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Closed-system manufacturing of CD19 and dual-targeted CD20/19 chimeric antigen receptor T cells using the CliniMACS Prodigy device at an academic medical center

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Abstract

Background aims. Multiple steps are required to produce chimeric antigen receptor (CAR)-T cells, involving subset enrichment or depletion, activation, gene transduction and expansion. Open processing steps that increase risk of contamination and production failure are required. This complex process requires skilled personnel and costly clean-room facilities and infrastructure. Simplified, reproducible CAR-T-cell manufacturing with reduced labor intensity within a closed-system is highly desirable for increased availability for patients. *Methods.* The CliniMACS Prodigy with TCT process software and the TS520 tubing set that allows closed-system processing for cell enrichment, transduction, washing and expansion was used. We used MACS-CD4 and CD8-MicroBeads for enrichment, TransAct CD3/CD28 reagent for activation, lentiviral CD8 TM-41BB-CD3 ζ-cfrag vectors expressing scFv for CD19 or CD20/CD19 antigens for transduction, TexMACS medium-3%-HS-IL2 for culture and phosphate-buffered saline/ethylenediaminetetraacetic acid buffer for washing. Processing time was 13 days. *Results.* Enrichment (N = 7) resulted in CD4/CD8 purity of 98 ± 4.0%, 55 ± 6% recovery and $CD3^+$ T-cell purity of 89 ± 10%. Vectors at multiplicity of infection 5–10 resulted in transduction averaging 37%. An average 30-fold expansion of 10^8 CD4/CD8-enriched cells resulted in sufficient transduced T cells for clinical use. CAR-T cells were 82–100% $CD3^+$ with a mix of $CD4^+$ and $CD8^+$ cells that primarily expressed an effector-memory or central-memory phenotype. Functional testing demonstrated recognition of B-cells and for the CAR-20/19 T cells, CD19 and CD20 single transfectants were recognized in cytotoxic T lymphocyte and interferon-γ production assays. *Discussion.* The CliniMACS Prodigy device, tubing set TS520 and TCT software allow CAR-T cells to be manufactured in a closed system at the treatment site without need for clean-room facilities and related infrastructure.

Key Words: *chimeric antigen receptor T cells, CliniMACS Prodigy, immunotherapy, lentiviral vectors*

Introduction

CD19 and CD20 are antigens expressed on both healthy B cells and a multitude of B cell–derived hematological malignancies including non-Hodgkin lymphomas (NHL), acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL). CD19 and CD20 are both highly attractive targets for immunotherapy of B-cell neoplasms. CD19 is expressed by most B-cell malignancies including NHL, ALL, CLL, hairy cell leukemia and a subset of acute myelogenous leukemia [1]. CD19 is a 95 kDa glycoprotein present on the B-cell surface from early development until differentiation into plasma cells, when it is lost. CD19 is a member of the immunoglobulin superfamily and a component of a cell surface signaling transduction complex that regulates signal transduction through the B-cell receptor. CD19 expression is restricted to B lineage cells and is not expressed by pluripotent blood stem cells or on most other normal tissues [2,3]. Several clinical trials demonstrated effectiveness of CD19 chimeric antigen receptor (CAR) modified T cells in patients with ALL, CLL or NHL [4–8].

CD20 is a non-glycosylated 33- to 37-kDa phosphoprotein that is expressed on both normal and malignant B cells. It normally functions as a component of signal transduction in growth regulation of B lymphocytes. CD20, like CD19, is a cell surface receptor restricted to B-cell precursors and mature B

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cells that is lost after plasma cell differentiation [9,10]. Monoclonal antibodies against CD20 are effective in treating B-cell malignancies. Unlike CD19 CAR, CD20 CAR-T-cell therapies are in early stages of development but have shown significant promise in some clinical trials [11,12].

