differentiated phenotype, expressing both CD45RA and CD62L. Preliminary characterization of metabolic phenotype showed that at the end of the 14-day process, cells were able to maintain a spare respiratory capacity with or without MTX selection. Initial studies showed that CD19CAR+ cells were able to produce cytokines in response to antigen-expressing target cells; preliminary analysis showed CAR+ cells respond markedly by the production of IFNγ alone or in combination with TNFα. CAR+ cells expressed the degranulation marker CD107a specifically in response to target antigen or TCR stimulation. We propose this process as a means to shorten the timeline and cost for production by using a nonviral method to engineer CAR T cells, avoid the use of feeder cells, and chemically select for cells of interest. Ultimately, this workflow is also applicable to CARs of any specificity and allows for multiplexing.

Figure 1. Schematic of 14-day nonviral process of CAR T cell production. Prior to starting a manufacturing process, T cells are isolated from a leukapheresis product and cryopreserved. Thaw, electroporation, and stimulation of the primary T cells occurs on day 0, where cells are electroporated with CAR-containing MC constructs that will be integrated into the genome by the SB transposase RNA. Chemical selection begins on day 2 of the process and occurs through the remaining 12 days, with a bead removal step at day 7. On day 14, cells are characterized and then cryopreserved for later studies.