Analysis of Inflammatory and Anemia-Related Biomarkers in a Randomized, Double-Blind, Placebo-Controlled Study of Siltuximab (Anti-IL6 Monoclonal Antibody) in Patients With Multicentric Castleman Disease

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Abstract

Purpose: Siltuximab (IL6 antibody) is approved for the treatment of multicentric Castleman disease (MCD). Effects of IL6 inhibition on the inflammatory milieu accompanying MCD have not been characterized.

Experimental Design: Trends in inflammatory- and anemia-associated markers, measured over the course of a placebo-controlled study of siltuximab (11 mg/kg q3w) in patients with MCD (n = 79), were characterized.

Results: Baseline IL6 and C-reactive protein (CRP) levels were significantly correlated (r = 0.708; P < 0.0001). CRP levels decreased (median, 92%) by cycle 1 day 8 (C1D8), remaining suppressed during siltuximab treatment while remaining stable in the placebo group. There were no associations between baseline CRP or IL6 and MCD symptom burden, histologic subtype, ethnicity, maximum CRP decrease, and response parameters. A hemoglobin response (change ≥ 15 g/L at week 13) was observed with siltuximab (61%; P = 0.0002). Median hepcidin decrease from baseline at C1D8 with siltuximab was 47% versus median 11% increase with placebo. Maximum post-baseline changes in hepcidin levels among siltuximab recipients were correlated with maximum changes for hemoglobin (r = –0.395; P = 0.00607), total iron-binding capacity (TIBC; r = –0.354; P = 0.01694), and ferritin (r = 0.599; P = 0.0001). Greater median changes from baseline in ferritin, hemoglobin, and TIBC were observed in anemic siltuximab-treated patients.

Conclusions: IL6 neutralization with siltuximab resulted in sustained CRP suppression and improvement of anemia, in part, by hepcidin pathway inhibition. Clin Cancer Res; 21(19); 4294–304. ©2015 AACR.

Introduction

Castleman disease was first described by Benjamin Castleman in 1956 as a solitary mediastinal tumor resembling thymoma and characterized by lymph node hyperplasia with germinal center formation and marked capillary proliferation (1). Castleman disease can be classified as hyaline vascular, plasmacytic, or mixed on the basis of lymphoid nodal architectural changes (2). Hyaline vascular Castleman disease features distinctive follicles with mantle zones of lymphocytes and vascularized germinal centers, whereas plasmacytic Castleman disease has less well-defined pathologic features (3). Multicentric Castleman disease (MCD) is associated with dysregulated overproduction of IL6 by B cells in germinal centers accompanied by inflammatory symptoms [e.g., fever, general fatigue, elevated C-reactive protein (CRP), hypalbuminemia, and hypergammaglobulinemia; ref. 4]. Typically, corticosteroids and immunosuppressants have been used to treat patients, with limited efficacy (5). Rituximab, an anti-CD20 monoclonal antibody, has shown efficacy in human immunodeficiency virus (HIV)–positive MCD patients (6). Tocilizumab, a humanized monoclonal antibody that targets the IL6 receptor (IL6R), is approved only in Japan for the improvement of symptoms and laboratory findings associated with Castleman disease (7, 8).

IL6 is known for its pleiotropic and proinflammatory functions, with a central role in the pathophysiology of MCD; therefore, IL6 has emerged as an important therapeutic target for this disease (9, 10). IL6 induces proliferation and differentiation of T cells and terminal differentiation of B cells, including autoantibody-producing cells (11). Overproduction of IL6 results in systemic and local inflammation and causes fever, general fatigue, and anorexia, along with other clinical manifestations (11). Therefore, inhibiting the activity of IL6 is believed to reduce inflammatory responses and improve MCD symptoms.
Siltuximab (CNTO 328) is a chimeric (human murine) immunoglobulin G1κ monoclonal antibody against human IL6 (12, 13). In the randomized, double-blind, placebo-controlled MCD2001 study in patients with HIV-negative and human herpes virus 8 (HHV-8)–negative MCD, siltuximab induced a durable tumor and symptomatic response rate of 34% versus 0% with placebo (P = 0.0012; ref. 14). The best tumor response rate by independent review was 38% versus 4% (P = 0.0022), and the median time to treatment failure was not reached [95% confidence interval (CI), 378 days to not evaluable] in the siltuximab arm versus 134 days (95% CI, 85 days to not evaluable) in the placebo arm (HR, 0.418; 95% CI, 0.214–0.815; P = 0.0084). Complete durable symptom responses were achieved by 25% of patients with siltuximab versus 0% with placebo (P = 0.0037; ref. 14). The overall incidences of grade ≥3 adverse events (47% vs. 54%) and serious adverse events (23% vs. 19%) were similar despite a longer median treatment duration with siltuximab compared with placebo (375 vs. 152 days; ref. 14). On the basis of these results, siltuximab (Sylvant) was recently approved by the FDA and the European regulatory authority and is indicated for the treatment of HIV-negative and HHV–8–negative MCD patients (15, 16).

