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Simplified process for the production of anti–CD19-CAR–engineered T cells

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Abstract

Background aims. Adoptive immunotherapy with the use of chimeric antigen receptor (CAR)-engineered T cells specific for CD19 has shown promising results for the treatment of B-cell lymphomas and leukemia. This therapy involves the transduction of autologous T cells with a viral vector and the subsequent cell expansion. We describe a new, simplified method to produce anti-CD19-CAR T cells. *Methods.* T cells were isolated from peripheral blood mononuclear cell (PBMC) with anti-CD3/anti-CD28 paramagnetic beads. After 2 days, the T cells were added to culture bags pre-treated with RetroNectin and loaded with the retroviral anti-CD19 CAR vector. The cells, beads and vector were incubated for 24 h, and a second transduction was then performed. No spinoculation was used. Cells were then expanded for an additional 9 days. *Results.* The method was validated through the use of two PBMC products from a patient with B-cell chronic lymphoblastic leukemia and one PBMC product from a healthy subject. The two PBMC products from the patient with B-cell chronic lymphoblastic leukemia contained 11.4% and 12.9% T cells. The manufacturing process led to final products highly enriched in T cells with a mean CD3+ cell content of 98%, a mean expansion of 10.6-fold and a mean transduction efficiency of 68%. Similar results were obtained from the PBMCs of the first four patients with acute lymphoblastic leukemia treated at our institution. *Conclusions.* We developed a simplified, semi-closed system for the initial selection, activation, transduction and expansion of T cells with the use of anti-CD3/anti-CD28 beads and bags to produce autologous anti-CD19 CAR–transduced T cells to support an ongoing clinical trial.

Key Words: *adoptive cellular immunotherapy, CD19 antigen, genetic engineering, genetic transduction, precursor cell lymphoblastic leukemia-lymphoma*

Introduction

Cancer is the leading cause of disease-related death in US pediatric patients (1), and acute lymphoblastic leukemia (ALL) represents the most common pediatric malignancy. Although disease-free survival rates are high, approximately 20% of patients die of their underlying disease, making ALL the leading cause of cancer death in childhood (2). For those who relapse, long-term prognosis is poor. Even when a second remission is achieved, long-term disease-free survival rates <50% are expected (3). New treatment approaches are therefore needed.

Adoptive immunotherapy is considered an attractive way to treat cancer (4–10), and genetic modification of T cells with genes encoding tumor-specific chimeric antigen receptors (CARs) represents a novel strategy to obtain large quantities of tumor-reactive T cells to be used for the treatment of adult (11–13) and pediatric patients with cancer (14).

CD19 has been chosen as a target for CAR T cells because it is universally expressed on ALL tumor cells and is not expressed on pluripotent hematopoietic stem cells (15–17). Anti-CD19-CAR T cells are under investigation in several institutions through the use of different viral constructs and various manufacturing processes and have shown promising clinical results (18–21).

For the production of CAR-engineered T cells, procedures and techniques enabling *ex vivo* gene modifications of cells followed by expansion to clinically relevant cell numbers are of key importance. For this purpose, cell culture systems have been developed in compliance of good manufacturing

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practice (GMP) criteria. Current manufacturing processes rely on the use of recombinant human fibronectin fragment CH296 (RetroNectin, Takara Bio Division, Shiga, Japan), which has been demonstrated to improve transduction efficiency by bringing together retroviral particles and cells. This has often been paired with spinoculation, a procedure that promotes gene transfer by pre-loading the retroviral vector stocks on the RetroNectin by a 2-h centrifugation, followed by a second centrifugation of target cells. Some of the processes use bags and are semi-closed, but others use plates and flasks and are open, presenting a large risk of contamination and spilling of vector.

