Eculizumab Therapy in Children with Severe Hematopoietic Stem Cell Transplantation–Associated Thrombotic Microangiopathy

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Abstract

We recently observed that dysregulation of the complement system may be involved in the pathogenesis of hematopoietic stem cell transplantation–associated thrombotic microangiopathy (HSCT-TMA). These findings suggest that the complement inhibitor eculizumab could be a therapeutic option for this severe HSCT complication with high mortality. However, the efficacy of eculizumab in children with HSCT-TMA and its dosing requirements are not known. We treated 6 children with severe HSCT-TMA using eculizumab and adjusted the dose to achieve a therapeutic level >99 μg/mL. HSCT-TMA resolved over time in 4 of 6 children after achieving therapeutic eculizumab levels and complete complement blockade, as measured by low total hemolytic complement activity (CH50). To achieve therapeutic drug levels and a clinical response, children with HSCT-TMA required higher doses or more frequent eculizumab infusions than currently recommended for children with atypical hemolytic uremic syndrome. Two critically ill patients failed to reach therapeutic eculizumab levels, even after dose escalation, and subsequently died. Our data indicate that eculizumab may be a therapeutic option for HSCT-TMA, but HSCT patients appear to require higher medication dosing than recommended for other conditions. We also

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observed that a CH50 level 4 complement activity enzyme units correlated with therapeutic eculizumab levels and clinical response, and therefore CH50 may be useful to guide eculizumab dosing in HSCT patients as drug level monitoring is not readily available.

INTRODUCTION

Hematopoietic stem cell transplantation–associated thrombotic microangiopathy (HSCT-TMA) is a challenging post-transplant complication associated with long-term morbidity and high mortality [1–3]. HSCT-TMA shares features with other TMAs where endothelial injury affects the kidney and other organs. Mild HSCT-TMA may have a benign course that requires no therapy or only modification of calcineurin inhibitor dosing [4,5]. However, a proportion of cases develop a systemic vascular injury that manifests as kidney damage, serositis, pulmonary hypertension, and multisystem organ failure [6–9]. In the most severe form of HSCT-TMA, mortality rates approach 90%, whereas milder cases have an increased risk of chronic kidney disease [3,8,10]. Targeted therapy is urgently needed for patients with severe HSCT-TMA in whom mortality is the highest.

Recently, we showed that patients with HSCT-TMA have evidence of complement dysregulation, including complement factor H autoantibodies and renal C4d deposition [11,12]. HSCT-TMA is a multifactorial disease in which either the classical or alternative complement pathways may become activated, resulting in tissue damage from micro-vessel thrombosis [13]. Currently available therapeutic options in patients with HSCT-TMA include therapeutic plasma exchange (TPE), rituximab, and withdrawal of calcineurin inhibitors. However, clinical response is often limited, especially in patients with severe HSCT-TMA. Furthermore, altering immunosuppressive therapy may increase the risk of graft-versus-host disease (GVHD), which itself is associated with high mortality [5,14,15].

Eculizumab, a humanized monoclonal antibody against the complement component C5 that prevents tissue damage by blocking formation of the membrane attack complex, is increasingly prescribed in the treatment of other diseases presenting with TMA [16–19]. Eculizumab has a low toxicity profile and has been well tolerated in patients with paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome (aHUS). It has been approved for use in children with aHUS, and a pediatric weight-based dosing schedule has been established [20]. It is unknown if eculizumab is effective in children with HSCT-TMA and if the existing dosing regimen adequately blocks complement in these patients. Therefore, we describe our clinical experience in a cohort of children treated with eculizumab for severe HSCT-TMA and propose an algorithm to adjust the dosage and monitor therapeutic response based on our observations.

METHODS

Patient Population

Six patients with severe HSCT-TMA were treated with eculizumab (Alexion, Cheshire, CT) at Cincinnati Children's Hospital Medical Center (CCHMC) between January 2012 and May 2013. The CCHMC Institutional Review Board approved retrospective chart review. Patient demographics, therapy characteristics, and HSCT complications were abstracted from the medical record. HSCT-TMA was diagnosed using current diagnostic criteria and included elevated lactate dehydrogenase above normal for age, haptoglobin below the lower limit of normal, schistocytes on peripheral blood smear, anemia, thrombocytopenia, a negative Coombs test, and acute kidney injury, defined as a doubling of the serum creatinine or a 50% decline in the cystatin C–estimated glomerular filtration rate (eGFR) from each subject's pre-HSCT baseline [5,21,22]. Proteinuria was identified using a random spot urine protein-to-creatinine ratio (normal <.2 mg/mg, nephrotic range >2 mg/mg) [23,24]. Kidney biopsy results, if available, were reviewed for histology of TMA.

Each subject's legal guardian signed informed consent for treatment with eculizumab. The decision to start eculizumab was at the discretion of the treating physician but generally included severe TMA presenting with multiorgan impairment, uncontrolled hypertension, worsening renal function, and a lack of response to TPE and withdrawal of calcineurin inhibitors in allogeneic transplant recipients.

Outcomes

A hematologic response to eculizumab was defined as normalization of lactate dehydrogenase and haptoglobin, resolution of the need for RBC and platelet transfusions, and disappearance of schistocytes. A complete responsewas defined as normalization of the hematologic parameters noted above combined with a doubling of the cystatin C–eGFR and improvement of proteinuria to values below the nephrotic range, as defined by a random spot urine protein-to-creatinine ratio <2 mg/mg [25].