Individuals with MCD often present with increases in acute-phase serum proteins such as CRP and fibrinogen, decreases in serum albumin, and an inflammatory anemia (5, 17). IL6 is the primary inducer of hepatic CRP production (18). Inhibition of IL6 activity by siltuximab treatment has been shown to cause strong suppression of systemic CRP levels, with fibrinogen decreases and albumin level increases (12, 14, 19). MCD2001 is the largest study to date in MCD and therefore provides a unique opportunity to evaluate consistently collected inflammatory- and anemia-associated markers (14). As the only placebo-controlled study in MCD, it also provides a unique opportunity to examine the effects of selective IL6 inhibition.

Several studies have shown that IL6 is the main inducer of hepatocyte-synthesized CRP (20, 21). Furthermore, a significant correlation between elevated serum CRP and IL6 levels was observed in patients with renal cell carcinoma (22); therefore, IL6 and CRP were measured in both treatment arms, and the association of baseline serum levels of IL6 with CRP levels was evaluated. Exploratory analyses of the association of these markers with clinical responses were also performed.

Hepcidin, an iron-regulating peptide hormone produced by the liver, is implicated in anemia of inflammation (23). It has been reported that increased IL6 upregulates hepcidin production, which binds and downregulates its cellular receptor, ferroportin, causing iron sequestration leading to anemia (24). Associations between hepcidin, ferritin, and soluble transferrin receptors have been observed in patients with chronic kidney disease (25).

In a phase I, dose-finding study in patients with multiple myeloma, B cell non–Hodgkin lymphoma, or Castleman disease (Study, C0328T03), evidence of decreased serum hepcidin and a general improvement in hemoglobin levels was observed following siltuximab treatment (12). An association analysis of hepcidin at baseline, and during treatment, with hemoglobin response and other iron parameters was therefore performed in MCD2001 to gain more insight into the effect of siltuximab on anemia resolution in patients with MCD. In addition, in this study of the largest published cohort of patients with MCD, further subgroup analyses were performed on the basis of histologic subtype and ethnicity (Asian and non-Asian) to investigate trends in these populations.

Materials and Methods
Patient and clinical study design
This study (ClinicalTrials.gov: NCT01024036) was conducted at 38 sites in 19 countries. The enrollment criteria and study design were previously described (14). Briefly, patients had symptomatic MCD confirmed by central pathology review (Department of Pathology, University of Washington School of Medicine, Seattle, WA), were aged ≥18 years, and were HIV- and HHV-8–negative. Symptomatic disease was defined clinically by the presence of grade ≥1 symptoms per National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) that were attributable to MCD and for which treatment was indicated. Patients were required to have measurable disease, which was not to be limited to cutaneous lesions. Patients were newly diagnosed or previously treated (excluding IL6-targeted therapy); those receiving corticosteroids must have been on a stable or decreasing dose of ≤1 mg/kg/d of prednisone or equivalent for >4 weeks before randomization.

Patients were centrally assigned in a 2:1 ratio, using randomly permuted blocks based on a computer-generated randomization schedule prepared under the supervision of the sponsor before the study; and stratified by baseline concomitant corticosteroid use, to receive intravenous siltuximab infusions (11 mg/kg) or placebo every 3 weeks until protocol-defined treatment failure. All patients were provided best supportive care (BSC), including treatment for anemia. At treatment failure [defined as a sustained increase in grade ≥2 disease-related symptoms persisting for ≥3 weeks; new disease-related grade ≥3 symptoms; sustained ≥1-point increase in Eastern Cooperative Oncology Group performance status persisting for >3 weeks; radiologic progression by modified Cheson criteria (26); or initiation of another MCD therapy], patients randomized to siltuximab discontinued study treatment and those randomized to placebo could cross-over and receive...
unblinded siltuximab until a second treatment failure. Patients who discontinued study treatment were followed for survival and subsequent therapy until the primary analysis.

The study was conducted according to the principles of the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice, with written informed consent obtained from all patients before study entry and with the approval of the study protocol by the institutional review board or independent ethics committee at each site.

Assessment of efficacy endpoints has been described previously (14). Briefly, the primary efficacy endpoint was durable tumor and symptomatic response; secondary endpoints included duration of tumor and symptomatic response, best tumor response, time to treatment failure, and hemoglobin response (≥15 g/L hemoglobin increase from baseline to week 13).

