SP192

Eculizumab Reduces Biomarker Levels Related to Thrombotic Microangiopathy in Patients With Atypical Hemolytic Uremic Syndrome: Correlation to Renal Function Improvement

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INTRODUCTION

- Atypical hemolytic uremic syndrome (aHUS) is a genetic, life-threatening disease of chronic, uncontrolled complement activation leading to chronic and systemic complement-mediated thrombotic microangiopathy (TMA)[1,2]
- Eculizumab (anti-C5 mAb) potently blocks terminal complement activation, inhibits chronic TMA, normalizes platelets, and improves renal function and quality of life in patients with aHUS[3]
- Sustained inhibition of complement-mediated TMA with eculizumab in patients with aHUS led to clinically meaningful improvements in key hematologic and renal parameters in a post-approval clinical trial in adult patients with aHUS (ClinicalTrials.gov Identifier: NCT01194973)[4]:
  - 73% of patients (30/41) achieved complete TMA response (normalization of platelets and lactate dehydrogenase [LDH], and <25% increase in serum creatinine)
  - 98% of patients (40/41) achieved platelet count normalization, with a mean increase in platelet count from baseline of 135 x 10^9/L (P<0.0001)
  - 83% of patients (20/24) on dialysis at baseline discontinued dialysis. Of these, 5 patients discontinued before the first eculizumab dose. Of the remaining 19 patients, 15 (79%) discontinued dialysis before Week 26 (median [range] dialysis duration, 29 [1–180] days)
  - Mean estimated glomerular filtration rate (eGFR) increased (n=41) from baseline of 29.3 mL/min/1.73 m^2 (P<0.0001)
- We previously reported biomarker data from this study, demonstrating elevated biomarkers at baseline in patients with aHUS compared with healthy volunteers (HV).[5] In this analysis, we evaluated the ongoing impact of complement dysregulation in patients with aHUS and biologic pathways potentially impacted by sustained terminal complement blockade by:
  - Measuring levels of markers of complement activation, inflammation, thrombosis, and endothelial cell activation and damage prior to and during treatment with eculizumab
  - Evaluating potential correlations between biomarker levels and renal function in these patients

METHODS

Clinical Trial Design

- Open-label, single-arm, multicenter, multinational trial of adult patients with aHUS aged ≥18 years treated with eculizumab
- All patients had confirmed aHUS at screening based on the following*:
  - Platelet count <150 x 10^9/L
  - Hemoglobin ≤ lower limit of the normal range (LLN)
  - Lactate dehydrogenase (LDH) ≥1.5 x upper limit of the normal range (ULN)
  - Serum creatinine ≥ULN
  - ADAMTS13 >5%
  - No positive Shiga-toxin test
- There was no requirement for identified complement mutation or antibody, and no specification for plasma exchange/plasma infusion (PE/PI) prior to enrollment
  - The mutational status of patients with no previously identified complement gene mutations/polymorphisms was confirmed by whole gene sequence analyses of CFH, CFI, CD46 (MCP), CFB, C3 genes and multiplex ligation-dependent probe amplification analyses to detect deletions or duplications of the CFHR3-CFHR1 region (University of Iowa and Assistance Publique–Hôpitaux de Paris, Hôpital Européen Georges Pompidou)
- Dosing of eculizumab was 900 mg intravenously once per week for 4 weeks, 1200 mg at Week 5, then 1200 mg every 2 weeks thereafter for 26 weeks
- Patients could continue to receive eculizumab in an optional extension phase

*Hemoglobin, LDH, and creatinine assays were performed at local laboratories.

Clinical Samples and Biomarker Analyses

- This exploratory analysis evaluated biomarker levels using standard methods in plasma, serum, and urine from patients with aHUS and HV
- Patient samples were taken at baseline, prior to eculizumab treatment, and longitudinally at Visits 3 (weeks 1-3), Visit 6 (weeks 4-6), Visit 12 (weeks 12-17), Visit 17 (weeks 26-33), Visit 18 (weeks 38-42), and Visit 19 (weeks 49-54) during eculizumab treatment. For simplicity, Visit 17 is referred to as Week 26 and Visit 19 as Week 52. Not every patient had samples available for evaluation at every time point
- Forty-one adult patients with aHUS were treated; 38 (93%) completed the initial 26-week clinical study period, and 21 (51%) continued treatment for 1 year during the optional extension period
- HV serum samples (n=20) were purchased (BioreclamationIVT, Westbury, NY). Plasma and urine samples (n=10) were freshly obtained from HV selected randomly from a donor pool, according to institutional guidelines. Donors were between the ages of 18 and 68 years. Pregnant donors or those with recent illness or surgery were excluded
- All biomarker assays were conducted in the Research Laboratories of Alexion Pharmaceuticals, Inc., Cheshire, CT