Complement Assessments

Assessment of the complement profile was performed at the time of HSCT-TMA diagnosis. Testing included measurement of complement proteins (Table 1), soluble terminal complement complex activity (sC5b-9), and autoantibodies to complement factor H (CFH) [11,26,27]. ADAMTS13 activity was measured to rule out thrombotic thrombocytopenic purpura. These studies were performed at the CCHMC clinical laboratory. Circulating CFH-related 1 protein (CFHR1) testing was performed on recipients' plasma to exclude a CFHR1 homozygous deletion. CFHR1 was detected by immunoblotting (Division of Nephrology, The Hospital for Sick Children, Toronto, Ontario, Canada), as previously described [28,29]. CFH-related genes 3 and 1 (*CFHR3-CFHR1*) were examined in stored pretransplant (recipient) DNA samples by multiplex ligation-dependent probe amplification testing (MORL Laboratory, University of Iowa, Iowa City, IA) [30,31].

Eculizumab Treatment and Monitoring

In patients receiving TPE, treatment was stopped before starting eculizumab so as not to remove the drug. The first dose of eculizumab was given according to recommendations for children with aHUS [20]. Eculizumab was infused via central venous access over 60 minutes. Because immunocompromised HSCT recipients do not respond to meningococcal vaccination, all patients were maintained on ciprofloxacin or penicillin VK prophylaxis until eculizumab was cleared and the CH50 levels normalized.

Because dosing recommendations to guide eculizumab therapy in HSCT recipients are not available, eculizumab serum levels were tested at least twice a week after each dose and always included a trough level drawn before each dose. Eculizumab drug levels were performed at Cambridge Biomedical, Inc. (Boston, MA), and we adjusted the dose to maintain a trough concentration >99 μg/mL, because this drug level has been considered therapeutic in patients with aHUS [16]. Patients weighing <40 kg started with 600 mg i.v., and patients weighing 40 kg started with 900 mg i.v. as a first dose. Subsequent dose adjustments were as follows: If a trough level was reported as therapeutic before the next weekly dose, the same induction dose was continued weekly. If the patient was due for the next weekly dose and the eculizumab trough concentration was subtherapeutic, the dose was increased by 300 mg/dose. If a subtherapeutic result was reported 4 to 5 days after the prior dose, an extra induction dose was given when the result was obtained. If results were not available for dose adjustment, the same eculizumab induction dose was continued weekly until the trough eculizumab concentration was documented to be above the therapeutic level. Laboratory markers of TMA were documented daily during inpatient stay and at least twice weekly in the outpatient setting.

Weekly induction therapy was continued until patients achieved a hematologic TMA response and had documented eculizumab levels >99 μg/mL, at which point a maintenance schedule was started (see Figure 3B). The criteria for stopping eculizumab included normalization of hematologic TMA parameters and improvement in renal function.

Pharmacokinetic and Pharmacodynamic Analyses

Total hemolytic complement activity (CH50) was measured in serum during eculizumab therapy as a pharmacodynamic marker of eculizumab-induced complement blockage, at the same time points as eculizumab drug levels. We correlated eculizumab drug levels with the degree of complement blockade as measured by CH50. A normal CH50 level was 60 to 144 complement activity enzyme (CAE) units (ARUP Laboratories, Salt Lake City, UT) [32].

The pharmacokinetic profiling of eculizumab was performed at the Division of Clinical Pharmacology, CCHMC, and was described by a 1-compartment model because the eculizumab concentrations showed an exponential decline over time. The elimination rate constant (k_{el}) and concentration at 0 (C_0) were estimated by linear least-squares regression of the log-transformed concentrations versus time in the log-linear phase of the disposition profile. Apparent volume of distribution (V_d) was estimated based on dose/C_0 in the first treatment. Apparent systemic clearance (CL) was calculated by the formula CL (L/h) = $k_{el} \times V_d$. Maximum eculizumab concentrations in the nth dose ($C_{max,n}$) were determined by the

following equation: $C_{max,n} = C_{trough, n-1} + dose/V_d$. Receiver operating characteristic curve analysis and Youden's index were used to find the best cut-off value of CH50 that predicted a therapeutic eculizumab trough level > 99 μg/mL.

RESULTS

Patient Characteristics

Six children with severe HSCT-TMA received eculizumab therapy; patient characteristics are shown in Table 1. Median patient age was 5 years (range, 2.4 to 10.9 years). HSCT-TMA was diagnosed within 100 days (range, 6 to 69 days) of transplant in 5 patients. One patient was diagnosed 1 year post-transplant after presenting with acute HSCT-TMA and renal failure requiring hemodialysis. At diagnosis of HSCT-TMA, all patients had impaired renal function with a median serum cystatin C–eGFR of 18.5 mL/min (range, 15 to 48 mL/min) and nephrotic range proteinuria with a median random urine protein-to-creatinine ratio of 11 mg/mg (range, 4.5 to 81.6 mg/mg). Two patients required renal replacement therapy. All patients had severe hypertension requiring 6 to 9 antihypertensive medications at initiation of eculizumab therapy. ADAMTS13 activity level was normal (>67%) in all patients, ruling out thrombotic thrombocytopenic purpura.