Biomarker evaluation
Hemoglobin was measured locally at the investigative sites. CRP and iron-related parameters were analyzed at a central laboratory (Covance Central Laboratory Services). CRP was analyzed using a high sensitive CRP assay with a lower limit of quantification (LLOQ) of 0.20 mg/L by an immunonephelometry method using the Behring Nephelometer II (Siemens Healthcare Diagnostics, Inc.). Serum CRP levels were assessed in all patients before study agent administration on day 1 of cycles 1 to 8, 12, and 16; at 2 and 4 hours after the end of infusion on cycle 1 days 1, 8, and 15; at 4, 8, and 12 weeks after the last dose of study agent; and at each radiologic evaluation during the follow-up period, until disease progression. Baseline serum IL6 levels were measured using the Quantikine Human IL6 ELISA (R&D Systems, Inc.) at the central laboratory, with an LLOQ of 0.156 pg/mL. Hepcidin was measured at the Intrinsic Life Sciences laboratory using a polyclonal antibody–based competitive ELISA (LLOQ of 19 ng/mL) that recognizes bioactive Hepcidin-25 (23). Serum samples for hepcidin analysis were collected before study agent administration on day 1 of cycles 1 to 8, and 15 and on cycle 2 day 1, cycle 3 day 1, and cycle 4 day 1. To avoid potential unblinding, results of CRP, IL6, and hepcidin analyses were kept blinded and not provided to the investigators or sponsor during the study.

Statistical analysis
Changes in analyte levels relative to baseline were calculated on the basis of a fixed visit across all patients. When required, data that were skewed were transformed using the log transformation. Associations between clinical responses and baseline biomarker values were assessed by fitting a logistic regression model. Associations between 2 variables were measured using Pearson correlation coefficients, and corresponding P values were determined.

Results
Patient characteristics
In total, 79 patients were randomly assigned to siltuximab (n = 53) or placebo (n = 26). Baseline demographics and disease characteristics have been previously described (14) and were well balanced with the exception of sex (57% vs. 85% male in the siltuximab and placebo groups, respectively). Median age was 48 years; approximately half of patients were Asian (51% siltuximab; 42% placebo) and about 40% were white (36% siltuximab; 46% placebo). On the basis of central pathology review, disease histology was 33% hyaline vascular, 23% plasmacytic, and 44% mixed. Anti-anemia medications, including folic acid, vitamin B12, and iron, were received by 18.9% and 19.2% of patients in the siltuximab and placebo arms, respectively. All patients in the intent-to-treat population with evaluable samples were included in the biomarker analyses.

Evaluation of baseline IL6
Serum IL6 levels were measured only at baseline because accurate quantification of IL6 in post-baseline samples was not possible, as IL6-neutralized siltuximab complexes interfere with currently available immunologic-based IL6 quantification methods (data not shown). Using a high sensitive IL6 assay, all patients in the placebo (n = 26) and siltuximab (n = 49) groups had IL6 levels above the LLOQ (0.156 pg/mL) at baseline, with mean (SD) baseline levels of 8.43 (9.14) pg/mL and a median value of 6.78 pg/mL (range, 0.38–50.64 pg/mL), indicating large interpatient variation that was also observed within each treatment arm (Fig. 1).

Association of baseline IL6 with clinical response
An analysis of the potential association between baseline IL6 levels and clinical response was performed for responders (n = 17; defined as durable tumor and symptomatic response) versus nonresponders (n = 32) in the siltuximab arm. Mean (SD) and median baseline IL6 levels in the siltuximab arm were 12.60 (13.10) pg/mL and 7.98 pg/mL for responders and 7.64 (8.89) pg/mL and 5.09 pg/mL for nonresponders, indicating a trend for higher IL6 levels in the responder subgroup (P = 0.0999 for log10-transformed values; Table 2). However, with respect to best tumor response, siltuximab responders did not differ significantly (P = 0.0780) from nonresponders in baseline IL6 values, although a trend toward higher IL6 levels being associated with best tumor response was observed (Table 2). Baseline IL6 levels of subjects with ≥grade 3 symptoms or <grade 3 symptoms were not associated with baseline overall symptom score (defined in ref. 14). At baseline, a weak correlation was observed (P = 0.0211) between baseline IL6 and general symptom score [combination of fatigue, malaise, hyperhidrosis (profuse sweating), night sweats, fever, weight loss, anorexia, tumor pain, dyspnea, pruritus] in all subjects. The correlation was not strong, but was significant (P = 0.0072), between baseline IL6 levels in subjects with <grade 3 symptoms and general symptom score at baseline.

CRP suppression with siltuximab treatment
A total of 79 patients were evaluated for baseline serum CRP, and 52 (98%) patients in the siltuximab arm and all 26 patients in the placebo arm showed levels above the LLOQ (>0.2 mg/L) at baseline. Median CRP levels at baseline were 17.60 mg/L in the siltuximab arm and 4.18 mg/L in the placebo arm (Fig. 1).
Interpatient variations in baseline CRP levels were observed in the siltuximab arm, with a mean (SD) CRP level of 43.18 (53.63) mg/L and a range of 0.10 to 181.00 mg/L (Fig. 1). In the histologic subgroups, baseline mean and median CRP levels were higher in patients with the plasmacytic subtype (Table 1). Mean and median baseline CRP levels were lower in non-Asian patients
than in Asian patients (Table 1). Further evaluations were performed to determine whether higher baseline CRP levels in the siltuximab arm were related to prior corticosteroid treatment; however, the difference between the siltuximab and placebo treatment arms with and without prior corticosteroid treatment was not statistically significant ($P = 0.3647$).