RESULTS

Baseline Disease Characteristics

- Patient demographics and clinical disease characteristics at baseline are shown in **Table 1**

Table 1. Patient Demographics and Clinical Characteristics at Baseline

Characteristic	N=41
Age, mean, years	40.3
Female, n (%)	28 (68.3)
Family history of aHUS, n (%)	6 (14.6)
Identified complement regulatory gene mutation/polymorphism, n (%)	20 (48.8)
Presented with first clinical manifestation, n (%)	30 (73.2)
Initiated eculizumab without using PE/PI, n (%)	6 (14.6)
On dialysis at baseline, n (%)	24 (58.5)
Renal transplant, n (%)	9 (22.0)
Platelet count <150 x 10^9/L, n (%)	27 (65.9)
LDH >ULN, n (%)	32 (78.0)
Haptoglobin concentration, mean (SD), g/L	0.6 (0.4)
Serum creatinine level, mean (SD), µmol/L[a]	411 (264.6)

[a]n=40.
aHUS, atypical hemolytic uremic syndrome; LDH, lactate dehydrogenase; PE/PI, plasma exchange/plasma infusion; SD, standard deviation; ULN, upper limit of normal.

Elevated Biomarker Levels at Baseline

- Levels of terminal complement activation, as measured by urine (U)-sC5b-9, were elevated in the majority (23/27; 85% of patients)
- All patients with aHUS showed elevated levels of Ba (alternative pathway activation) and soluble tumor necrosis factor receptor-1 (sTNFR1; inflammation) at baseline
- More than 94% of patients with aHUS demonstrated a combined biomarker signature at baseline in which markers of complement alternative pathway (CAP) activation, inflammation, thrombosis, and endothelial cell activation and damage were significantly elevated compared with levels measured in adult HV **(Table 2)**

Table 2. At BL, Prior to Eculizumab, Markers of CAP Activity, Vascular Inflammation, Endothelial Activation and Damage, and Coagulation Were Significantly Elevated in aHUS Patients Compared with HV

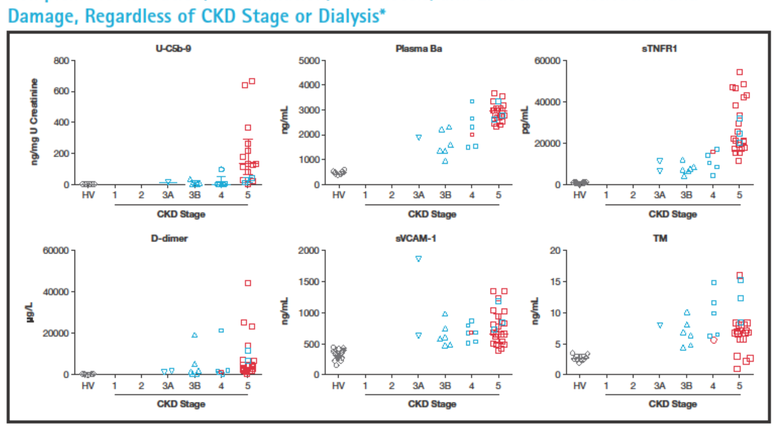
Disease Process	Biomarker	Function/Association With Complement	HV Range	Median Levels at BL (*P* Value vs. HV*)	n/N (%) Elevated at BL	Fold Increase Over HV at BL
Terminal complement activation	U-sC5b-9	• Marker of C5 activation,[6,7] blocked by eculizumab • Mediates endothelial cell activation,[8] glomerular injury,[9] ischemic injury leading to organ damage[10] • Stimulates vWF multimer secretion,[11] endothelial cell prothrombinase activity[12] and TF expression[13]	0.0–0.6 ng/mg U-creatinine	30.50 (<0.0001)	23/27 (85.2)	305
CAP activation	Ba	• Alternative pathway biomarker upstream of C5[14] • Alternative pathway stimulated by damaged endothelial cells[15] and activated platelets[16]	388.0–588.0 ng/mL	2676.4 (<0.0001)	35/35 (100)	5.53
Inflammation	sTNFR1	• Surrogate marker for TNF-α[17] • TNF-α is pro-inflammatory; associated with vascular[18] and chronic renal inflammation and progression of renal failure[17,19,20] • TNF-α upregulated by complement activation[21]	407.3–1391.3 pg/mL	17616.85 (<0.0001)	38/38 (100)	18.71
Thrombosis	D-dimer	• Fibrin degradation product indicating fibrinolysis[22]	157.0–395.9 µg/L	2735 (<0.0001)	34/36 (94.4)	9.84
Endothelial activation	sVCAM-1	• Adhesion molecule released by activated endothelial cells[23] • Upregulated by TNF-α and terminal complement[23,24]	159.2–444.7 ng/mL	659.75 (<0.0001)	36/38 (94.7)	1.99
Endothelial damage	TM	• Protective against thrombotic risk when membrane bound[25] • Released by damaged endothelial cells[26] • Upregulated by TNF-α[27]	2.0–3.6 ng/mL	10 (<0.0001)	33/34 (97.1)	3.64