More specifically, patient 1 was treated with eculizumab without attempting TPE because she had acute development of HSCT-TMA with uncontrolled hypertension and was not considered a suitable candidate for apheresis line placement under anesthesia. Patient 2 had progressive renal function decline and was approaching the need for renal replacement therapy. Renal biopsy showed >90% of glomeruli affected by TMA. Diffuse C4d staining was evident in TMA affected renal arterioles indicating complement deposits, as previously described [12]. Patient 3 had exacerbation of HSCT-TMA symptoms and progressive hypertension with each attempt to wean TPE. Patient 4 developed seizures and posterior reversible encephalopathy syndrome and was on 9 antihypertensive medications with blood pressures still above 99% for her age and height before initiation of eculizumab. Patients 5 and 6 were critically ill with acute GVHD, viral infections, and multiorgan failure in addition to HSCT-TMA before initiation of eculizumab.

Cyclosporine was discontinued in all 4 allogeneic transplant recipients at diagnosis of HSCT-TMA due to kidney injury or the development of posterior reversible encephalopathy syndrome/seizures and was replaced with steroids and/or mycophenolate mofetil. Three of these patients had active acute GVHD at diagnosis of TMA. Patient 3 had acute stage III skin GVHD (overall grade II GVHD) and was treated with mycophenolate mofetil and methylprednisolone 1 mg/kg/day. GVHD improved sufficiently by the end of eculizumab therapy to allow weaning of anti-GVHD medications. Patient 5 had acute ocular GVHD, acute stage III skin GVHD, and acute stage II gut GVHD (overall grade III GVDH) at TMA diagnosis and was treated with mycophenolate mofetil, methylprednisolone 2 mg/kg/day, and infliximab with resolution of all but ocular GVHD by the time he died from multiorgan failure. Patient 6 had acute stage II gut GVHD with intermittent intestinal bleeding at HSCT-TMA diagnosis and was treated with methylprednisolone 2 mg/kg/day and infliximab. Intestinal bleeding persisted during eculizumab therapy until patient's family opted to

withdraw clinical support. At autopsy, intestinal bleeding was attributed to gut GVHD coincident with severe intestinal TMA.

Complement Assessments

Terminal complement complex activity (sC5b-9) was elevated in 5 of 6 patients (Table 1). The patient with a normal sC5b-9 level was receiving TPE when the blood sample was obtained. CFH autoantibody was not detected in any patient. Two patients had decreased C4 levels and 1 decreased CFH levels. All patients had detectable CFHR1 protein, ruling out a homozygous deletion of *CFHR3-CFHR1*. One of 3 patients tested with multiplex ligation-dependent probe amplification testing had a heterozygous *CFHR3-CFHR1* deletion. Renal biopsy performed on patient 2 showed severe TMA with diffuse C4d staining in affected arterioles [12]. Patient 6 had renal TMA confirmed on autopsy.

Clinical Response to Eculizumab

Patients 1 to 4 had complete clinical responses to eculizumab after achieving therapeutic eculizumab levels (Table 2). A median of 7 doses (range, 4 to 13 doses) of eculizumab were required to resolve HSCT-TMA. All surviving patients weighing < 40 kg received eculizumab 600 mg i.v. and those weighing 40 kg received 900 mg i.v. at least weekly for at least 4 weeks before any consideration was given to advance the dosing schedule. A hematologic TMA response in these 4 patients was observed a median of 28.5 days (range, 15 to 45 days) after initiation of eculizumab.

All 4 responders had a dramatic improvement in hypertension with a reduction in the number of antihypertensive medications from 6 to 9 at the start of eculizumab therapy to 0 to 2 medications at eculizumab therapy completion. The median time for a complete response of all HSCT-TMA parameters, including doubling in cystatin C–eGFR and improvement of proteinuria below 2 mg/mg, was 69.5 days (range, 29 to 141 days) after eculizumab initiation. All responders were doing well at a median of 47.5 weeks (range, 38 to 72 weeks) post-diagnosis of HSCT-TMA. Patients 1 and 4 completely recovered renal function as evidenced by normal cystatin C–eGFR >100 mL/min and the absence of proteinuria and hypertension. Patients 2 and 3, both of whom had prolonged severe kidney injury due to HSCT-TMA at the start of eculizumab therapy, remain with chronic kidney disease and a cystatin C–eGFR <50 mL/min but are off hemodialysis and normotensive on losartan therapy.

Patients 5 and 6 died from multisystem organ failure and active HSCT-TMA while receiving eculizumab. These 2 patients were older (and weigh more) than the 4 patients who responded to treatment. Both patients were critically ill with severe multiorgan injury at the time of starting eculizumab therapy, and neither achieved sustained therapeutic eculizumab trough levels or complement blockade, as measured by CH50 (Figure 1). All 6 patients tolerated eculizumab therapy without any side effects or reactions, and there were no meningococcal infections or other bacterial infections.