The median CRP level decreased from 17.60 at baseline to 1.04 mg/L (median percent decrease of 92%) as early as cycle 1 day 8 in the siltuximab arm but increased from 4.18 to 5.63 mg/L (median percent increase of 3%) in the placebo arm (Fig 3A). CRP suppression was sustained in the siltuximab arm throughout the entire treatment period, with the median decrease from baseline ranging from 72% to 88% until cycle 16 day 1 (the last time point tested) and the end-of-treatment visit. CRP reductions of $\geq 80\%$ were observed in 40 of 52 (77%) patients in the siltuximab arm compared with 0 of 25 patients in the placebo arm at cycle 1 day 8. This level ($\geq 80\%$) of CRP suppression from baseline was maintained at time points after cycle 1 day 1 in 15 of 34 (44%) to 30 of 50 (60%) patients in the siltuximab arm compared with 0 of 25 (0%) to 1 of 7 (14%) patients in the placebo arm.

### Association of CRP with clinical response

Serum CRP concentrations greater than 8 to 10 mg/L are considered to be elevated (27, 28); therefore, a correlation analysis of CRP levels with either durable tumor and symptomatic response or best tumor response was performed by subgrouping responders and nonresponders based on baseline CRP levels of $<8$ versus $\geq 8$ mg/L. Although the majority ($n = 15$ of 18) of
responders had baseline CRP levels ≥8 mg/L and all responders showed CRP suppression during treatment, the correlation of baseline CRP levels with durable tumor and symptomatic response \( (r = 0.3616) \) or best tumor response \( (r = 0.4188) \) was not strong; however, the correlations were statistically significant \( (P = 0.0078 \text{ and } P = 0.0018, \text{ respectively}) \).

Association analyses with clinical response to siltuximab and log-transformed CRP values (performed because of large inter-patient variability and non-normalized distribution) were consistent with this finding. There was a trend in baseline CRP levels between responders and nonresponders by durable tumor and symptomatic response \( (\text{median}, 34.50 \text{ vs. } 6.32 \text{ mg/L}; \text{log } P = 0.0522) \) and best tumor response \( (\text{median}, 38.00 \text{ vs. } 5.09 \text{ mg/L}; \text{log } P = 0.0118) \) in the siltuximab arm \( (P \text{ values refer to the log-transformed values; Table 2}) \). However, mean (SD) baseline CRP levels overlapped between responders and nonresponders, with values of 58.06 (49.32) mg/L versus 35.53 (54.82) mg/L for durable tumor and symptomatic response and 65.01 (51.60) mg/L versus 29.95 (51.14) mg/L for best tumor response \( (\text{Table 2}) \). Baseline CRP values were not predictive of clinical response based on a logistic regression model with durable tumor and symptomatic response as the dependent variable and log\(_{10}\) baseline CRP as the independent continuous variable. The sensitivity was the proportion of responders who were correctly classified as a responder \( (5 \text{ of } 18; 28\%) \) and the specificity was the proportion of nonresponders who were correctly classified as a nonresponder \( (29 \text{ of } 35; 83\%) \). In a similar model with a cutoff of 8 mg/L for baseline CRP, the area under the ROC curve was 0.69. The sensitivity and specificity were 83% and 54%, respectively.

Correlations of clinical response with decreases in CRP levels from ≥8 mg/L at baseline to <8 mg/L at any post-baseline time point were also investigated, but no statistical significance was found to support an association with either durable tumor and symptomatic response \( (r = 0.0914; P = 0.6249) \) or best tumor response \( (r = 0.0482; P = 0.5511) \). Also, maximum post-baseline changes in CRP levels (irrespective of baseline levels) were not found to have meaningful associations with independently reviewed durable tumor and symptomatic responses or best tumor responses in the siltuximab arm.

**Baseline CRP and IL6 levels and symptom burden**

Associations of CRP levels with MCD-related symptom scores were evaluated, with patients assigned a score on the basis of their overall symptoms. Assessed by investigators, the scores were the sum of NCI-CTCAE version 4.0 severity grades of 34 MCD-related signs and symptoms, including a general symptom score (fatigue, malaise, hyperhidrosis, night sweats, fever, weight loss, anorexia, tumor pain, dyspnea, and pruritus) and skin disorders, neuropathy, autoimmune phenomena, and fluid retention \( (14) \). Association of log\(_{10}\)-transformed baseline CRP levels \( (\text{with or without prior corticosteroid treatment}) \) with the baseline overall symptom score showed no significant correlation \( (r = 0.0681; P = 0.5511) \). In addition, no correlation was found between CRP change from baseline to cycle 1 day 8 and maximum change in symptom score in each treatment arm \( (P = 0.6249) \). Also, maximum post-baseline corticosteroid treatment; \( (r = 0.0681; P = 0.5511) \).