aHUS, atypical hemolytic uremic syndrome; BL, baseline; CAP, complement alternative pathway; HV, healthy volunteers; sTNFR1, soluble tumor necrosis factor receptor-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TF, tissue factor; TM, thrombomodulin; TNF-α, tumor necrosis factor-α; vWF, von Willebrand factor.
**P* values were calculated using Wilcoxon rank sum test, testing for a difference between groups.

Impact of CKD and Dialysis on Baseline Biomarker Levels

- Twenty-four patients (58.5%) were on dialysis at baseline, and no patients had a baseline chronic kidney disease (CKD) stage of less than 3 **(Figure 1)**
- Regardless of CKD stage, all patients (35/35; **Table 2**) demonstrated elevated plasma Ba levels at baseline relative to HV levels (P<0.005 for all), while only 85% of patients demonstrated elevated U-C5b-9 levels (P<0.02 for all)
- Compared with HV levels, patients with aHUS also demonstrated elevated sTNFR1, D-dimer, soluble vascular cell adhesion molecule-1 (sVCAM-1), and thrombomodulin (TM) (P<0.02 for all groups vs. HV) at baseline, regardless of CKD stage or dialysis use **(Figure 1)**
- When controlling for CKD stage among aHUS patients who were on or not on dialysis, there was no difference in levels of Ba, sTNFR1, D-dimer, sVCAM-1, and TM at baseline (P>0.26 for all)
- Worse baseline renal function was correlated (correlation coefficient [CC]; P value) with higher absolute levels of Ba (–0.7448; P<0.0001), sTNFR1 (–0.6030; P<0.0001), and TM (–0.3774; P=0.0278) at baseline
- Baseline eGFR was not correlated (CC; P value) with baseline levels of D-dimer (–0.1609; P=0.3415) or sVCAM-1 (0.1686; P=0.3115)
- These results suggest that elevated Ba levels in all patients reflect ongoing CAP dysregulation, regardless of CKD stage, and absolute levels of measures of CAP, inflammation, and endothelial damage are related to the severity of renal disease

Figure 1. Patients With aHUS Show Elevated Levels of Markers of Proximal and Terminal Complement Activation, Inflammation, Thrombosis, and Endothelial Activation and Damage, Regardless of CKD Stage or Dialysis*



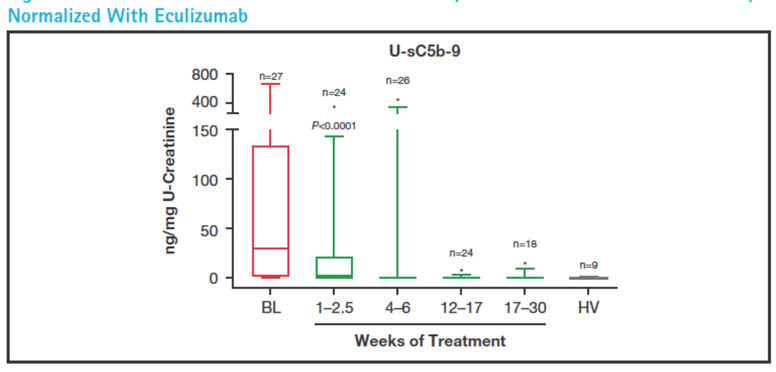
aHUS, atypical hemolytic uremic syndrome; CKD, chronic kidney disease; HV, healthy volunteers; sTNFR1, soluble tumor necrosis factor receptor-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TM, thrombomodulin.
Individual patient data are shown. *No patient had CKD stage 0-2 at baseline; red symbols indicate patients on dialysis.

