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Variable Eculizumab Clearance Requires Pharmacodynamic Monitoring to Optimize Therapy for Thrombotic Microangiopathy after Hematopoietic Stem Cell Transplantation

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Thrombotic microangiopathy (TMA) after hematopoietic stem cell transplantation (HSCT) associated with terminal complement activation, as measured by elevated plasma terminal complement (sC5b-9) concentrations, has a very high mortality. The complement inhibitor eculizumab may be a therapeutic option for HSCT-associated TMA. We examined the pharmacokinetics and pharmacodynamics (PK/PD) of eculizumab in children and young adult HSCT recipients with TMA and activated complement to determine drug dosing requirements for future efficacy trials. We analyzed prospectively collected laboratory samples and clinical data from 18 HSCT recipients with high-risk TMA presenting with complement activation who were treated with eculizumab. We measured eculizumab serum concentrations, total hemolytic complement activity, and plasma sC5b-9 concentrations. Population PK/PD analyses correlated eculizumab concentrations with complement blockade and clinical response and determined interindividual differences in PK parameters. We also compared transplant survival in patients treated with eculizumab (n = 18) with patients with the same high-risk TMA features who did not receive any targeted therapy during a separate prospective observational study (n = 11). In the PK analysis, we found significant interpatient variability in eculizumab clearance, ranging from 16 to 237 mL/hr/70 kg in the induction phase. The degree of complement activation measured by sC5b-9 concentrations at the start of therapy, in addition to actual body weight, was a significant determinant of eculizumab clearance and disease response. Sixty-one percent of treated patients had complete resolution of TMA and were able to safely discontinue eculizumab without disease recurrence. Overall survival was significantly higher in treated subjects compared with untreated patients (56% versus 9%, $P = .003$). Complement blocking therapy is associated with improved survival in HSCT patients with high-risk TMA who historically have dismal outcomes, but eculizumab pharmacokinetics in HSCT recipients differ significantly from reports in other diseases like atypical hemolytic uremic syndrome and paroxysmal nocturnal hemoglobinuria. Our eculizumab dosing algorithm, including pr-treatment plasma sC5b-9 concentrations, patient's actual body weight, and the first eculizumab dose (mg), accurately determined eculizumab concentration-time profiles for HSCT recipients with high-risk TMA. This algorithm may guide eculizumab treatment and ensure that future efficacy studies use the most clinically appropriate and cost-efficient dosing schedules.

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INTRODUCTION

Thrombotic microangiopathy (TMA) is a common complication after hematopoietic stem cell transplantation (HSCT) [1-4]. The reported incidence of TMA after HSCT varies from 0 to 74% in retrospective studies [5]. Our recent prospective observational study, using rigorous monitoring for microangiopathy, identified TMA in 39% of patients [6]. Consistent with the literature, the clinical presentation of TMA ranged from mild (laboratory test changes only) to severe life-threatening disease [7-11]. Half of the patients with TMA in our study had severe multivisceral disease, contributing to dismal transplant outcomes in untreated patients.

Traditional risk factors for TMA include endothelial injury from conditioning chemotherapy, radiation, calcineurin inhibitors, or infections [12-15]. However, there is increasing evidence that complement is involved in the pathophysiology of HSCT-associated TMA and ensuing renal injury, similar to what occurs in atypical hemolytic uremic syndrome (aHUS). In our recently published prospective study [16], HSCT recipients with proteinuria and terminal complement activation, defined as elevated plasma concentrations of the soluble terminal complement complex (sC5b-9), in addition to hematologic markers of TMA, had very poor survival (<20%) and were classified as having high-risk TMA. In contrast, patients with hematologic TMA markers but without evidence of complement activation or proteinuria all survived despite not receiving any targeted interventions and were classified as having low-risk TMA. After this prospective study, all HSCT recipients at our center with high-risk TMA were offered eculizumab therapy in light of the known poor prognosis.

Eculizumab is a humanized monoclonal antibody against the complement component C5 that prevents endothelial damage by blocking formation of the membrane attack complex (Supplementary Figure 1). In our initial report of the first 6 treated patients, we noted a lag in clinical response, despite achieving an eculizumab serum concentration expected to be therapeutic of >99 μg/mL [17]. In the current prospective study we performed population-based pharmacokinetic and pharmacodynamic (PK/PD) analyses in an extended cohort of children and young adults receiving HSCT who were treated with eculizumab, using plasma sC5b-9 concentrations as a marker of TMA disease activity, to establish dosing and monitoring regimens for future prospective efficacy studies.

METHODS

Study Subjects

All consecutive HSCT recipients who received eculizumab [18] for high-risk TMA at our center from January 2012 to June 2014 were included in the PK/PD analyses. All study subjects had high-risk TMA features, including plasma sC5b-9 concentrations above normal (>244 ng/mL) and nephrotic range proteinuria (random urine protein/creatinine ratio > 2 mg/mg) present at the time of TMA diagnosis, in addition to hematologic TMA markers (schistocytes, elevated lactate dehydrogenase, reduced haptoglobin, de novo anemia, and thrombocytopenia) as previously determined in our prospective observational study [6]. Clinical and laboratory data were prospectively captured from the electronic medical record into HSCT databases. The institutional review board at our center approved the study. Informed consent was obtained from all study subjects.

