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CD25 Blockade Delays Regulatory T Cell Reconstitution and Does Not Prevent Graft-versus-Host Disease After Allogeneic Hematopoietic Cell Transplantation

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Daclizumab, a humanized monoclonal antibody, binds CD25 and blocks formation of the IL-2 receptor on T cells. A study of daclizumab as acute graft-versus-host disease (GVHD) prophylaxis after unrelated bone marrow transplantation was conducted before the importance of $CD25^{+}FOXP3^{+}$ regulatory T cells (Tregs) was recognized. Tregs can abrogate the onset of GVHD. The relation between Tregs and a graft-versus-malignancy effect is not fully understood. An international, multicenter, double-blind clinical trial randomized 210 adult or pediatric patients to receive 5 weekly doses of daclizumab at 0.3 mg/kg (n = 69) or 1.2 mg/kg (n = 76) or placebo (n = 65) after unrelated marrow transplantation for treatment of hematologic malignancies or severe aplastic anemia. The risk of acute GVHD did not differ among the groups ($P = .68$). Long-term follow-up of clinical outcomes and correlative analysis of peripheral blood T cell phenotype suggested that the patients treated with daclizumab had an increased risk of chronic GVHD (hazard ratio [HR], 1.49; 95% confidence interval [CI], 1.0 to 2.3; $P = .08$) and a decreased risk of relapse (HR, 0.57; 95% CI, 0.3 to 1.0; $P = .05$), but similar survival (HR, 0.89; 95% CI, 0.6 to 1.3; $P = .53$). T cells from a subset of patients (n = 107) were analyzed by flow cytometry. Compared with placebo, treatment with daclizumab decreased the proportion of Tregs among CD4 T cells at days 11-35 and increased the proportion of central memory cells among CD4 T cells at 1 year. Prophylactic administration of daclizumab does not prevent acute GVHD, but may increase the risk of chronic GVHD and decrease the risk of relapse. By delaying Treg reconstitution and promoting immunologic memory, anti-CD25 therapy may augment alloreactivity and antitumor immunity.

INTRODUCTION

Daclizumab is a humanized monoclonal antibody that targets the p55 subunit of the IL-2 receptor α (IL-2Rα, or CD25) on human lymphocytes. On binding of CD25, daclizumab (like the similar monoclonal antibody basiliximab) blocks IL-2 binding to the high-affinity IL-2 receptor and impairs IL-2–mediated activation of T lymphocytes. Several investigators

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have reported on the activity of daclizumab in adults and children as primary [1] or secondary [2-9] therapy for acute graft-versus-host disease (aGVHD). Favorable safety and pharmacokinetic data prompted the design of a multicenter, randomized, double-blind clinical trial adding daclizumab to cyclosporine/methotrexate prophylaxis in unrelated donor bone marrow transplantation.

At the time that this trial was designed, it was thought that daclizumab would block T cell activation and prevent aGVHD. Since that time, however, the central role of regulatory T cells (Tregs) as negative regulators of immune responses has been established [10-15]. Tregs, characterized by CD25 cell surface expression and the nuclear transcription factor FOXP3, depend on IL-2 [16,17], and blockade of CD25 could inhibit their expansion and function, thereby augmenting the immune response. Tregs play a key role in preventing and abrogating the severity of GVHD in both murine and human models [18,19].The study failed to meet the primary endpoint of decreasing the risk of aGVHD, and the results were reported in abstract form [20].

We recently hypothesized that IL-2 blockade with daclizumab after immune ablation and transplantation of hematopoietic cells hindered repopulation by Tregs while allowing the expansion of memory T cells, preventing the expected clinical response. Treg biology was unknown at the time of initial study design; however, banked samples allowed us to address this question. In the present study, we obtained long-term follow-up clinical data from this trial and analyzed the corresponding stored patient samples for Treg markers.

MATERIALS AND METHODS

Study Design and Objectives

Protocol NO14348 was a randomized, multicenter, double-blind, placebo-controlled study designed to evaluate the efficacy of 5 weekly doses of 0.3 mg/kg or 1.2 mg/kg of daclizumab when administered with cyclosporine and methotrexate for the prevention of aGVHD in recipients of a first unrelated allogeneic bone marrow transplant. The study's primary endpoint was the 100-day cumulative incidence of aGVHD necessitating steroid therapy. Secondary endpoints included the incidence of chronic GVHD (cGVHD), disease relapse, and survival.

Blood samples were obtained throughout the first 101 days and at 1 year post-transplantation to evaluate daclizumab binding to CD25, the number of free CD25 binding sites, and the overall expression of CD25 on T cells. The long-term follow-up protocol was designed to evaluate Treg and memory T cell phenotype by flow cytometry and to evaluate overall survival and the incidence of cGVHD. The trial protocol and updated analysis were approved by the relevant Institutional Review Boards.

Eligibility and Transplantation Therapy

Adult and pediatric patients undergoing bone marrow transplant for any malignancy or severe aplastic anemia with total body irradiation at a dose of 1200 to 1440 cGy as part of the conditioning regimen were eligible for this study. HLA matching was determined by serologic typing for HLA-A and HLA-B and by allele typing by PCR for HLA-DRB1. A single mismatch within the 6 A, B, or DRB1 loci was permitted. Exclusion criteria were patient age over 55 years or under 1 year, previous autologous or allogeneic marrow transplantation, cardiac disease or arrhythmia, active or suspected pulmonary infection, serum glutamic oxalocetic transaminase more than twice the normal level or direct bilirubin >2 mg/dL, serum creatinine more than twice the normal level, involved field radiation to the chest within the previous 6 months or at any time if the total exposure exceeded 1500 cGy, and human immunodeficiency virus infection.

