"Assessment of human antihuman antibodies to eculizumab after long-term treatment in patients with paroxysmal nocturnal hemoglobinuria."

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**References**


**Assessment of human antihuman antibodies to eculizumab after long-term treatment in patients with paroxysmal nocturnal hemoglobinuria**

*To the Editor:* Eculizumab (Soliris®; Alexion Pharmaceuticals, Cheshire, CT, USA) is a humanized monoclonal antibody (mAb) that displays high-affinity binding to complement protein C5 has been approved by US, European, and other regulatory agencies for the treatment of paroxysmal nocturnal hemoglobinuria (PNH).

The administration of any large-molecular therapeutic, such as a mAb, potentially induces an unwanted immune response through the development of antibody and subsequent immune-mediated adverse reactions (ADRs). In clinical studies of eculizumab in patients with PNH, the development of ADAs was transient and very infrequent with minimal impact on clinical response [4–6]. This current laboratory study, M07-003 (registered at http://www.clinicaltrials.gov as NCT01412047), addresses a postapproval commitment to the FDA and the European Medicines Agency (EMA) to examine immunogenicity of eculizumab after long-term treatment. The development of neutralizing and non-neutralizing HAHAs associated with eculizumab use was assessed in patients with PNH who participated in the open-label, long-term study E05-001 [7]. Briefly, the study included 195 patients with PNH who participated in three prospective trials: a phase two pilot study [3]; and two phase three studies [4,6]. The E05-001 study comprised a 104-week treatment period and a 16-week post-treatment follow-up period for patients who terminated treatment early [7]. The M07-003 study included patients from E05-001 who had eculizumab-naïve blood samples for comparison. The primary study endpoint was the proportion of patients who developed neutralizing HAHAs during treatment with eculizumab or who had discontinued treatment. Secondary endpoints were the proportion of patients who developed non-neutralizing HAHAs and the proportion that developed neutralizing HAHAs with evidence of increased hemolysis. Study procedure-related adverse events (AEs), defined as any AE not present prior to protocol-required blood draw and occurring as a result of the blood draw, were monitored and recorded from informed consent until site notification of final HAHAs results. AEs were coded using current Medical Dictionary for Regulatory Activities (MedDRA) terminology.

The full analysis set included patients with informed consent who had a blood sample collected; the safety analysis included patients with informed consent. Analyses were based on the full analysis set using descriptive statistics for patient disposition and demographics. A validated electrochemiluminescence (ECL) ADA detection method tested for evidence of anti-eculizumab antibody formation—BTM-0048, analysis of human anti-bodies against eculizumab in human serum using the Meso Scale Discovery® SECTOR Imager 2400 Validation (Meso Scale Discovery, Rockville, MD) assay.

A total of 75 patients were enrolled in the current study; the mean age (standard deviation [SD]) was 48.4 (13.14) years, and most were female (57.3%). At the initial visit, 97.3% (73/75) of patients were receiving eculizumab, most (88.9%; 67/75) at the maintenance dosage of 900 mg infused biweekly; six patients were receiving eculizumab 1200 mg biweekly. The median (range) duration of treatment from the initial dose in study E05-001 to the last treatment before Visit 1 of the current study was 90.2 (77.3–129.9) months, or 7.5 (6.5–10.8) years, for the 72 patients who had treatment data available. Most patients (88%) on treatment received their previous infusion 13–15 days before Visit 1.

Antieculizumab HAHAs assay findings showed no positive test results for HAHAs. Thus, no assay for confirmation of antibody formation was performed, and no follow-up visits or samples were needed for assessment of hemolysis or pharmacodynamics. Serum samples from 72 patients currently receiving eculizumab and from two patients no longer receiving eculizumab were assayed for serum eculizumab levels (Table I). Blood samples from two patients on treatment were not collected at trough per protocol and were considered out-of-window. Exclusion of patients with out-of-window measurements had no meaningful impact on results. The median trough level for the on-treatment group, excluding patients with out-of-window measurements, was 113.3 μg/ml (range 31.1–427.4).

75 patients were included in the safety population. A total of three patients experienced eight AEs. One patient experienced sepsis, a serious AE, with no cultured organism. The second patient experienced four AEs: an arterial hemorrhage, a serious AE and mild epistaxis, moderate wound infection, and a skin lesion. A third patient experienced three mild AEs: fatigue, arthralgia, and headache. None of the eight AEs was considered by the investigators to be related to study procedure.

The current study demonstrated that long-term eculizumab treatment (>6.6 years) at 900 or 1200 mg biweekly resulted in no development of HAHAs. This analysis provides sufficient evidence to support long-term lack of immunogenecity of commercial eculizumab in the treatment of PNH [8]. Moreover, these findings are consistent with shorter-term studies in which HAHAs were infrequent, occurring in 2% (3/196) of patients [9]. No sequelae, including rebound hemolysis, were linked to the detection of ADAs in these studies or HAHAs in the present study. Additionally, in the extension study, E05-001, 5 of 161 patients (3.1%) experienced transient positive results on HAHAs assays after a follow-up of >66 months, with no discernible impact on clinical response or pharmacodynamic effects of eculizumab, which continued to block complement activity [7,9]. Of these five patients, three have participated in the present HAHAs study; testing for antibody formation was negative at the time of enrollment. The frequencies of humanized antibodies against eculizumab observed in other FDA-approved biologics range from <1 to 9% [10].