Impact of Sustained Eculizumab Treatment on Biomarkers of Disease Activity in Patients with aHUS

Complement Activation

- Levels of U-C5b-9 were significantly elevated in 85% of patients at baseline (P<0.0001; **Table 2**)
- Patients demonstrated immediate and sustained reductions in U-C5b-9 following the first dose of eculizumab, with up to 100% reduction by Week 2.5 (P<0.0001 versus baseline by 2.5 weeks and at all later time points) to levels consistent with those of HV (P=0.2552) **(Figure 2)**

Figure 2. Elevated Levels of Markers of Terminal Complement Activation Are Immediately Normalized With Eculizumab

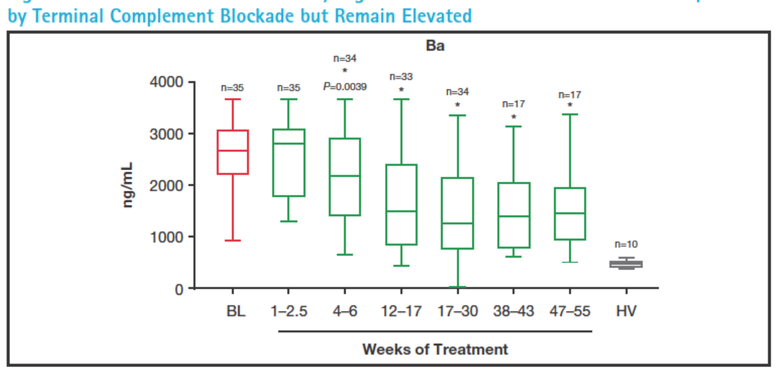


Longitudinal median biomarker levels are shown. Changes in biomarker levels with ongoing eculizumab treatment are displayed using box-whisker graphs showing median, 25th, and 75th percentiles, and range. Change from BL at each time point was statistically analyzed using a restricted maximum likelihood-based, repeated measures approach (mixed effect analysis of variance model) with the *P* value of first significant reduction shown. Significant reduction from BL is indicated at each timepoint (*).

Persistent CAP Activation

- There was evidence of significant CAP activation in 100% of patients with aHUS at baseline (P<0.0001; **Table 2**)
- Ongoing terminal complement blockade with eculizumab for 1 year reduced Ba levels (mean 30% reduction; **Figure 3**)
- Significant reduction in Ba occurred after Week 6 (P=0.0039) and was sustained at all later time points (*P<0.0001 for all) with ongoing terminal complement blockade
- These data indicate that proximal complement activation in aHUS is amplified by terminal complement activation
- Though reduced from baseline, Ba levels remain elevated compared with HV levels after 1 year of eculizumab treatment, reflecting chronic CAP activation upstream of C5
- Continued elevated Ba levels reflect ongoing dysregulated CAP activation in aHUS

Figure 3. Patients With aHUS Show Dysregulated CAP Activation: Ba Levels are Impacted by Terminal Complement Blockade but Remain Elevated

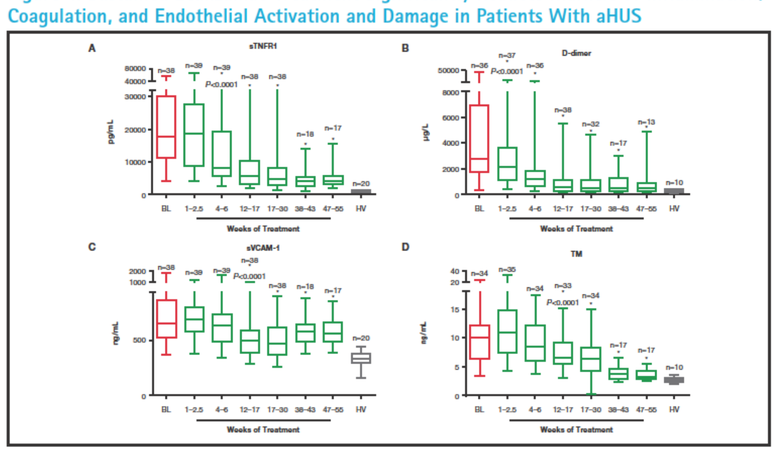


aHUS, atypical hemolytic uremic syndrome; CAP, complement alternative pathway.
Analysis was performed and presented as detailed in the legend to **Figure 2**.

