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Visilizumab with Tacrolimus and Methotrexate for Graft-Versus-Host Disease Prevention After Allogeneic Hematopoietic Cell Transplantation From Mismatched Unrelated Donors

Lia Elena Perez[1,2], **Hugo Fernandez**[1,2], **Ernesto Ayala**[1,2], **Francisca Beato**[1], **Anthony Neuger**[3], **Joseph Pidala**[1,2], **Michael J. Schell**[4], and **Claudio Anasetti**[1,2]

[1]Department of Blood and Marrow Transplantation, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[2]Department of Oncologic Sciences, H. Lee Moffitt Cancer Center and Research Institute/ University of South Florida College of Medicine, Tampa, FL, USA

[3]Translational Research Core, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

[4]Department of Biostatistics, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

Effective graft-versus-host disease (GVHD) prevention after T-cell-replete, HLA-mismatched transplant is needed and has incentivized alternative approach development.[1] CD3-specific antibodies induce immunotolerance by rapidly clearing pathogenic T cells and promote tolerance by sparing regulators.[2] Their success in experimental GVHD prevention, human autoimmune disease treatment, and posttransplant rejection has established the basis to test visilizumab for GVHD prophylaxis in humans.[3, 4] Visilizumab (Abbott), an anti-CD3 monoclonal antibody with T-cell-receptor partial agonist ligand function, enhances activation-induced cell death (AICD), leading to T-cell apoptosis.[5,6] We reasoned that visiluzimab, with safety and biological activity previously described in clinical studies of glucocorticoid-resistant GVHD treatment,[3, 4] could prevent GVHD by depleting activated T cells yet allow immune reconstitution. Here, we report results from our prospective pilot trial using visilizumab for GVHD prevention in combination with tacrolimus (from day +4 at 0.02 mg/kg/day) and methotrexate (day +1 at 15 mg/m^2 and then at 10 mg/m^2 on days +3, +6, and +11) after unrelated donor allogeneic hematopoietic cell transplant (HCT) mismatched for 1 or 2 HLA-A, -B, -C, or DRB1 loci.

We used a Simon two-stage clinical trial design planned for 15 patients in stage 1. If serious toxicities were >2% (defined as grade 4/5 reaction to visiluzimab, HHV6 encephalitis, or PTLD within 100 days or any grade 4/5 adverse event unexpected with HCT) and <20% had

Corresponding Author: Lia Elena Perez, Associate Professor of Medicine, Department of Blood and Marrow Transplantation, Moffitt Cancer Center, 12902 Magnolia Dr. FOB-3, Tampa, FL 33612, Phone: (813) 745-2557; Fax: (813) 745-5820; lia.perez@Moffitt.org.

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GVHD grade 3/4, patients would proceed to stage 2, with groups randomized 1:1 with ATG or visiluzimab (30/group). GCSF-mobilized peripheral blood CD34+ cell dose/kg of 5–10 × 10^6 was used to minimize high-risk rejection compared with bone marrow. Conditioning regimen included fludarabine (40 mg/m^2 over 4 days) and busulfan (145 mg/m^2 IV over 4 days) to a steady-state concentration of 900 ± 100 ng/mL (AUC of 5300 ± 500 μmol/min). Prophylaxis with foscarnet (60 mg/kg/day on day +1 until ANC >500) followed by ganciclovir (5 mg/kg/day from ANC >500 to day + 100 if CMV-positive or to day +42 if CMV-negative) or valganciclovir (900 mg/day orally from ANC >500 if able to tolerate oral administration) days +2 to +42 was used.[7]

Eight patients (6 women/2 men) were enrolled (median age 46 years; range, 23–50). Three patients had AML, 2 had ALL, 1 had MDS, 1 had follicular NHL, and 1 had severe aplastic anemia, with HLA mismatched 6/8 (n=2) or 7/8 (n=6). We hypothesized that visilizumab ≥2000 ng/mL might be optimal to prevent GVHD. A single 3-mg/m^2 visilizumab dose immediately resulted in goal levels[3] in the first 7 patients. Visilizumab depleted blood T cells for <14 days. Using a non-compartmental ELISA with a murine anti-M291 monoclonal antibody (PDL, Fremont, CA, USA), we determined mean maximal concentration (±SD) at 1–2 hours of 1564 ± 428 ng/mL and terminal half-life of 157 ± 48 hours (Figure 1A). We surmised that repeat antibody administration was necessary to produce T lymphopenia for >14 days. Patient 8 received four 3-mg/m^2 doses on days 0, 3, 10, and 17 before premature study closure due to lack of efficacy,[8] and a two-compartment model was used to analyze that patient's pharmacokinetics (Figure 1B). All 8 patients had similar maximal concentration and alpha half-life parameter estimates; however, patient 8 showed a prolonged beta half-life (335 vs. 187 hours for multiple vs. single dose) and a significantly slower clearance rate (0.02 vs. 0.05 L/hour for multiple vs. single dose).