Eculizumab Pharmacokinetics

Eculizumab dosing and drug levels during the first 3 weeks of therapy are shown in Figure 1. Four of 6 patients had subtherapeutic eculizumab through levels after the first dose. Notably, the first eculizumab dose for patient 3 was higher (600 mg) than recommended (300 mg) for his weight of 9 kg, producing a therapeutic level after the first dose. This child was the only patient who achieved and maintained a therapeutic eculizumab level after the first dose, which may have been associated with a faster clinical response. Therapeutic trough levels of eculizumab >99 μg/mL were eventually achieved in all 4 responders (patients 1 to 4) using either extra doses or doses that were higher than currently recommended (Figure 1). Eculizumab trough levels remained subtherapeutic in the 2 nonresponding cases (patients 5 and 6). Patient 5 did not achieve a therapeutic trough level during 5 weeks of therapy, despite significant dose escalation to 900 mg twice weekly, starting on day 24 of therapy (data not shown in Figure 1). Patient 6 received 2 weekly induction doses of 900 mg, as recommended for his weight, before he died. In both nonresponders, the eculizumab level dropped below therapeutic 3 days after the first dose, but results were pending so dosing could not be adjusted in a timely manner. Elimination rate constants and systemic clearance of eculizumab showed significant variability among patients, with the most rapid clearance in patients 5 and 6 who were most ill at the time of treatment and perhaps more highly catabolic than the responding patients (data not shown).

Relationship between Eculizumab Levels and CH50

Eculizumab and CH50 levels that were measured at the same time points strongly correlated with each other (Figures 1 and 2). Specifically, a CH50 level of 0 to 3 CAE units corresponded with an eculizumab concentration >99 μg/mL, except for 1 measurement from patient 6, drawn during an extremely rapid elimination phase in the first week. Conversely, at all sampling time points with a CH50 count 4 CAE units, the patients' eculizumab concentrations were below therapeutic at <99 μg/mL ($P = .0001$).

Complete blockade of complement activity (CH50 < 3 CAE units) was achieved in all 4 responders (patients 1 to 4) after the second dose of therapy (Figure 1). Complement blockade was incomplete in the 2 nonresponding cases (patients 5 and 6).

DISCUSSION

We report the first use of the terminal complement inhibitor eculizumab in children with severe HSCT-TMA. We observed that 4 of 6 children had complete resolution of severe HSCT-TMA after achieving steady therapeutic eculizumab levels. The 2 remaining patients, who were critically ill when therapy was initiated, died without achieving therapeutic drug levels or complement blockade, despite dose escalation. The 2 nonresponders were also older than the 4 responders, an observation that deserves future study. All children with HSCT-TMA required higher eculizumab dosing and/or more frequent drug administrations to reach and maintain therapeutic eculizumab trough levels > 99 μg/mL compared with the dosing regimen currently recommended for children with aHUS [20].

Measurement of eculizumab levels in our patient cohort was of significant value in understanding the drug's pharmacokinetics in children after HSCT. Pharmacokinetic analysis showed that 3 of 6 patients had eculizumab subtherapeutic drug concentrations < 99 μg/mL as soon as 4 to 5 days after the first eculizumab infusion and required an additional eculizumab dose to achieve a therapeutic drug trough level. Also, the rate of eculizumab elimination was not constant after every treatment but decreased over time and stabilized as represented by a declining slope of the log-transformed eculizumab concentration, which became shallower after multiple treatments indicating that doses could be administered with longer intervals. After steady therapeutic eculizumab serum concentrations were achieved, younger patients in our cohort were able to maintain a much higher drug level than targeted (>99 μL/mL) without any adverse events and with notable improvement in TMA-related symptoms. The current literature reports no significant adverse events attributed to high eculizumab levels, outside the known risk of infection. Unfortunately, eculizumab drug levels are only available from 1 laboratory in the United States (Cambridge Biomedical, Inc.), and results are often not available for 1 to 2 weeks. This long turnaround time may delay needed dose adjustment and prevent the achievement of complement blockade in HSCT patients treated with eculizumab. We speculate that earlier adjustments to dosing may have allowed treatment to be more effective in controlling TMA and preserving organ function in some of our patients.

In contrast, CH50 represents a pharmacodynamic measure of complement activity and is more readily available in most institutions, with a result turnaround time of 1 to 2 days. Therefore, CH50 testing during eculizumab therapy in HSCT patients may offer a more rapid assessment of complement blockade, allowing for practical dose adjustments in a more timely fashion. Our proposed dosing schedule modification based on CH50 level is displayed in Figure 3. In our study, only a CH50 level < 4 CAE units strongly correlated with a therapeutic eculizumab level > 99 μg/mL and clinical TMA response, indicating successful complement blockade and adequate drug dosing. Our observations suggest that low CH50 levels of 0 to 3 CAE units (or CH50 activity below 5%) should be maintained during eculizumab treatment to ensure the best therapeutic response.

Our study also provides important data regarding the time to clinical response with eculizumab in children with HSCT-TMA. Even though resolution of severe TMA took several weeks in responding patients, similar to aHUS, the fastest and most complete response was achieved in patients who started eculizumab therapy promptly after the diagnosis of HSCT-TMA and sustained steady therapeutic eculizumab levels and complement blockade [16]. However, we were able to achieve a hematologic response and control of hypertension even in patients with a prolonged course of HSCT-TMA that was previously refractory to TPE. In cases with prolonged HSCT-TMA before eculizumab therapy, renal recovery was incomplete and the time to recovery was longer, but all responders exhibited continuous, time-dependent improvement in renal function, suggesting that early use of eculizumab may maximize benefit in cases of severe HSCT-TMA.