Inflammation, Thrombosis, and Endothelial Activation and Damage

- Levels of the inflammatory marker sTNFR1 were markedly elevated in 100% of patients with aHUS at baseline **(Table 2)**
- Ongoing treatment with eculizumab significantly reduced sTNFR1 levels by up to 94% **(Figure 4A)**
  - Reduction occurred quickly, by Week 6 after initiation of treatment, and was sustained and significant across all later time points **(Figure 4A; *P<0.0001 for all)**
- Levels of the coagulation marker D-dimer were significantly elevated at baseline in >94% of patients. D-dimer levels were quickly reduced by up to 99% by Week 6 and reduction was sustained **(Figure 4B; *P<0.0001 for all time points)** with ongoing eculizumab treatment, remaining modestly above HV levels at Week 52
- sVCAM-1 was elevated in 95% of aHUS patients at baseline **(Table 2)**, suggesting significant endothelial cell activation
  - Significant reduction in sVCAM-1 levels was first observed at Weeks 12-17 **(Figure 4C)**. This reduction was sustained (*P≤0.0039 for all later time points), but remained elevated **(Figure 4C)**
- TM levels were elevated in 97% of patients with aHUS at baseline **(Table 2)**, indicating significant endothelial cell damage
  - Significant reduction in TM levels was first observed at Weeks 12-17 **(Figure 4D; P<0.0001)** and continued to reduce to near normal levels during eculizumab treatment
  - After Week 17, reduction in TM levels was significant at all later time points **(Figure 4D; *P<0.0001 for all)**

Figure 4. Sustained Eculizumab Treatment Significantly Reduces Markers of Inflammation, Coagulation, and Endothelial Activation and Damage in Patients With aHUS

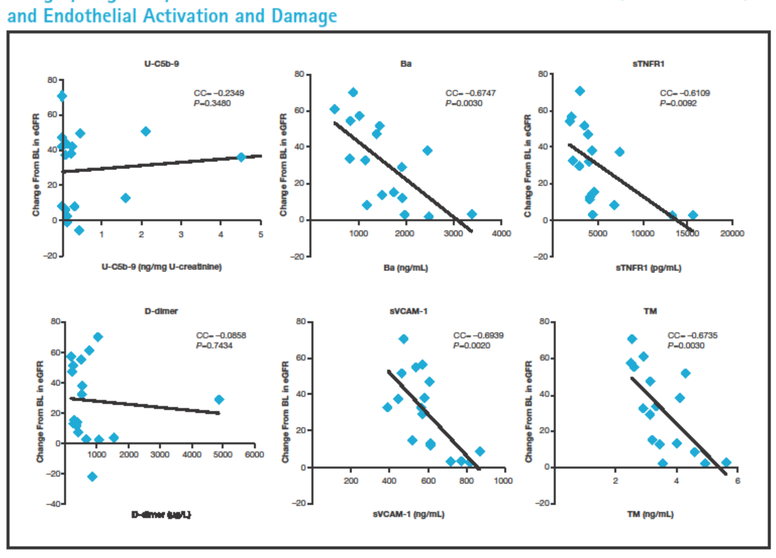


aHUS, atypical hemolytic uremic syndrome; BL, baseline; HV, healthy volunteers; sTNFR1, soluble tumor necrosis factor receptor-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TM, thrombomodulin.
Analysis was performed and presented as detailed in the legend to **Figure 2**.

Correlations with Clinical Outcome

- Ba, sTNFR1, sVCAM-1, and TM levels are highly negatively correlated with clinical improvement in renal function at Week 52 **(Figure 5)**
- Terminal complement activation (U-C5b-9) and D-dimer levels quickly reached their nadir by 2.5 and 6 weeks, respectively **(Figures 2 and 4)**; therefore, a correlation between levels and improved renal function at later time points could not be established

Figure 5. Clinical Improvement in Renal Function During 1 Year* of Eculizumab Treatment Is Highly Negatively Correlated With Levels of Markers of CAP Activation, Inflammation, and Endothelial Activation and Damage

