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Off the shelf T cell therapies for hematologic malignancies

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ABSTRACT

Adoptive transfer of autologous CAR-T cells can induce durable remissions in patients with relapsed/refractory hematologic malignancies. However, multiple challenges exist for manufacturing CAR-T cells from patients with advanced disease including inability to manufacture a product, disease progression or death while waiting for the CAR-T product to be available, and heterogeneity among autologous CAR-T products that contributes to unpredictable and variable clinical activity. Healthy donor T cells can provide a source for production of universal CAR-T cells when combined with gene editing to prevent expression of endogenous TCRs and avoid generation of GvHD in HLA mismatched recipients. Additional gene edits can be included to impart resistance to immunosuppression or improve trafficking to tumor sites. Recent advances in cell manufacturing and analytics technology can provide for consistent batch to batch manufacturing of gene edited allogeneic CAR-T cells in sufficient quantity to treat thousands of patients when needed as off the shelf products.

1. Autologous CAR-T therapies for treatment of hematologic malignancies

Chimeric antigen receptor (CAR) T cell therapies have produced significant and durable clinical responses in patients with refractory and relapsed hematologic malignancies. The majority of CAR-T therapies that have been studied in clinical trials and proven to provide a clinical benefit have been autologous products directed against the B cell antigen, CD19, in patients with B-cell acute lymphoblastic leukemia (B-ALL) [1–3], chronic lymphocytic leukemia (CLL) [4,5], and non-Hodgkin lymphoma (NHL) [6,7]. More recently CAR-T cells directed against B-cell maturation antigen (BCMA) [8,9] and CD123 [10] have shown activity in patients with relapsed or refractory multiple myeloma or acute myeloid leukemia, respectively. Manufacturing of autologous CAR-T therapies requires a leukapheresis procedure followed by activation and transduction of patient T cells using a lenti- or γ-retrovirus vector, or in some cases adenoviral vectors or a transposon/transposase system such as Sleeping Beauty [11] or PiggyBAC [12], to introduce a CAR transgene into the genome of the cell. Following transduction, T cells are expanded in culture, washed, resuspended in a solution compatible with i.v. administration to humans, and reinfused into the patient or cryopreserved for subsequent thawing and administration. Due to the advanced stage of disease and history of previous chemotherapies in patients with relapsed/refractory disease, it is sometimes not possible to produce an efficacious CAR-T product due to low T cell counts and/or poor quality of the T cells obtained from the patients at the time of leukapheresis. Several weeks are required from leukapheresis of the patient through manufacturing and shipping of autologous CAR-T product to the clinical site for infusion during which time patients may experience disease progression or die while waiting for their autologous product. In addition, in the case of autologous CAR-T therapies, a single manufacturing campaign generates a product that can be used to dose a single patient resulting in a significantly high cost of goods. The high costs associated with manufacturing of autologous CAR-T products places a burden on health care systems and restricts

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broad patient access to these novel therapies.

Autologous CAR-T products are inherently heterogenous due to differences in T cell quantity and phenotypes obtained from patients. In addition, the use of lenti- and retroviral vectors, which integrate semi-randomly within transduced T cells, introduces variability in CAR expression levels due to differences in copy number and expression of the CAR transgene within the genomes of the transduced cells. Turtle et al. [13] recently described autologous anti-CD19 CAR-T cells manufactured from defined $CD4^+$ and $CD8^+$ T cell subsets and administered in a defined CD4:CD8 composition to patients with B-ALL. The authors reported that this defined composition CAR-T product enabled identification of factors that correlated with CAR-T cell expansion, persistence and toxicity and facilitated determination of potential correlations between cell dose and efficacy or toxicity. Refinements in manufacturing procedures, such as pre-selection of T cell subsets, separate manufacturing streams for isolation, transduction, expansion, and remixing of $CD4^+$ and $CD8^+$ T cells can help to reduce the heterogeneity inherent in manufacturing of autologous CAR-T products. However, collection and shipping of leukapheresis products to a centralized manufacturing facility, and return of manufactured CAR-T product for patient infusion continue to represent logistical challenges that contribute to the complexity and costs associated with individualized manufacturing of autologous CAR-T products. Lastly, it is not possible to guaranty immediate availability of product when needed by the patient, some of whom cannot wait several weeks for the manufacture of an autologous CAR-T therapy.