Based on our clinical and pharmacologic observations, we suggest at least 4 to 6 weeks of induction therapy with a documented therapeutic eculizumab trough level or adequate complement blockade (low CH50) before calling a patient a nonresponder. If a patient is

determined to be a rapid eliminator after the first induction dose (ie, 3 to 4 days after therapy initiation), an additional eculizumab induction dose should be considered to achieve a therapeutic trough level as quickly as possible. The CH50 level should be measured daily during induction therapy. In patients who fail to achieve full complement blockade, we suggest increasing the eculizumab dose by 300 mg above the currently recommended induction dose (Figure 3). As demonstrated by our 2 nonresponding subjects, those with additional complications such as GVHD and viral infections may be highly catabolic with concomitant activation of terminal complement. It may be challenging to achieve therapeutic levels of eculizumab needed to block complement and control TMA. Administration of eculizumab in critically ill HSCT patients without proper monitoring may provide only intermittent or no complement blockade, leading to the conclusion that eculizumab does not work in controlling TMA while in reality inadequate dose is being administered.

Five patients in our cohort had elevated sC5b-9, indicating an activated terminal complement pathway that could be caused by either alternative or classic complement dysregulation as previously described by our group of patients with HSCT-TMA [11,12]. Differences in the pathogenesis of HSCT-TMA occurring after allogeneic and autologous HSCT remain to be examined. We observed that autologous HSCT recipients present with evidence of classic pathway activation with a low C4 level, C4d deposition in the kidney, and severe multiorgan injury that may result from direct damage to the vascular endothelium from conditioning chemotherapy. Conversely, after allogeneic HSCT, we observed that some patients have evidence of alternative pathway activation with the presence of CFH antibodies. Nevertheless, complement activated by either the alternative or classic pathway can be blocked by the terminal complement blocker eculizumab, making this medication an appropriate therapy regardless of the complement pathway involved in pathogenesis of HSCT-TMA (Figure 4) [20,33].

Although patients with paroxysmal nocturnal hemoglobinuria and aHUS are often committed to life-long therapy with eculizumab, some HSCT patients may develop TMA due to infections or temporary immune dysregulation without a genetic predisposition, suggesting that eculizumab therapy can be discontinued in these patients after TMA is controlled. We were able to stop eculizumab therapy in all 4 responders after achieving a hematologic and renal response. The shortest successful course was 4 weeks, and the longest therapy was 15 weeks. There were no recurrences of HSCT-TMA in the 4 responders after stopping therapy.

To consider stopping eculizumab therapy, we first suggest achieving full resolution of hematologic TMA markers during induction therapy with the steady therapeutic eculizumab level and full complement blockade documented by low a CH50 level. After achieving hematologic TMA control and improvement in renal function, eculizumab can be advanced to a maintenance schedule. Hypertension must be well controlled in all patients with a target systolic blood pressure below the 95th percentile for age and height [34]. Only after ensuring TMA is well controlled on a maintenance dosing regimen, eculizumab may be stopped while TMA markers are closely monitored (Figure 3).

In conclusion, eculizumab is a promising therapeutic option for pediatric patients with severe HSCT-TMA. In our practice, we consider eculizumab as a first-line therapy in patients who present with acute-onset HSCT-TMA with significant microangiopathic hemolysis, severe hypertension, or evidence of multiorgan involvement or in patients with subacute TMA whose symptoms progress within 2 weeks of observation and supportive care. We believe that early therapy initiation with eculizumab may prevent irreversible organ damage, and toxicity is low. Eculizumab dosing in children with HSCT-TMA should be guided by pharmacokinetic or pharmacodynamic testing, and more aggressive dosing schedules need to be explored in the most ill and catabolic patients. CH50 monitoring may aid in timely dose adjustments, especially in highly catabolic patients, likely requiring drug administration more than once a week. Controlled studies of this approach in both children and adults after HSCT are warranted to further examine clinical response and the benefit of therapeutic monitoring.