GVHD prophylaxis consisted of i.v. methotrexate 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11. Administration of cyclosporine 1.5 mg/kg i.v. every 12 hours began on day -1 and continued until recovery from gastrointestinal toxicity of the conditioning regimen. Thereafter, cyclosporine was administered orally at 6.25 mg/kg every 12 hours until day +50. Dosage reduction was allowed: methotrexate for impaired liver function as evaluated by elevated bilirubin and both cyclosporine and methotrexate for impaired renal function based on serum creatinine. Tapering of cyclosporine doses at 5% per week began on day +51 for patients who did not require systemic corticosteroids and had no GVHD. Patients developing grade II, III, or IV aGVHD were eligible for prednisone therapy. Concomitant enrollment onto GVHD therapy trials was allowed. Patients who received any study drug were evaluated for the study endpoints. The use of hematopoietic growth factor was at the discretion of the principal investigator. Fluconazole prophylaxis was to continue from conditioning until day +75.

Study Treatment

Randomization was stratified by donor HLA (matched versus mismatched), patient age (<20 versus ≥20 years), and center (Fred Hutchinson Cancer Research Center (FHCRC) versus United States non-FHCRC versus non–United States). Therapy consisted of 5 weekly i.v. doses of placebo (arm A), 0.3 mg/kg daclizumab (arm B), or 1.2 mg/kg daclizumab (arm C) to a maximum of 100 mg beginning on the day before transplantation. Assessments were performed no less than weekly following transplantation up to day +55 and then regularly until day +100, with cGVHD assessment at days +180 and +365. Weekly clinical assessments for aGVHD onset were recorded until day +100. Staging of aGVHD was based on the Glucksberg scale [21]. cGVHD was classified as limited (localized skin or hepatic dysfunction not accompanied by liver histology changes or other organ involvement) or extensive [22]. Adverse events were defined as any adverse change from the patient's baseline (pretreatment) condition, including intercurrent illness, occurring during the course of the study.

Data Collection and Sample Analysis

Data from all 210 originally randomized patients were available for a summary of baseline characteristics and reported trial primary outcome measure (aGVHD requiring steroid therapy). Additional outcome data (grade II-IV aGVHD, grade III-IV aGVHD, cGVHD, relapse, death) were available from the original trial data for only 209 patients (data missing for 1 patient on the placebo arm). From these original 209 patients, additional long-term clinical data (beyond the data available at study closure through full duration of surviving patient follow-up) were secured from 95% of the patients at risk for events (obtained for 99 of the 104 patients alive at trial closure). Investigators at the various sites were contacted to update study records with the following long-term data: duration of follow-up, maximal grade of aGVHD, maximal grade of cGVHD, attainment of complete remission from primary disease, primary disease relapse, date of death, cause of death, and date of last contact.

Blood samples were collected at 5 distinct time windows from patients treated at FHCRC: before conditioning, on therapy (days +11 to +35), off therapy (days +36 to +80), washed (days +81 to +101), and long-term follow-up (day +365 ± 30). These samples were incubated with fluorescent-tagged antibodies or with primary antibodies and secondary fluorescent-tagged antibodies and analyzed to determine the fraction of T cells expressing CD25, to quantify free CD25 daclizumab-binding sites, and to determine the effectiveness of daclizumab binding to CD25$^+$ cells. Cell aliquots were cryopreserved for later studies.

Permission from FHCRC and Moffitt Internal Review Board approval were obtained to analyze cryopreserved samples so that Treg and T cell memory phenotype could be evaluated. It was hypothesized that differences in T cell repopulation due to daclizumab would be most evident in comparisons between placebo (arm A) and daclizumab 1.2 mg/kg (arm C). Cells were stained with L/D yellow, and surfaces were stained with CD3 PB, CD4 Alexa Fluor 700, CD15 PerCp-Cy5.5, CD25 PE-Cy7, and CD127-Alexa Fluor 647 (BD Biosciences, San Jose, CA). The cells were then fixed and permeabilized using the Foxp3 buffer set (BD Biosciences), and then stained with Foxp3-PE. In another panel, cells were surface-stained with L/D yellow, CD3 PB, CD4 Alexa Fluor 700, CD15 Percp-Cy5.5, CD8 APC-H7, CD127 Alexa Fluor 647, CD45RA FITC, and CCR7 PE-Cy7. Cells were analyzed by flow cytometry (LSRII; BD Biosciences). Results from each post-transplantation time window (on therapy [days +11 to +35], washed [days +81 to +101], and long-term follow-up [day +365 ± 30) were compared between arms A and C. Neither absolute lymphocyte count nor T cell count data were available, and thus the absolute number of Treg or central memory subsets could not be derived.

Statistical Analyses

All study endpoints were analyzed according to the principle of intent-to-treat analysis. The study was designed to detect a decrease in the incidence of aGVHD (grade II-IV, requiring treatment with corticosteroids to day +100) from 80% to 55% with 80% power and 5% type 1 error. Baseline characteristics were summarized using descriptive statistics, including mean, median, standard deviation, and range for continuous measures and counts and frequencies for categorical measures. The cumulative incidence rates of aGVHD, grade III-IV aGVHD, cGVHD, primary disease relapse, and nonrelapse mortality were estimated using standard methods for competing-risks analysis and compared using Wald tests in a Cox regression model [23]. Overall survival was analyzed using the Kaplan-Meier method and compared using the