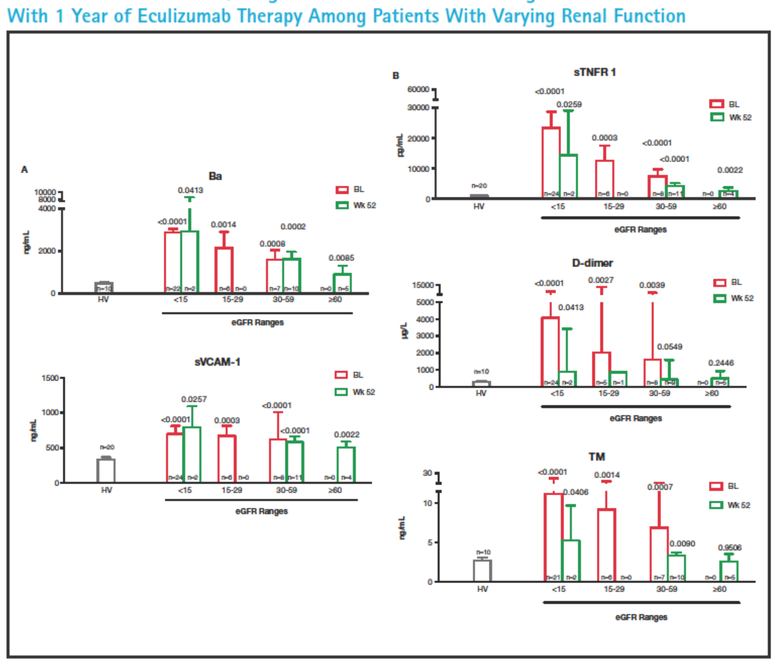


BL, baseline; CC, correlation coefficient; eGFR, estimated glomerular filtration rate; sTNFR1, soluble tumor necrosis factor receptor-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TM, thrombomodulin.
Individual patient data are represented. *U-C5b-9 correlations are at 6 months.

Eculizumab Reduces Measures of Inflammation, Coagulation, and Endothelial Damage Regardless of Renal Function

- Ba and sVCAM-1 **(Figure 6A)** levels remained similarly elevated among patients regardless of eGFR, demonstrating profile of dysregulated CAP and the propensity for endothelial cell activation in aHUS
  - Although patients with aHUS showed improvement in renal function with ongoing eculizumab treatment, they continued to manifest CAP and endothelial cell activation
- Patients showed continued reduction in measures of inflammation, coagulation, and endothelial damage at Week 52 regardless of eGFR status **(Figure 6B)**
  - Including those with very poor renal function (<15 eGFR) at baseline
  - Despite ongoing proximal complement activation upstream of C5
- Persistent endothelial activation does not translate into endothelial damage with continued terminal complement blockade

Figure 6. Despite (A) Ongoing Proximal Complement and Endothelial Activation, (B) Measures of Inflammation, Coagulation and Endothelial Damage Reach Near Normal Levels With 1 Year of Eculizumab Therapy Among Patients With Varying Renal Function



eGFR, estimated glomerular filtration rate; sTNFR1, soluble tumor necrosis factor receptor-1; sVCAM-1, soluble vascular cell adhesion molecule-1; TM, thrombomodulin.

CONCLUSIONS

- Prior to eculizumab treatment, >94% of patients with aHUS showed a significant elevation in biomarker levels of CAP activation, inflammation, thrombosis, and endothelial activation and damage
  - Levels of these markers were elevated regardless of CKD stage or dialysis use
  - Absolute levels of measures of CAP activation, inflammation, and endothelial damage increased with the severity of renal disease
- Ongoing terminal complement blockade with eculizumab:
  - Significantly reduced and normalized measures of terminal complement activation
  - Markedly reduced all biomarkers of inflammation, thrombosis, and endothelial damage to near normal levels
- Terminal complement blockade during 1 year of treatment with eculizumab continues to be associated with improved renal function, and improvement was associated with reductions in overall biomarker signature
  - Terminal complement blockade significantly inhibits systemic CAP activation, inflammation, and endothelial activation and damage that drives renal injury in aHUS
- Ongoing terminal complement blockade with eculizumab reduced levels of proximal complement activation upstream of C5, but Ba and sVCAM-1 levels remained significantly elevated after 1 year of treatment, regardless of level of renal function
  - Amplification of proximal complement activation in aHUS is mediated by terminal complement activation and blocked by eculizumab
  - Persistent proximal complement and endothelial activation underscore the underlying genetic dysregulation of the alternative pathway[28] in patients with aHUS and the propensity for endothelial cell activation in these patients
- Although patients with aHUS continued to manifest proximal complement and endothelial activation, terminal complement–mediated endothelial damage was successfully reduced
  - Sustained terminal complement blockade with eculizumab protects patients from the potentially devastating consequences of demonstrated ongoing proximal complement and endothelial activation, leading to improvement in renal function
- These data demonstrate a strong relationship between terminal complement activation and endothelial damage leading to tissue injury
- This exploratory analysis suggests correlations between biomarkers and improvement in eGFR in patients with aHUS; however, these research assays are not intended or validated for use in diagnostic or clinical treatment decisions

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