An exploratory analysis was also performed to understand whether patients with a high overall symptom score also had higher log\(_{10}\)-transformed baseline IL6 levels \( (\text{with or without prior corticosteroid treatment}) \); however, no significant

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**Table 2. Summary of baseline CRP and IL6 by tumor response**

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<th>Parameter</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Median</th>
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<th>Mean (SD)</th>
<th>Median</th>
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<tr>
<td>Responders</td>
<td>18</td>
<td>58.06 (49.32)</td>
<td>34.50</td>
<td></td>
<td>20</td>
<td>65.01 (51.60)</td>
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<tr>
<td>Nonresponders</td>
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<td>6.32</td>
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<td>33</td>
<td>29.95 (51.14)</td>
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<td>CRP, log(_{10})-transformed</td>
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<tr>
<td>Responders</td>
<td>18</td>
<td>1.43 (0.76)</td>
<td>1.54</td>
<td>0.0522</td>
<td>20</td>
<td>1.50 (0.74)</td>
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<td>Nonresponders</td>
<td>35</td>
<td>0.97 (0.79)</td>
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<tr>
<td>Responders</td>
<td>17</td>
<td>12.60 (13.10)</td>
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<td>Nonresponders</td>
<td>32</td>
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<td>Responders</td>
<td>17</td>
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**Note:** Data shown are for patients in the ITT population who were treated with siltuximab during the blinded treatment period. Both durable tumor and symptomatic responses and best tumor responses were independently reviewed. Abbreviation: ITT, intent-to-treat.

*Parameters measured at baseline.
correlation was observed ($r = 0.1739; P = 0.1357$), indicating baseline IL6 levels were not clearly associated with symptom scores. An additional analysis indicated that there was no association between baseline CRP-levels and prior corticosteroid dose (data not shown). No significant correlations were observed between baseline IL6 levels and baseline overall symptom score, or with general symptom score in the newly diagnosed subgroup (i.e., no prior therapy) from both treatment groups combined (data not shown).

Evaluation of hepcidin

Serum hepcidin levels were measured before and during treatment to potentially identify baseline hepcidin levels that were reflective of hemoglobin improvement and to investigate the association of post-baseline changes in hepcidin with changes in hemoglobin and iron parameters. At baseline, 44 of 51 (86%) patients in the siltuximab arm and 25 of 26 (96%) patients in the placebo arm had hepcidin levels ≥ 19 ng/mL (Fig. 1). Mean and median baseline hepcidin levels were similar among the histologic subtypes and were lower in non-Asian patients than in Asian patients (Table 1).

Mean (SD) and median hemoglobin values were 128.4 (20.42) g/L and 134.0 g/L (range, 69.0–166.0 g/L), respectively, in patients with hyaline vascular MCD and 116.6 (27.32) g/L and 113.0 g/L (range, 65.0–181.0 g/L), respectively, in the combined plasmacytic and mixed MCD patient group. Hepcidin values were higher in anemic patients. At baseline, in the 41 patients with anemia, mean (SD) hepcidin values were 182.9 (168.30) ng/mL compared with 101.6 (77.94) ng/mL in the 36 non-anemic patients ($P = 0.0075$). Median hepcidin values at baseline were 141.7 ng/mL (range, 9.5–762.4 ng/mL) for patients with anemia compared with 86.6 ng/mL (range, 9.5–368.0 ng/mL) in non-anemic patients ($P = 0.0305$).

Figure 3.
Median percent change from baseline in CRP (A) and hepcidin (B) by visit. The maximum median percent decrease in CRP occurred at cycle 1 day 8 (84.6%) in the siltuximab arm compared with an increase in the placebo arm (26.7%) at this time point. The maximum median percent decrease in hepcidin occurred at cycle 1 day 8 (10.9%) in the siltuximab arm compared with an increase in the placebo arm (26.7%) at this time point. Data shown are for the ITT population. C, cycle; D, day; ITT, intent-to-treat.
Hepcidin levels showed a median percent decrease from baseline of 47% at cycle 1 day 8 in the siltuximab arm, compared with an 11% increase from baseline in the placebo arm. The median percent decrease of hepcidin at other tested time points (cycle 2 day 1 to cycle 4 day 1) ranged from 15% to 22% in the siltuximab arm compared with 2% to 11% in the placebo arm (Fig. 3B). The large median percent decrease in hepcidin levels in the siltuximab arm as early as cycle 1 day 8 was observed in all patients irrespective of histology, and reductions from baseline were present for the study duration (data not shown).

**Association of hepcidin with changes in hemoglobin**

In the hemoglobin response–evaluable population (anemia at baseline), a hemoglobin response (defined as ≥15 g/L) hemoglobin improvement from baseline at week 13 was reported for 61% of patients on siltuximab and 0% on placebo (P = 0.0002). In the total study population, a significant correlation of higher increases in hemoglobin with lower baseline hepcidin levels was observed (r = 0.562; P < 0.0001).