2. Gene editing for production of allogeneic CAR-T cells from unrelated healthy donor cells

Recent advances in gene editing technology allow for the manufacture of CAR-T cell therapies starting from healthy donor leukapheresis material in which the quantity and quality of T cells can be pre-selected. However, the use of unrelated healthy donor T cells for production of allogeneic CAR-T cell products requires the use of gene editing technology to prevent expression of endogenous T cell receptors (TCRs) in order to minimize the potential to cause graft-versus-host disease (GvHD) in HLA mismatched recipients. Several different gene editing technologies have been used to prevent expression of endogenous TCRs. Zinc-finger nucleases (ZFN) [14], CRISPR/Cas9 [15,16], transcription activator-like effector nucleases (TALEN) [17,18], and engineered homing endonucleases [19] have been described that target an exon within the TCRα constant (TRAC) or TCRβ constant 1 (TRBC1) or 2 (TRBC2) loci for genetic knockout thereby preventing expression of α/β TCRs on the cell surface. ZFN, CRISPR/Cas9, or TALEN are two component systems in which sequence-specific DNA targeting and DNA cleavage functions are provided by separate molecular entities. For gene editing using CRISPR/Cas9 the components are introduced into T cells via electroporation of mRNAs encoding guide RNA (gRNA) or Cas9 nuclease, although other methods such as the use of lipid nanoparticles are being developed to deliver the gRNA and Cas9 nuclease. In the case of ZFN and TALEN, T cells are electroporated with mRNA or plasmid DNA encoding chimeric proteins consisting of either a pair of Zn-finger binding domains, or a pair of TAL effector (TALE) DNA binding domains, linked to the DNA cleavage domain of FokI nuclease. The DNA cleavage domain of FokI and Cas9 nucleases introduce a DSB at the target site specified by the Zn-finger, TALE, or gRNA which, in the absence of a homology repair template, is repaired by nonhomologous end joining (NHEJ), an error prone cellular repair pathway that results in insertion or deletion of nucleotides at the cleavage site resulting in loss of functional gene expression [20,21]. In the case of the engineered homing endonuclease technology such as described by MacLeod et al. [19], a guide sequence is not required in order to target nuclease activity to the intended genomic DNA sequence. T cells are electroporated with an mRNA that encodes the engineered nuclease which directly binds the DNA target sequence and makes a staggered cut resulting in 4 bp 3′ overhangs, a feature that may favor targeted insertion of a transgene via homology-directed repair (HDR) in the presence of a homology repair template (described below).

Gene edited (TCR knockout) allogeneic CAR-T cells are often produced by isolating and activating healthy donor T cells, most often using soluble or bead-bound anti-CD3 and anti-CD28 antibodies followed by transduction of the activated cells with a lenti- or γ-retroviral vector to deliver the CAR transgene (or using a transposon incorporating the CAR transgene and a transposase) resulting in random integration of the CAR transgene at several sites within the genome of the T cell [18,22]. The transduced T cells are subsequently gene edited to prevent expression of endogenous TCRs. The cells are then expanded in culture, depleted of remaining TCR positive (i.e. unsuccessfully gene edited) cells, washed and frozen in cryopreservation medium. By starting with healthy donor T cells in which the number, ratio of CD4 and CD8, and percentage of cells with naïve and central memory phenotypes can be pre-selected, a CAR-T product with desirable properties for use in adoptive cellular immunotherapy can be manufactured provided that the process has been optimized to maintain the desired T cell phenotypes in the final product. The percentage of cells that express the CAR transgene but do not express α/β TCRs at the cell surface is dependent on the efficiency of transduction and gene editing. Transduction efficiency increases with the multiplicity of infection (MOI) used to transduce the cells with the viral vector. However, with increasing MOI comes cellular toxicity and increased number of randomly integrated copies of the viral genome, thereby increasing the potential for mutagenic events [23,24]. Gene editing efficiency is affected by the efficiency and toxicity associated with transfection of T cells and the efficiency and specificity (related to nuclease-associated toxicity) of the guide/nuclease. Efficiency and toxicity associated with transfection of large numbers of T cells in a single batch is a major challenge in the manufacturing of gene edited CAR-T products. In recent years a number of manufacturers have introduced electroporation devices that can efficiently transfect up to several billion T cells in less than 1 hour using a closed system that is amenable to GMP manufacturing but optimization of electroporation conditions is a challenging task that has high impact within the manufacturing process of gene edited CAR T cells (see below in Allogeneic CAR-T Manufacturing). Target specificity of guide/nuclease, or just the nuclease in the case of homing endonucleases, may impact the efficiency of gene editing by determining the amount of nuclease that is available and active at the intended target site as well as toxicity that may be associated with off-target nuclease activity. In addition to the contribution to gene editing efficiency, nuclease specificity for the intended target is of paramount importance as off-target DSBs can negatively impact CAR T cell phenotype and function, create the potential for translocations and/or result in undesirable genotoxicities that may