Visiluzimab infusion toxicity was CTC grade 1–2 in 6 patients and grade 3 in 2 patients. Median time to neutrophil engraftment was reached at 14 days (12–17 days), and median time to platelet engraftment was 11.5 days (10–22 days) with >95% donor chimerism at day 30. The cumulative incidence of grade II–IV acute GVHD score at 100 days was 100%, with 6 having grade I–II and 2 having grade III–IV. Median onset of GVHD was 14 days (range, 7–21). Seven patients developed protracted acute overlapping with chronic GVHD, 3 having mild-to-moderate and 4 having severe. Chronic GVHD affected the skin (n=5), gut (n=4), and lung (n=2). Two patients were alive after median of 2818 days (2831-2806 days), and 6 died after median of 197 days (150–643 days) due to GVHD with or without infection. Prophylaxis resulted in no CMV reactivation. Six patients had EBV reactivation that responded to rituximab.

Flow cytometry showed that visilizumab resulted in immediate and fast clearance of CD4+ and CD8+ lymphocytes, followed by a gradual count recovery at days 14–28 posttransplant, which could explain the poor results (Figure 2A). T-regulatory cells were detected as early as day 28 but did not translate to a positive outcome;CD56+/CD16+ cell recovery occurred by day +30 and CD19+ cells steadily fell after HCT (Figure 2A). The proportion of host-reactive interferon-γ-producing cells measured by ELISPOT was significantly increased versus donor control at day 90 post-HCT (P=0.030) (Figure 2B). We used either PMA/Iono polyclonal stimulation or antigen-presenting cells from a third-party donor as positive

stimulation controls. The Th17 lineage, measured by IL-17 production in ELISPOT supernatants at day 90, suggested increased IL-17 production by donor-derived host-reactive Th17 cells (P=0.4) (Figure 2C). We detected significantly increased IL-10 but not TGF-β production at day 90 post-HCT by host-reactive donor-derived T cells (P=0.03) (Figure 2C).

EBV-specific CD8+ T cells were measured by tetramers in 5 HLA-A*0201 patients with positive EBV serology pre-HCT, with positive EBV DNA titers shown post-HCT (limit of detection ≤0.6 cells/μL). By day 90 post-HCT, 4/5 patients had detectable EBV-specific CD8+ T cells measured with phycoerythrin (PE)-HLA-A*0201 EBV (GLCTLVAML) peptide, indicating persistence after visilizumab, although this did not translate to EBV reactivation prevention. CMV-specific CD8+ T cells measured with PE-HLA-A*0201 CMV PP65 (QYDPVAALF) peptide were undetectable in the 2 HLA-A*0201-positive patients. Flow cytometry analysis of the CD4+ T-cell receptor repertoire using 25 anti-Vβ monoclonal antibodies showed minimal if any skewing from the normal donor repertoire, suggesting polyclonal alloresponse (data not shown).

Here, visilizumab prior to methotrexate and tacrolimus was ineffective in preventing GVHD after HLA-mismatched unrelated transplant compared with other established prophylaxis methods. Tacrolimus was initiated at day +4 to avoid calcineurin blockade as delayed administration is effective in other settings.[9, 10] Although there was no GVHD liver involvement, all 8 patients developed acute GVHD of the gut and skin. Immune endpoint failure was observed as residual Th1, and possibly Th17 responses against host were still identified.

Despite dose accumulation observed with multi-dose visilizumab, we observed intolerable GVHD, warranting no further testing. Drug interactions that could account for results are calcineurin inhibitors inhibiting T-cell AICD by down-modulation of CD95L,[11] glucocorticoids preventing cytokine release, and/or methotrexate preventing division of T cells required for subsequent AICD.[12]

CD3-specific antibodies are promising modalities, but challenges remain for universal clinical use as effectiveness depends on numerous variables. CD3-specific antibodies have been able to halt active autoimmunity in mice but have been less effective in preventing disease, suggesting different mechanisms of actions according to disease state. Furthermore, results depend on administration route/dose, with intravenous formulations more suitable for active autoimmune disease and oral formulations more potent for disease prevention.[13]

Immunosuppressive pharmacological interactions in preclinical models is warranted, and primate models developed since our trial was conceived may contribute to better trial design.[14] We cannot exclude that a potential beneficial effect of anti-CD3 therapy may have been counteracted by concomitant immunosuppressive drugs and/or this intervention may not be effective in GVHD prevention as opposed to GVHD treatment.