Baseline hepcidin values ranged from below the LLOQ to 249.3 ng/mL in siltuximab-treated patients who had >15 g/L hemoglobin improvement from baseline at week 13 (hemoglobin responders) and from below the LLOQ to 762.4 ng/mL in those who had ≥20 g/L hemoglobin improvement from baseline. These results indicate that baseline hepcidin values were not predictive of hemoglobin improvements ≥15 or ≥20 g/L.

An exploratory correlation analysis that was performed to understand the relationship between post-baseline changes in hepcidin and hemoglobin levels showed no appreciable association in the siltuximab arm (r = –0.298; P = 0.336). Among the 31 patients evaluable for hemoglobin response in the siltuximab arm, 25 (81%) patients had a post-baseline hepcidin reduction; of these, 16 (54%) patients also showed hemoglobin increases ≥15 g/L and 11 (44%) showed hemoglobin increases ≥20 g/L. The median hepcidin change was –56.01% (range, –94.0% to –3.9%) in the siltuximab group and –26.56% (range, –60.9% to –1.0%) in the placebo group. However, hepcidin decreases alone were not predictive of hemoglobin improvement, as 9 patients in the siltuximab arm and none of the 8 patients in the placebo arm who had post-baseline hepcidin reductions showed hemoglobin improvements ≥15 g/L, suggesting that hepcidin decreases in these patients are possibly caused by IL6-independent pathways.

**Association of hepcidin with iron parameters**

Ferritin levels consistently decreased from baseline at each time point for patients in the siltuximab group (median percent change of –28%, –30%, and –41% at cycles 2, 3, and 4, respectively), with minimal change in the placebo group (median percent change of –1%, –3%, and –1%, respectively). Total iron-binding capacity (TIBC) increased from baseline in patients receiving siltuximab (median percent change of 11%, 14%, and 13% at cycles 2, 3, and 4, respectively) but remained nearly unchanged in patients receiving placebo (4%, 0%, and 0%, respectively). An exploratory correlation analysis of hepcidin changes from baseline and iron parameters was performed, using the time point at which the maximum median change from baseline was observed for each parameter (i.e., cycle 1 day 8 for hepcidin and cycle 2 day 1 for hemoglobin and iron parameters). No significant correlations were observed between maximum median hepcidin changes and either iron or transferrin saturation in both treatment arms. However, consistent with the findings described in the previous paragraph, a weak but significant negative correlation was observed between maximum median hepcidin changes and hemoglobin in the siltuximab arm (r = –0.395; P = 0.00607), but not the placebo arm. Similarly, a weak but significant negative correlation was observed between the maximum median changes in hepcidin and TIBC in the siltuximab arm (r = –0.354; P = 0.01694), but not the placebo arm. As expected, on the basis of the known relationship between hepcidin and ferritin (29), the change in ferritin levels followed the same trend as the change in hepcidin levels in the siltuximab arm (r = 0.599; P = 0.00001).

An additional analysis indicated a positive, statistically significant correlation between the maximum median changes in hepcidin and ferritin in hemoglobin nonresponders