Acknowledgments

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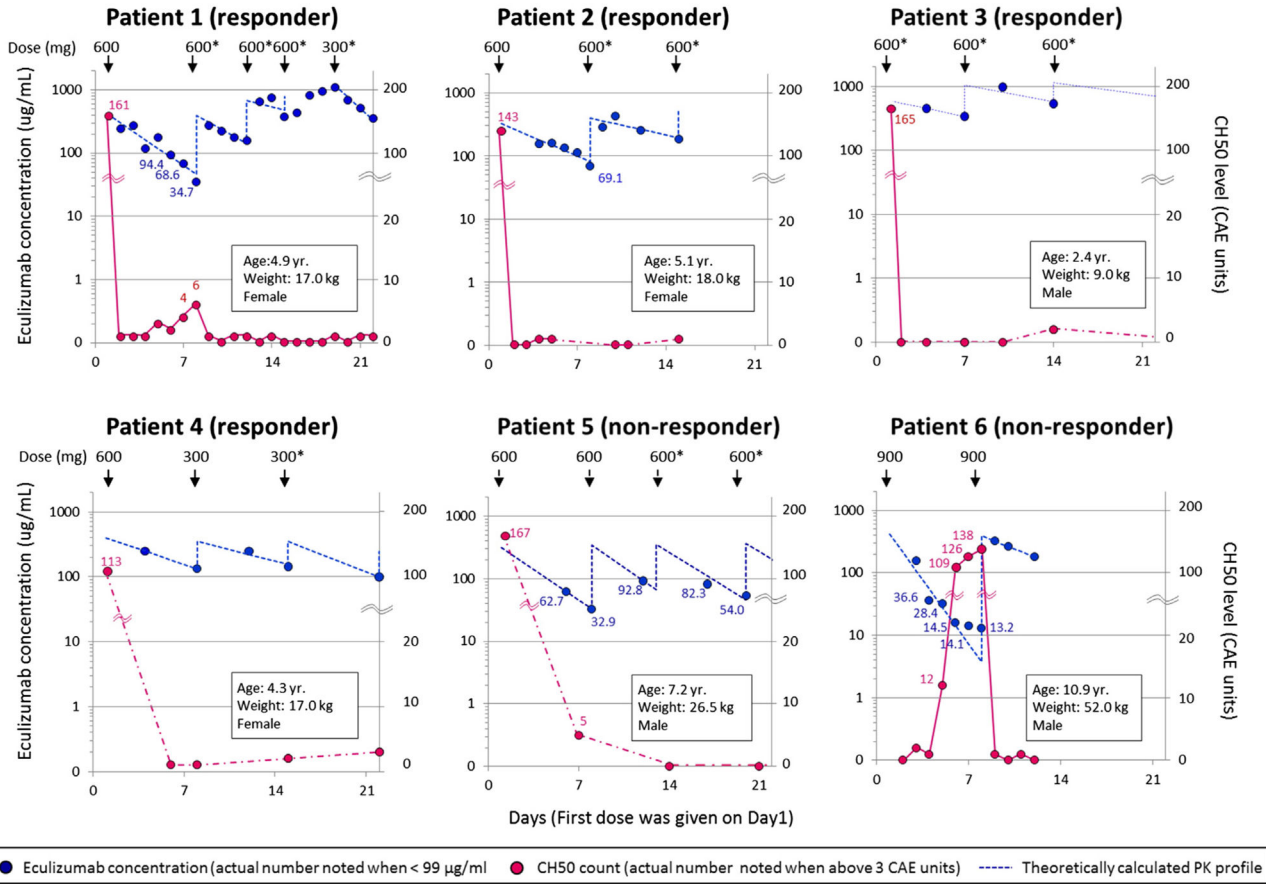
Figure 1.