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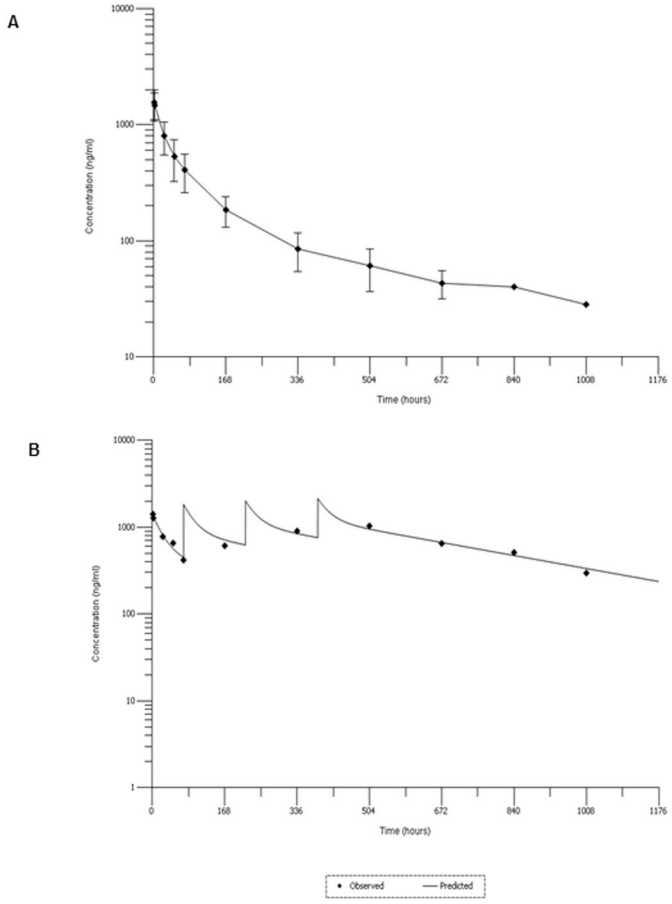

Figure 1. Visilizumab Pharmacokinetics

(A) Mean ± SD concentration (ng/mL) vs time profile for 7 patients receiving a single visilizumab dose of 3 mg/m^2. **(B)** Concentration vs. time profile for 1 patient receiving 4 consecutive doses of visilizumab at 3 mg/m^2 on days 0, 4, 11, and 18.

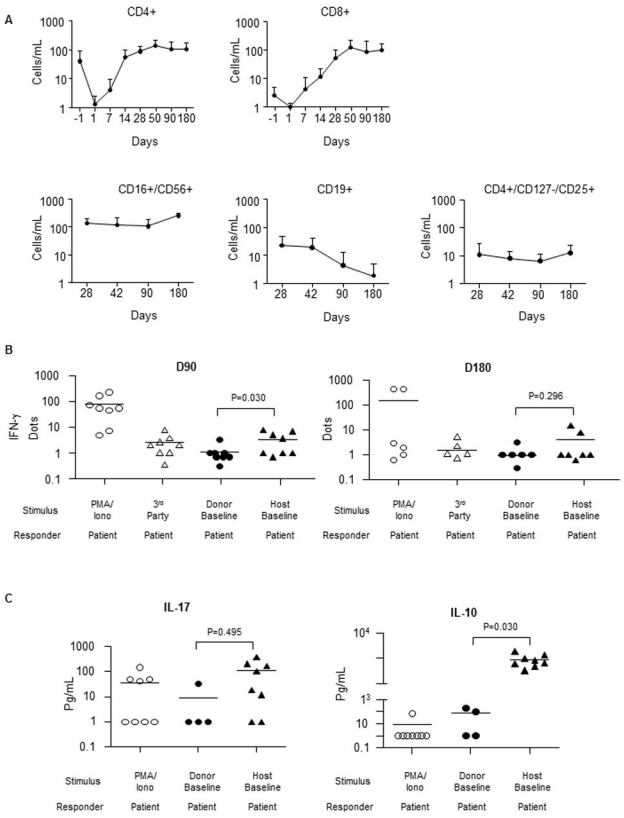

Figure 2. (A) Immune Recovery After Visilizumab Treatment

Flow cytometric analysis of peripheral blood leukocytes from patients before (day −1) and after visilizumab administration (days 1–180) (top graphs). Bottom panels show kinetics of recovery of natural killer cells (CD16+/CD56+), B cells (CD19+), and T-regulatory cells (CD4+/CD127−/CD25+) from days 28 to 180 posttransplant.. **(B) Analysis of Specific Alloreactive T-Cell Subsets After Visilizumab Treatment.** Interferon production by ELISPOT assay in blood at 90 and 180 days after HCT. PMA/Iono polyclonal stimulation or antigen-presenting cells from a third-party donor were used as positive stimulation controls. **(C) IL-17 and IL-10 cytokine testing by ELISA in ELISPOT supernatants at day 90 after HCT.**