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<th>Parameter</th>
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<th>Placebo Median change (SD)</th>
<th>Placebo Median change p*</th>
<th>Siltuximab Mean (SD)</th>
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<td>No baseline anemia</td>
<td>14 92.6 (64.3)</td>
<td>89.9 (61.4)</td>
<td>–2.73 (33.8)</td>
<td>2.25</td>
<td>18 126.2 (83.9)</td>
<td>78.0 (50.7)</td>
</tr>
<tr>
<td>Baseline anemia</td>
<td>11 134.4 (63.3)</td>
<td>154.6 (79.5)</td>
<td>20.2 (49.5)</td>
<td>16.6</td>
<td>30 200.6 (191.0)</td>
<td>98.8 (111.1)</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No baseline anemia</td>
<td>15 146.7 (16.65)</td>
<td>144.47 (17.23)</td>
<td>–2.27 (8.36)</td>
<td>–4.00</td>
<td>22 113.72 (10.50)</td>
<td>119.68 (15.29)</td>
</tr>
<tr>
<td>Baseline anemia</td>
<td>10 107.06 (16.16)</td>
<td>106.50 (16.30)</td>
<td>–0.564 (3.33)</td>
<td>–1.00</td>
<td>30 100.33 (19.21)</td>
<td>110.43 (16.23)</td>
</tr>
<tr>
<td>Iron, mol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No baseline anemia</td>
<td>11 6.29 (3.10)</td>
<td>5.63 (2.75)</td>
<td>–0.645 (1.85)</td>
<td>–0.890</td>
<td>29 6.48 (3.36)</td>
<td>9.64 (4.89)</td>
</tr>
<tr>
<td>Baseline anemia</td>
<td>10 Transferin saturation (fraction)</td>
<td>6.53 (2.65)</td>
<td>–0.645 (1.85)</td>
<td>–0.890</td>
<td>29 6.48 (3.36)</td>
<td>9.64 (4.89)</td>
</tr>
<tr>
<td>No baseline anemia</td>
<td>14 0.21 (0.077)</td>
<td>0.22 (0.084)</td>
<td>0.0170 (0.074)</td>
<td>0.0250</td>
<td>18 0.26 (0.105)</td>
<td>0.31 (0.159)</td>
</tr>
<tr>
<td>Baseline anemia</td>
<td>10 0.15 (0.065)</td>
<td>0.14 (0.066)</td>
<td>–0.00832 (0.0599)</td>
<td>–0.0102</td>
<td>28 0.16 (0.073)</td>
<td>0.19 (0.091)</td>
</tr>
<tr>
<td>TIBC, mol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No baseline anemia</td>
<td>13 55.00 (15.44)</td>
<td>55.04 (15.40)</td>
<td>0.0468 (11.7)</td>
<td>1.00</td>
<td>22 52.21 (13.77)</td>
<td>52.39 (11.69)</td>
</tr>
<tr>
<td>Baseline anemia</td>
<td>10 44.93 (17.55)</td>
<td>44.53 (18.42)</td>
<td>–0.402 (6.9)</td>
<td>1.80</td>
<td>28 47.25 (24.87)</td>
<td>53.98 (17.22)</td>
</tr>
<tr>
<td>Ferritin, pmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No baseline anemia</td>
<td>14 526.78 (526)</td>
<td>369.19 (276)</td>
<td>–0.58 (524)</td>
<td>–19.2</td>
<td>22 410.24 (499)</td>
<td>354.71 (475)</td>
</tr>
<tr>
<td>Baseline anemia</td>
<td>11 418.73 (322)</td>
<td>487.97 (363)</td>
<td>69.2 (148)</td>
<td>25.8</td>
<td>28 961.08 (1429)</td>
<td>600.36 (1020)</td>
</tr>
</tbody>
</table>

NOTE: Baseline anemia was defined as patients with a hemoglobin reference range indicator of low at the baseline measurement. The hepcidin and iron parameter changes from baseline were measured using the time point at which the maximum median change from baseline was observed for each parameter (i.e., cycle 1 day 8 for hepcidin and cycle 2 day 1 for hemoglobin and iron parameters).

Abbreviation: TIBC, total iron-binding capacity.

*p* value siltuximab versus placebo.
(r = 0.607; P = 0.00011) and a positive but nonstatistically significant correlation in hemoglobin responders (r = 0.337; P = 0.34039) in the siltuximab arm. No significant associations were observed between maximum median change in hepcidin and other iron parameters in hemoglobin responders or nonresponders (data not shown). Among the iron parameters evaluated, higher median and mean changes in ferritin, hemoglobin, and TIBC were observed in anemic patients compared with non-anemic patients in the siltuximab arm (Table 3). There were no reported adverse events of polycythemia reported in either treatment group during the study.

**Discussion**

This randomized, double-blind clinical study of patients with MCD showed that siltuximab was superior to placebo, as measured by durable tumor response, MCD symptom alleviation, and laboratory parameter improvement (14). This was the largest and only placebo-controlled study in MCD to date and recruited a global patient population with comprehensive representation of race and histologic subtypes. The study also provided a unique opportunity to prospectively investigate the effects of selective IL6 inhibition and to evaluate inflammatory and inflammatory anemia-associated markers and identified relevant post-baseline changes among siltuximab-treated patients.

A strong, rapid, and sustained CRP suppression was observed in the siltuximab arm but not in the placebo arm. IL6 has been shown to induce CRP expression by activating the transcription factors STAT3 and C/EBP beta; ref. 30), and prior evidence indicated that CRP is a relevant biomarker for indirectly measuring IL6 bioactivity (12, 19). CRP suppression in siltuximab-treated patients is consistent with prior observations in patients with MCD who were treated with the same dose regimen in Study C0328T03 (12). IL6 pathway inhibition in patients with MCD by tocilizumab, an anti-IL6R antibody, has also been reported to induce CRP suppression (7). This study further highlighted the relationship between IL6 and CRP, shown by the positively correlated baseline levels, and was reinforced by the observation that siltuximab, an IL6 antibody, rapidly and stably suppressed CRP levels.

While these and previous data clearly support siltuximab-induced CRP suppression, the associations between CRP levels and tumor responses are less clear. The study indicated that there was an association between log10-transformed median baseline CRP levels and durable tumor and symptomatic response and best tumor response in the siltuximab group. However, no cutoff systemic CRP value predicted response, with no association between maximum on-treatment CRP decreases and response parameters. It should be noted that this study was a registration trial and it is possible that some clinical responses may not have met the defined endpoint.