Pharmacokinetic and pharmacodynamic analyses of eculizumab therapy in 6 patients with HSCT-TMA during the first 3 weeks of treatment are displayed. The left and right y axes show eculizumab concentrations and total complement activity (CH50) levels, respectively. The x axis shows time as days from the start of eculizumab therapy with the first eculizumab dose given on day 1. Dosage (mg) and the timing of administration are indicated with arrows on the top of each figure. Asterisk (*) marks eculizumab doses that are either higher than currently recommended for children with aHUS based on dosing weight or given more frequently [20]. Blue circles represent observed eculizumab concentrations. Actual measured values are noted beside the circles only when eculizumab concentration is below 99 μg/mL. Blue dashed lines represent predicted eculizumab pharmacokinetic profiles based on a 1-compartment analysis (see Methods for detail). Red circles represent the CH50 level. The actual values are listed on the graph only when the CH50 level is above 3 CAE units. Red circles are connected with red solid or dashed lines when measurement of CH50 levels are continuous (daily) or intermittent, respectively. In patient 6, the first 3 measurements days 2, 3, and 4 were used to estimate pharmacokinetic parameters (k_{el}, CL) because concentrations of eculizumab irregularly showed a bi-exponential decline with very low concentrations at the last 3 measurements on days 5, 6s and 7. The V_d for this patient was calculated based on the second treatment.
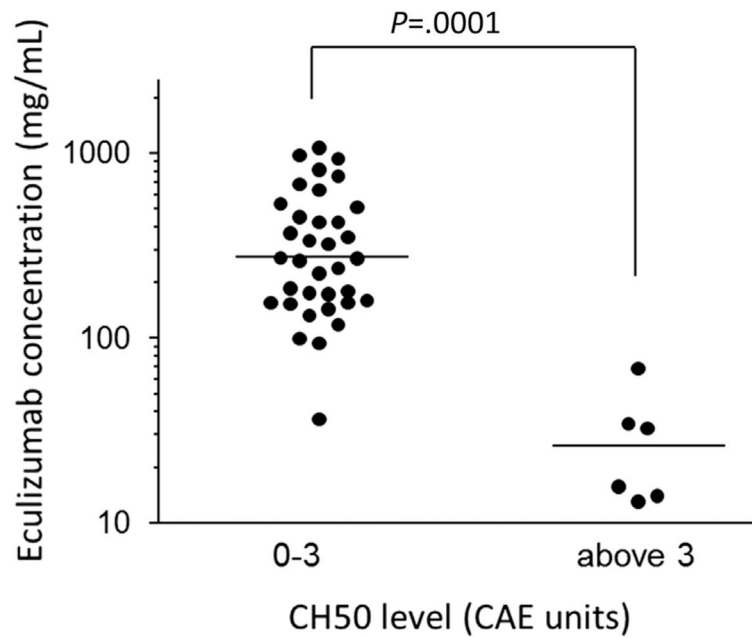
Figure 2.
Relationship between eculizumab concentration and total complement activity (CH50) level. Using the reported therapeutic eculizumab trough cut-off level of >99 or 99 μg/mL, the receiver operating characteristic curve and Youden's Index were used to determine the optimal CH50 cut-off level of 3.5 CAE units. The *y* axis shows eculizumab concentrations in log-scale. The *x* axis shows the CH50 level as a group. Horizontal lines represent medians. A CH50 level of 0 to 3 CAE units corresponded with an eculizumab concentration of >99 μg/mL in all except 1 measurement (drawn from patient 6 during an extremely rapid elimination phase in the first week of therapy). Conversely, at all sampling time points, a CH50 level >3 CAE units corresponded with a subtherapeutic level < 99 μg/mL (*P* = .0001, Mann-Whitney U test).
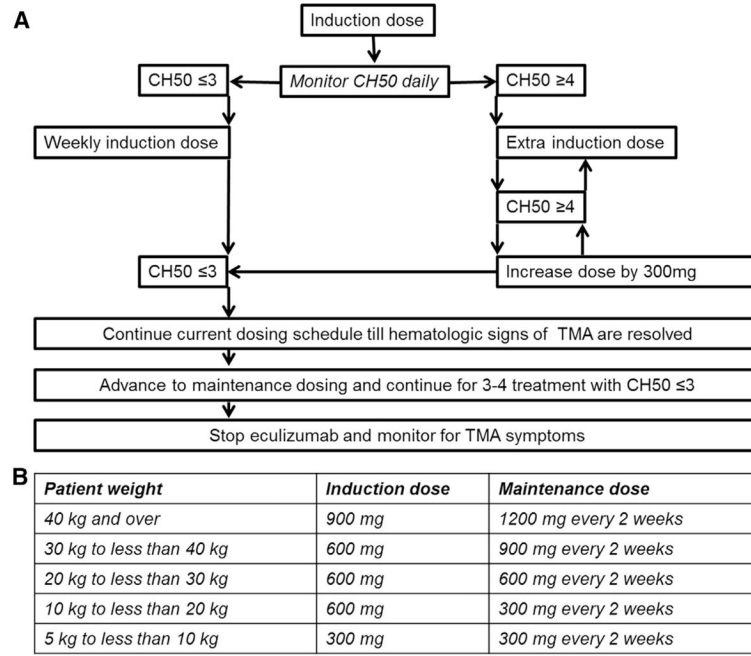
Figure 3.
Eculizumab dosing optimization in HSCT patients with TMA. (A) Suggested eculizumab dose adjustments based on CH50 level and clinical TMA response. (B) Eculizumab induction and maintenance doses based on patient's weight (modified from dosing schedule for children with aHUS [20]). CH50 should be monitored daily after the first eculizumab induction dose is given with the goal to maintain CH50 level of 0 to 3 CAE units that corresponds to a therapeutic eculizumab dose >99 μg/mL. If CH50 remains 0 to 3 CAE units after start of therapy, the induction dose should be given weekly until full resolution of hematologic parameters of TMA before advancing to maintenance schedule. If CH50 is 4 CAE units earlier than 7 days after starting therapy, an additional induction dose should be given. If CH50 raises again to 4 CAE units, the next dose should be increased by 300 mg to achieve a steady therapeutic level as soon as possible. The dose of eculizumab that maintains a CH50 level 3 CAE units is the required weekly induction dose for the particular patient. During maintenance therapy, CH50 should be monitored at least twice a week to ensure a therapeutic drug level.
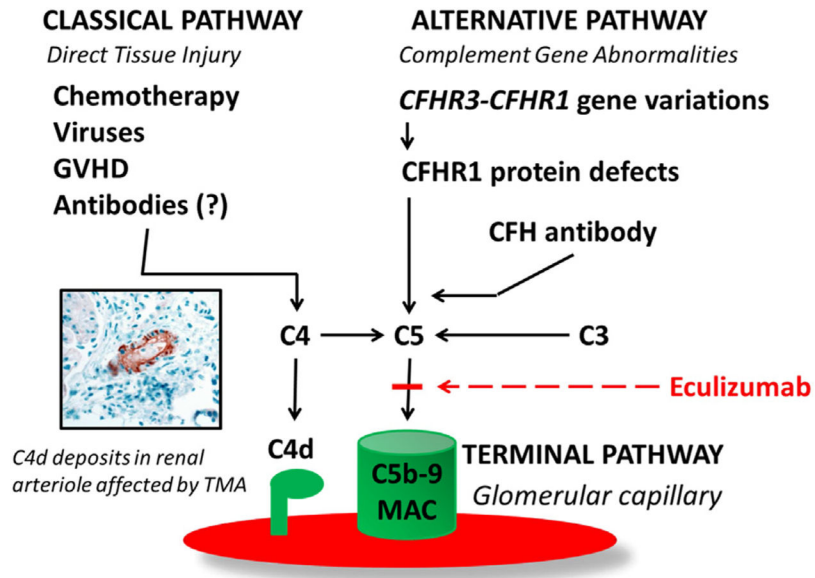
Figure 4.
Complement activation in pathogenesis of HSCT-TMA. Factors involved in classic and alternative pathway activation in patients with HSCT-TMA. The classic pathway can be activated by direct tissue injury from high-dose chemotherapy, viruses, and GVHD. C4d deposits, as the evidence of classic complement pathway activation, can be detected in renal arterioles of HSCT patients affected with TMA [12]. Alternative pathway dysregulation might present as a formation of the pathogenic autoantibody against complement factor H (CFH ab) due to either defects in *CFHR3-CFHR1* genes associated with defective or absent CFHR1 protein or immune dysregulation after HSCT without genetic predisposition [11]. Dysregulation in either the classic or alternative pathway of complement results in terminal complement activation detected as elevated sC5b-9 (MAC) that can be blocked by eculizumab, which inhibits the cleavage of C5 to C5a and C5b by the C5 convertase, which prevents the generation of the terminal complement complex C5b-9 and tissue injury.

