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Antitransgene Rejection Responses Contribute to Attenuated Persistence of Adoptively Transferred CD20/CD19-Specific Chimeric Antigen Receptor Redirected T Cells in Humans

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Immunotherapeutic ablation of lymphoma is a conceptually attractive treatment strategy that is the subject of intense translational research. Cytotoxic T lymphocytes (CTLs) that are genetically modified to express CD19- or CD20-specific, single-chain antibody–derived chimeric antigen receptors (CARs) display HLA-independent antigen-specific recognition/killing of lymphoma targets. Here, we describe our initial experience in applying CAR-redirected autologous CTL adoptive therapy to patients with recurrent lymphoma. Using plasmid vector electrotransfer/drug selection systems, cloned and polyclonal CAR^+ CTLs were generated from autologous peripheral blood mononuclear cells and expanded in vitro to cell numbers sufficient for clinical use. In 2 FDA-authorized trials, patients with recurrent diffuse large cell lymphoma were treated with cloned $CD8^+$ CTLs expressing a CD20-specific CAR (along with NeoR) after autologous hematopoietic stem cell transplantation, and patients with refractory follicular lymphoma were treated with polyclonal T cell preparations expressing a CD19-specific CAR (along with HyTK, a fusion of hygromycin resistance and HSV-1 thymidine kinase suicide genes) and low-dose s.c. recombinant human interleukin-2. A total of 15 infusions were administered (5 at 10^8cells/m^2, 7 at 10^9cells/m^2, and 3 at 2×10^9cells/m^2) to 4 patients. Overt toxicities attributable to CTL administration were not observed; however, detection of transferred CTLs in the circulation, as measured by quantitative polymerase chain reaction, was short (24 hours to 7 days), and cellular antitransgene immune rejection responses were noted in 2 patients. These studies reveal the primary barrier to therapeutic efficacy is limited persistence, and provide the rationale to prospectively define T cell populations intrinsically programmed for survival after adoptive transfer and to modulate the immune status of recipients to prevent/delay antitransgene rejection responses.

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INTRODUCTION

Although conventional chemotherapy, radiation therapy, and antibody therapy can be efficacious in treating lymphoma, relapse and progressive disease are the major sources of patient morbidity and mortality [1,2]. Experimental evidence that the cellular immune system can eradicate lymphoma provides a basis for the development of therapies aimed at amplifying antitumor immune responses [3,4]. The adoptive transfer of lymphoma-specific T cells is one strategy to augment antilymphoma immunity. A significant challenge to executing this strategy is the isolation of T cells specifically reactive to lymphoma. Alternately, the ex vivo derivation of tumor-specific T lymphocytes by genetic modification to express tumor-targeting chimeric antigen receptors (CARs) is a rapidly evolving focus of translational cancer

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immunotherapy [5,6]. Antibody-based CARs are HLA-unrestricted and thus can be used in patient populations with target-antigen–positive tumors.

We have constructed 2 CARs specific for the B cell lineage antigens CD20 and CD19 for the purpose of targeting lymphomas and leukemias [7,8]. When expressed in cytotoxic T lymphocytes (CTLs), these CARs redirect effector cells to lyse B-lineage lymphoma targets [7,8]. Here we report our initial clinical experience in manufacturing and infusing autologous T cells expressing CD20R or CD19R in patients with relapsed B cell lymphoma under City of Hope–held FDA-authorized trials BB-IND-8513/IRB 98142 and BB-IND-11411/IRB 01160, respectively.

MATERIALS AND METHODS

Patients

City of Hope Internal Review Board (IRB) protocols 98142 and 01160 were activated for patient accrual following IRB and Institutional Biological Safety Committee approval, FDA authorization (BB-IND-8513 and BB-IND-11411, respectively), and National Institutes of Health Office of Biotechnology Activities registration (9907-330 and 0207-543, respectively). In brief, for IRB 98142, patients were eligible if they had immunohistopathologically documented $CD20^+$ diffuse large cell lymphoma (DLCL) with a history of recurrent or refractory disease and did not have central nervous system metastases. After leukapheresis, patients began salvage/mobilization chemotherapy, then underwent hematopoietic stem cell transplantation (HSCT). The first of 3 escalating-dose T cell infusions was given at 28 days post-HSCT. For IRB protocol 01160, patients were eligible if they had pathologically documented follicular lymphoma (FL) with evidence of progression after previous rituximab therapy and did not have central nervous system metastases or a history of allogeneic HSCT. These patients were enrolled no sooner than 3 weeks after their most recent cytotoxic chemotherapy.

Plasmid Vectors

The plasmid expression vectors encoding the CD20R chimeric immunoreceptor and the neomycin phosphotransferase cDNAs and the CD19R chimeric immunoreceptor and the selection-suicide HyTK (a fusion of hygromycin resistance and HSV-1 thymidine kinase suicide genes) cDNAs have been described previously [7,8] (Figure S1A). In brief, the chimeric construct consists of V_H and V_L gene segments of the CD20-specific Leu-16 or CD19-specific FMC63 monoclonal antibodies (mAbs), an IgG hinge-C_{H2}-C_{H3} region, a CD4 transmembrane region, and the cytoplasmic domain of the CD3ζ chain (Figure S1B).

Isolation, Transfection, Selection, Cloning, and Expansion of T Cells

The methods for OKT3 stimulation of peripheral blood mononuclear cells (PBMCs), and for PBMC electroporation, selection, cloning (IRB 98142 only), and subsequent growth using the rapid expansion method (REM), consisting of recursive 14-day cycles of activation with OKT3, recombinant human interleukin (rHuIL)-2, and PBMC/lymphoblastoid cell line (LCL)-irradiated feeders, have been described previously [9]. The overall T cell product manufacturing schemas for each trial are depicted in Figure S1C.

Cell Product Quality Control Tests

The cell product quality control tests (QCTs) performed and the requisite test results for product release are summarized in Table S1.

Confirmation of Plasmid Vector Integration (IRB 98142 Only)

A single site of plasmid vector chromosomal integration was confirmed by Southern blot analysis of *XbaI*/*Hind*III-digested T cell genomic DNA using a 420-bp NeoR-specific probe generated using the pcDNA3.1(-) plasmid as a template [9]. The pass criterion of this test was defined as detection of a single band.

Confirmation of CAR Expression

Western blot analysis for CAR expression has been described previously [10]. In brief, reduced whole-cell lysates are subjected to Western blot analysis with an anti-human CD3-ζ (cytoplasmic tail)-specific mAb 8D3 (BD Pharmingen, San Diego, CA). This probe detects both the 16-kDa endogenous ζ and the 66-kDa CAR ζ. Pass criteria were defined as visualization of both the 16-kDa and 66-kDa bands. Flow cytometry analysis for surface CAR expression was determined using a fluorescein isothiocyanate (FITC)-conjugated Fc-specific antibody (Jackson ImmunoResearch, West Grove, PA). Pass criteria were defined as unimodal positive staining for Fc compared with the FITC-conjugated isotype control (BD Biosciences, San Jose, CA).

Surface Phenotype Determination

T cell products were evaluated for cell-surface phenotype using standard staining and flow cytometric procedures with FITC-conjugated mAbs (BD Biosciences), followed by analysis on a FACScaliber analyzer (BD Biosciences). The pass criterion was ≥90% positive staining for TCR-αβ and CD8 (IRB 98142) or CD3 (IRB 01160) compared with the isotype control. Independent of the QCT guidelines, other correlative surface markers included CD4 for IRB 98142 and both CD4 and CD8 for IRB 01160.

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