Response Assessment

A hematologic response to eculizumab was defined as normalization of lactate dehydrogenase, resolution of the need for RBC and platelet transfusions, and disappearance of schistocytes. A complete clinical response was defined as resolution of organ failure, normalization of the hematologic parameters noted above combined with a doubling of the cystatin C-estimated glomerular filtration rate, and improvement of proteinuria to values below the nephrotic range, as defined by a random spot urine protein-to-creatinine ratio < 2 mg/mg and normalization of plasma sC5b-9 [17]. Discontinuation of therapy was considered successful if there was no TMA recurrence 8 weeks after the last eculizumab dose with normal sC5b-9 and CH50 values.

Eculizumab Blood Concentration and Complement Testing

Soluble terminal complement complex activity (sC5b-9) was measured in plasma by ELISA (normal plasma concentration < 244 ng/mL). CH50 was measured in serum using a hemolytic assay (normal, 101 to 300 units). ADAMTS13 activity (normal > 67%) was measured at the time of TMA diagnosis to rule out thrombotic thrombocytopenic purpura [19]. All assays used for this study are validated and available for clinical use at our institution. Eculizumab serum concentrations were measured at Cambridge Biomedical, Inc. (Boston, MA) [20]. Recommended therapeutic eculizumab concentrations during eculizumab induction therapy was >99 μg/mL based on recent publications in patients with aHUS [21] and by Cambridge Biomedical laboratory recommendations for clinical testing.

Eculizumab Treatment Protocol

Eculizumab dosing was performed using CH50 monitoring as previously published by our group [1]. CH50 was measured before starting eculizumab to ensure patients did not have underlying hypocomplementemia that would preclude the use of CH50 for complement blockade monitoring and was then measured daily during therapy. The first eculizumab dose was based on weight as recommended for children with aHUS [20]. In brief, patients weighing < 40 kg started with 600 mg intravenously and patients weighing ≥ 40 kg started with 900 mg intravenously. Subsequent dose adjustments were as follows: If CH50 after the first eculizumab dose remained suppressed (<10% of normal) for at least 6 days, the subsequent dose was administered on the seventh day and then weekly while maintaining CH50 <10% [17,22]. If CH50 increased above 10% of normal sooner than 6 days, the next dose was given when CH50 elevation above 10% normal was documented. If there was no adequate CH50 suppression after intensifying dosing interval or there was no hematologic response for longer than 10 days, the eculizumab dose was increased by 300 mg/dose.

After establishing the required dosing schedule to maintain adequate CH50 suppression, induction therapy was continued until patients achieved a hematologic TMA response and had a documented eculizumab serum concentration > 99 μg/mL, at which point a maintenance schedule was started [17]. Complete blood counts (including schistocytes) and lactate dehydrogenase were monitored daily. Haptoglobin, urinalyses, random urine protein-to-creatinine ratio, and cystatin C-estimated glomerular filtration rate were monitored weekly. Eculizumab serum concentrations were measured daily during induction therapy, and plasma sC5b-9 was monitored at least 3 times per week during therapy. In addition, we measured plasma sC5b-9 weekly starting before HSCT therapy until clinical TMA diagnosis to evaluate the relationship between the first plasma sC5b-9 elevation and appearance of hematologic signs of TMA. Eculizumab serum concentration results were not available in real-time for drug dose adjustments but were used later for PK/PD analysis to correlate eculizumab serum concentrations with sC5b-9 and CH50 values and clinical response.

Eculizumab induction dosing was continued until hematologic TMA response was achieved and CH50 remained suppressed below 10% of normal for 4 weeks, at which point a maintenance schedule was started by giving the same dose every 2 weeks while maintaining CH50 < 10% [1]. When CH50 remained suppressed for longer than 2 weeks during the maintenance therapy without drug redosing and without active TMA signs, eculizumab therapy was stopped. TMA laboratory markers, serum CH50, and plasma sC5b-9 continued to be monitored 2 to 3 times per week for at least 8 weeks. All patients received antibacterial prophylaxis against *Neisseria meningitidis* until at least 8 weeks after discontinuation of eculizumab or until normalization of CH50, because meningococcal vaccination does not provide protection in severely immunocompromised HSCT patients [23].

Eculizumab PK/PD Analyses

Standard PK analyses were performed using a 1-compartment model to obtain eculizumab PK parameters such as systemic clearance and volume of distribution, as previously described [17]. Population PK modeling was performed using NONMEM version 7.2 (ICON Development Solutions, Ellicott City, MD) to characterize population PK parameters, focusing on the induction phase (first dose), and to identify significant covariates for eculizumab PK parameters (see Supplementary Methods). A 1-compartment PK model was used as the structural base model. Total body weight and plasma sC5b-9 concentration at initiation of the therapy were tested as potential covariates for each PK parameter in the covariate analysis. Selection of covariates was based on a significant reduction of the objective function value by stepwise forward inclusion ($P < .05$), backward elimination ($P < .01$), and graphic evaluation of goodness-of-fit plots. The eculizumab serum concentration required to suppress CH50 to <10% of normal