We found no strong relationship between baseline IL6 and response and no association between baseline CRP or IL6 and symptomatic scores. There was large variation in systemic levels of baseline IL6 and CRP in both treatment arms, possibly due to differences in disease manifestation or prior systemic or concomitant treatments. It is also important to note that due to the clinical trial design, participants on a stable dose of corticosteroids below 1 mg/kg were eligible for participation. Thus, the relationship between inflammatory markers and disease severity, Castleman subtype, or anemia biomarkers may have been confounded by the likely higher exposure and dose of corticosteroids among patients perceived to have a greater burden of disease. An increased prevalence of certain IL6 receptor polymorphisms may be associated with the development of MCD (31). These genetic factors may also contribute to the response to an IL6-targeted therapy. DNA samples were not collected during the MCD2001 study and SNP analyses of the IL6 response pathway components were not conducted.

Physicians may have understandably excluded patients with more severe MCD from the clinical trial due to possible assignment to placebo and BSC. In this study, subject-reported symptoms identified by an MCD Symptom Scale, a novel patient-reported outcome instrument, were generally mild to moderate (submitted for publication). Thus, the study may not have captured patients who could have possibly mounted more robust siltuximab responses due to greater disease severity. However, it is unclear whether inclusion of these patients in the biomarker analysis would have affected the findings of this study.

In the MCD2001 study, subgroup analyses of durable tumor and symptomatic responses were also performed (14). Responses were greater in siltuximab-treated non-white patients, the majority of whom were Asian. In the present study, mean and median baseline IL6 and CRP levels were higher in Asian patients than in non-Asian patients. Patients with plasmacytic histology, which is considered to be the more severe form of MCD, showed greater responses to siltuximab, compared with the mixed subtype. Mean and median baseline IL6 and CRP levels were higher in the plasmacytic subtype than in mixed histology patients. No patients with hyaline vascular MCD showed a durable tumor and symptomatic response by independent review. The implications of these novel findings in the racial and histologic subgroups merit further investigation with larger patient populations.

Higher median changes in ferritin and TIBC were observed in anemic patients versus non-anemic patients in the siltuximab arm, supporting the observed clinical benefits of increased hemoglobin and resolution of inflammatory anemia in siltuximab-treated MCD patients. IL6 is a known potent hepcidin inducer, the iron regulatory peptide hormone that contributes to hemoglobin homeostasis. Neutralization of IL6 by siltuximab is hypothesized to decrease hepcidin releasing sequestered iron leading to improvement in hemoglobin levels (32). Hepcidin decreases were seen in siltuximab-treated patients in the hemoglobin response–evaluateable population, hemoglobin responses (≥15 g/l hemoglobin improvement from baseline at week 13) were achieved in >60% of patients in the siltuximab arm compared with none in the placebo arm, supporting this hypothesis. Greater hemoglobin increases were observed in patients with lower hemoglobin levels at baseline.

In terms of the association between hepcidin and hemoglobin, during treatment with siltuximab, decreases in hepcidin levels were associated with hemoglobin improvement; however, hepcidin reductions alone were not predictive of hemoglobin improvement. Although IL6 is the primary inducer of hepcidin production by hepatocytes, IL6-independent pathways may also decrease hepcidin levels with or without concomitant increases in hepcidin (33, 34). Thus, factors other than hepcidin may have contributed to the positive effects on iron parameters in patients with anemia who were treated with siltuximab.

Overall, prospective biomarker analyses indicated strong and sustained suppression of CRP following siltuximab treatment, indicating inhibition of IL6 activity. Improvements in
hemoglobin levels and anemia, partly through inhibition of the IL6/hepcidin pathway, were observed following siltuximab treatment.

Disclosure of Potential Conflicts of Interest

C. Casper reports receiving grants and speakers bureau honoraria from Janssen, and is a consultant/advisory board member for GlaxoSmithKline, Janssen, and Tepotmte. N. Munshi and F. Van Rhee are consultant/advisory board members for Janssen. R. Wong reports receiving a commercial research grant and speakers bureau honoraria from and is a consultant/advisory board member for Janssen. H. van de Velde has ownership interest (including patents) in Johnson & Johnson. No potential conflicts of interest were disclosed by the other authors.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Casper, S. Chaturvedi, N. Munshi, R. Wong, F. van Rhee

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Casper, S. Chaturvedi, N. Munshi, R. Wong, M. Qi, M. Schaffer, R. Bandekar, H. van de Velde, J. Vermeulen, M. Reddy, F. van Rhee

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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Casper, S. Chaturvedi, F. van Rhee

Study supervision: C. Casper, H. van de Velde, J. Vermeulen, M. Reddy, F. van Rhee

Other (responsive for incorporation of biomarker strategy, testing methods, data review, analysis, and interpretation): M. Reddy

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Clinical Cancer Research

Analysis of Inflammatory and Anemia-Related Biomarkers in a Randomized, Double-Blind, Placebo-Controlled Study of Siltuximab (Anti-IL6 Monoclonal Antibody) in Patients With Multicentric Castleman Disease


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