Table 1

Patient Demographics and Disease Characteristics

	Responders				Non-responders	
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Gender	F	F	M	F	M	M
Age at HSCT, y	4.9y	5.1y	2.4y	4.3y	7.2y	10.9y
Weight, kg	4.9	5.1	2.4	4.3	7.2	10.9
Diagnosis	NBL	NBL	WAS	CID	WAS	CID
Stem cell source	Autologous	Autologous	Allogeneic	Allogeneic	Allogeneic	Allogeneic
HSCT conditioning regimen	MA	MA	MA	RIC	MA	RIC
Day post-HSCT when TMA was diagnosed	13	68	390	6	41	57
Cystatin C-eGFR (mL/min) at TMA diagnosis	48	20	16	30	15	17
Renal replacement therapy at TMA diagnosis	No	No	Yes	No	No	Yes
Urine protein-to-creatinine ratio (normal <2 mg/ mg, nephrotic >2 mg/mg)	81.6	11.3	4.5	10.6	14.1	6.4
HSCT-TMA–related complications	HTN, PH, pericardial effusion	HTN, pericardial effusion	HTN, pericardial tamponade	HTN, PRES, seizures, pericardial effusion	HTN, pulmonary bleeding	HTN, pericardial effusion
Complement profile *	↑C2, ↑C6, ↑CFB	↓C4	↓C4	↑C5, ↑C7, ↑C4BP	↑C8, ↑C1Inhib, ↓CFH	↑C1Q, ↑C4, ↑C8
sC5b-9 (normal, 119 to 17 5 ng/mL)	283	307	139 (on TPE)	328	432	375
CFH antibody	Absent	Absent	Absent	Absent	Absent	Absent
CFHR3-CFHR1 (by MLPA)	Not tested	Normal	Heterozygous deletion	Normal	Not tested	Not tested
CFHR1 protein	Present	Present	Present	Present	Present	Present
Renal biopsy/autopsy	Not done	TMA, C4d deposits in arterioles	Not done	Not done	Not done	TMA
Infections	None	None	None	None	None	Adenovirus, BK, and HSV viremia
Acute GVHD	n/a	n/a	Yes (skin)	No	Yes (skin, gut)	Yes (skin, gut)
Eculizumab start from HSCT-TMA diagnosis, days	3	40	122	26	24	97

| | Responders | | | | Non-responders | |
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Number of eculizumab doses given	9	13	6	4	7	2
Number of TPE sessions done before starting eculizumab	None	32	79	15	24[†]	17[†]

NBL indicates neuroblastoma; WAS, Wiskott-Aldridge syndrome; CID, combined immunodeficiency; MA, myeloablative regimen; RIC, reduced intensity regimen; HTN, systemic hypertension; PH, pulmonary hypertension; PRES, posterior reversible encephalopathy syndrome; HSV, herpes simplex 1 virus; MLPA, multiplex ligation-dependent probe amplification; Del (CFHR3-CFHR1), heterozygous deletion of CFH gene 3 and 1; n/a, not applicable.

[*] Complement profile includes C1inhib, complement component 1 inhibitor; C1Q, complement component 1q complex; C2–C9, complement components 2–9; C4BP, C4d binding protein; complement factors (CF) F, B, H, and I.

[†] Died while on eculizumab therapy.

Table 2

Response to Eculizumab Therapy in Patients with HSCT-TMA

	Responders				Non-responders	
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Response to eculizumab						
Therapeutic eculizumab level (trough) after first dose	No	No	Yes	Yes	No	No
Therapeutic eculizumab level (trough) after subsequent doses	Yes	Yes	Yes	Yes	No[*]	No[*]
Normalization of LDH, d	16	18	27	15	NR	NR
Normalization of haptoglobin, d	25	43	15	15	8	NR
Disappearance of schistocytes, d	30	45	14	7	NR	NR
Normalization of hemoglobin without transfusions, d	20	43	23	7	NR	NR
Recovery of platelets to 20,000 K/μL, d	18	43	14	7	NR	NR
Recovery of platelets to 50,000 K/μL, d	30	45	14	7	NR	NR
Doubling of cystatin C–eGFR, d	31	95	141	16	NR	NR
Urine protein-to-creatinine ratio <2 mg/mg, d	19	108	63	29	NR	NR
Number of anti-HTN medication at the start of eculizumab therapy	7	6	6	9	6	7
Number of anti-HTN medication at the end of eculizumab therapy	0	1	2	2	4	2
Hematologic HSCT-TMA response, d	30	45	27	15	NR	NR
Complete resolution of HSCT-TMA, d	31	108	141	29	NR	NR
Outcomes						
Current status	Alive	Alive	Alive	Alive	Dead	Dead
Time from staring eculizumab therapy to the last encounter, weeks	38	55	40	72	5	<2
Most current cystatin C–eGFR, mL/min	138	48	41	150	n/a	n/a
Most current random urine protein-to-creatinine ratio (normal <.2 mg/mg, nephrotic range >2 mg/mg)	.2	.9	.48	.14	n/a	n/a

LDH, lactate dehydrogenase; HTN, hypertension; NR, no response.

[*] Did not achieve therapeutic eculizumab level even with the dose escalation.