The primary advantage of adoptive immunotherapy using CAR-T cells compared with alternative modalities lies in the ability of T cells to expand and persist upon antigen binding of the target [13]. Persistence of CAR-T cells in patients with refractory NHL and ALL has correlated with sustained progression-free survival, likely due to the long-term surveillance these cells provide against recurrent malignancy [4]. However, despite encouraging results, not all patients respond to this treatment, and even those who have achieved remission may relapse. An important mechanism of relapse to both targeted monoclonal antibody treatment and CAR-T-cell therapy was shown to be down-regulation of the targeted receptor allowing tumor cells to escape destruction [5,14]. Given the overlap of CD19 and CD20 expression on B cells, we speculate that simultaneous targeting of these two separate B-cell receptors may result in a more complete B-cell ablation and a reduced risk of tumor cell escape.

The ability to produce CAR-T-cell lines suitable for clinical use requires manufacturing processes that are reproducible, scalable and that maintain sterility. Most CAR-T-cell trials have involved complex manual processing procedures that include enrichment and/or depletion of cellular subsets from starting cells, activation and exposure to viral vectors to introduce the chimeric antigen receptors, and cell expansion steps that may require weeks in culture. There have been attempts to automate individual steps or to close the system, but open steps remain [15]. The CliniMACS Prodigy device (Miltenyi Biotec) is the first computer-controlled unit that automates each of the required processing steps while in a completely closed system [15–18]. The Prodigy device has been approved for use in cell separation procedures and for the selection and culture of antigen reactive T cells [19,20]. Here we describe an approach for improving the efficiency and efficacy of CAR-T-cell therapy by using dual targeted CD19/CD20 CAR-T cells produced in a closed system with the CliniMACS Prodigy device.

Methods

Starting material

Cells used for preclinical testing were obtained from leukoreduction filters used for apheresis platelet donors (N = 7) [21]. Cell processing was begun within 24 h of product collection.

Instrumentation and tubing sets

CAR-T cells were prepared entirely within the CliniMACS Prodigy (Miltenyi Biotec) device (Prodigy) using the TCT software program and TS520 tubing set. The main components of the Prodigy include bag hangers for product and reagents, pinch valves to control the fluid path, a peristaltic pump, liquid sensors, a gas mix unit, the CentriCult Unit where the cells are washed and cultured, a magnet unit for cell separation, a bag compartment and an integrated microscope that allows for visual observation of cells during culture. All of the components are controlled by a touchscreen computer that runs software to automate the manufacturing steps. The TS520 set includes filters and columns for cell separation and an integrated centrifugation and culture chamber (CentriCult Chamber) for cell washing and culture and a disposable heat exchange cartridge. Sample pouches are part of the tubing set and allow for closed-system sampling. During culture the temperature and atmosphere is maintained at 37°C with 5% CO_2. Initially the chamber is static, but as medium is progressively added or exchanged, it is programmed to shake to allow for a higher cell density. Additional information and diagrams of the CliniMACS Prodigy are available at the Miltenyi website. A sterile connecting device (TSCDII, TerumoBCT) was used to make all connections to the tubing and a heat sealer; that is, part of the Prodigy was used to seal off fluid pathways and disconnect sampling pouches, waste bags and cell collection bags. Reagent preparation was performed within a Biosafety cabinet. All processing was performed within the Lymphocyte Propagation Laboratory at the Medical College of Wisconsin.

Lentiviral vectors

Two vectors were used for the preclinical experiments, the LTG1494 CAR19 vector and the LTG1497 CAR 20/19 vector (Lentigen Technology). Both vectors have an identical CD8-derived hinge and transmembrane region, 4-1BB/CD137 and CD3-zeta chain intracellular signaling domain. The LTG1494 vector contains an scFv from the antibody FMC63 specific for the CD19 antigen. The LTG1497 vector contains the same CD19 scFv in tandem with a scFv from the Leu16 antibody specific for the CD20 antigen, with the CD20 scFv distal and the CD19 scFv proximal to the hinge and transmembrane region [22]. The research-grade CD19 vector and the research grade CD20/CD19 vector were provided with only a quantitative polymerase chain reaction (qPCR) titer of virus. We used this information as a starting point to calculate the MOI to be used for all experiments, even though an infectious titer was provided for the clinical grade CD20/19 product.