In the present study, we describe the process that has been developed at our institution for the production of therapeutic doses of autologous anti−CD19-CAR T cells to support a phase I clinical trial for the treatment of B-cell malignancies in pediatric patients, through the use of a replication-defective mouse stem cell virus−based γ-retroviral vector 1 (MSGV-FMC63-28z) encoding a chimeric receptor containing the signaling domains of CD28 and CD3-ζ. The goal of this study was to test the level of the transduction efficiency that we could achieve through the use of tissue culture bags as vessel for transduction and cell expansion process, avoiding spinoculation. We made use of GMP paramagnetic beads coated with anti-CD3 and anti-CD28 to isolate CD3+ cells from peripheral blood mononuclear cell products (PBMCs) collected by apheresis and at the same time stimulate sufficient CD3+ cell proliferation to facilitate transduction and subsequent expansion. The process described allowed us to generate anti−CD19-CAR T cells through the use of a semi-closed system without the spinoculation step, maintaining acceptable transduction efficiency.

Methods

Construction and GMP production of the MSGV-FMC63-28z recombinant retroviral vector

Clinical grade MSVG-FMC63-28z recombinant retroviral vector was prepared and cryopreserved in the Surgery Branch, National Cancer Institute, National Institutes of Health Vector Production Facility. The construction and production of the MSGV-FMC63-28z vector has been described elsewhere by Kochenderfer *et al.* (22). These studies were approved by a National Institutes of Health institutional review board.

Culture media

T-cell initiation medium was composed of AIM V medium (Gibco, Grand Island, NY, USA), supplemented with 5% heat-inactivated human AB Serum (Valley Biomedical, Winchester, VA, USA), 1% Gluta-Max (Gibco) and 40 IU/mL interleukin (IL)-2 (Novartis Vaccines and Diagnostics, Inc, Emeryville, CA, USA).

T-cell expansion medium was composed of AIM V medium (Gibco), supplemented with 5% heat-inactivated human AB Serum (Valley Biomedical), 1% Gluta-Max (Gibco) and 300 IU/mL IL-2 (Novartis Vaccines and Diagnostics, Inc).

Generation of clinical grade anti−CD19-CAR−transduced T cells

The process was optimized with the use of PBMC products collected by apheresis from healthy subjects. The final process for the manufacture of clinical grade anti−CD19-CAR T cells was validated with the use of a PBMC product from one healthy subject (VR1) and two PBMC products from a patient with B-cell chronic lymphoblastic leukemia (B-CLL) (VR2 and VR3). Clinical anti−CD19-CAR T-cell products were generated to treat four patients with ALL who were enrolled in the phase I clinical study NCT01593696, sponsored by the National Cancer Institute.

On day 0, fresh PBMC products collected by apheresis were enriched for CD3+ cells with the use of anti-CD3 and anti-CD28 antibodies bound to paramagnetic beads (Dynabeads ClinExVivo CD3/CD28, Invitrogen, Camarillo, CA, USA) at a ratio of 3:1 (beads:cells). The enrichment was performed with the use of cells diluted to a total nucleated cell (TNC) concentration of $20-30 \times 10^6$/mL in PermaLife bags made of fluorinated ethylene propylene (OriGen Biomedical, Austin, TX, USA). The cells and beads were co-incubated for 2 h at room temperature, and CD3+ cell enrichment was performed with the use of the Dynal ClinExVIVO magnetic particles concentrator (MPC) magnet (Invitrogen). Cells in the CD3+ fraction were resuspended in initiation media at a concentration of 1×10^6 cells/mL in PermaLife bags (OriGen Biomedical).

On day 1, clinical grade RetroNectin was reconstituted in sterile water at 1 mg/mL and used to coat PermaLife bags (OriGen Biomedical) at a concentration of 2 μg/cm^2 in a solution of 10 μg/mL in phosphate buffer solution (PBS) (Lonza, Walkerville, MD, USA). The coated bags were incubated overnight at 4°C. On day 2, the RetroNectin solution was aspirated from bags, and the same volume of blocking solution, consisting of 2.5% human serum albumin (Baxter, Westlake Village, CA, USA) in PBS, was added to each bag and incubated at room temperature for 30 min. The blocking solution was aspirated, and each bag washed with a solution of 2.5% HEPES (Lonza) diluted in Hank's balanced salt solution (Lonza).

On day 2, retroviral supernatant was rapidly thawed and added to each bag at the final volume that