Enzyme-linked immunosorosent assay (ELISA) was used to assess HAHAs in the three pivotal studies, and an ECL bridging assay was used in the extension and present study [4–6]. ELISA has important limitations when assessing HAHAs reactivity, as it lacks an acceptable detection reagent for humanized mAbs and is considered complex and time consuming [3]. The current study utilized a validated and quantitative ECL bridging assay that was specifically designed to allow detection of an antibody response to any region of the eculizumab molecule, buttressing confidence in the study findings.

No study procedure-related AEs occurred. Our study was not designed to assess non-study procedure-related AEs. However, investigators reported three mild AEs (fatigue, arthralgia, and headache) in one patient as probably related to eculizumab; these AEs were not unexpected since they have been reported in previous clinical studies [4–7]. In

**Table I. Summary of Eculizumab Trough Level Serum Concentrations in Patients Still Treated with Eculizumab at Visit 1.**

<table>
<thead>
<tr>
<th>Parameter On treatment (n = 72) * (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
</tr>
<tr>
<td>137.0 (85.17)</td>
</tr>
<tr>
<td>Median</td>
</tr>
<tr>
<td>113.3</td>
</tr>
<tr>
<td>Minimum, maximum</td>
</tr>
<tr>
<td>31.1, 427.4</td>
</tr>
</tbody>
</table>

SD: standard deviation; HAHAs: human antihuman antibody.

* One patient who was found to be ineligible due to no eculizumab-naïve sample and was not included in the on-treatment analysis.
Efficacy of ibrutinib in the treatment of Bing–Neel syndrome

To the Editor: Waldenström macroglobulinemia (WM)/lymphoplasmacytic lymphoma (LPL) is a rare low-grade lymphoproliferative disorder of B-cell origin characterized by serum monoclonal M-immunoglobulin (IgM) and bone marrow involvement with lymphoplasmacytic cells. WM represents approximately 2% of monoclonal gammopathies, with neurological complications occurring in 25–47% of cases. Polynuropathies due either to IgM deposition, IgM with anti-MAG activity, IgM reacting with other gangliosides or cryoglobulinemic neuropathy are the most frequent. Central nervous system (CNS) manifestations are rare and mostly related to serum hyperviscosity syndrome. Direct malignant lymphoid infiltration of the CNS constitutes a pretty rare and poorly defined entity known as Bing–Neel syndrome (BNS) [1]: about 100 cases have been so far reported, including 44 patients recently collected and analyzed by the French Innovative Leukemia Organization [2]. Diagnosis of BNS is suspected in patients with CNS symptoms, which are heterogeneous and mainly represented by balance disorder or disturbed gait. BNS is the first manifestation of WM in up to 36% of cases, or follows the WM diagnosis with a median delay of 8.9 years. BNS frequency has been reported with a male predominance (8/10 cases), and a median age at diagnosis of 63 years. Brain magnetic resonance imaging (MRI) is abnormal in 78% of the cases. Cerebrospinal fluid (CSF) analysis usually shows an increased protein level (95% of the cases), and evidence for CNS infiltration by WM monoclonal tumor cells. Moreover, BNS CSF-infiltrating tumor cells have recently been shown to carry the MYD88 L265P mutation, adding this molecular tool in our diagnostic arsenal [3]. Due to the small number of available data in the literature, no consensus exists for BNS treatment. Remission has been reported either with intrathecal and/or systemic chemotherapies, including high-dose methotrexate or cytarabine, due to their good blood–brain barrier penetration. Front-line intensification with autologous stem-cell transplantation seems to be associated with long-term remissions. Rituximab has been used in about half of the patients of the largest series [2], with no effect on response rate nor survival, consistent with its presumed low blood–brain barrier penetration. Whole brain radiation therapy (20–40 Gy), alone or in combination with chemotherapy, also could bring some responses with short duration. Ibrutinib, a Bruton’s tyrosine kinase inhibitor, has shown remarkable efficacy in WM, especially in MYD88 L265P carrying WM [4], and has recently been approved by the FDA (January 29, 2015) and the EMEA (October 17, 2015) for WM. Recently, ibrutinib has been shown to penetrate through the blood–brain barrier and to induce response in some CNS lymphoid tumor infiltrations [5].

Herein, we report the first two cases of patients with a refractory/relapsed MYD88 L265P BNS, in whom control of neurological symptoms was achieved with ibrutinib.

Patient 1. A 72-year-old man was diagnosed with WM/LPL on an axillary lymph node biopsy associated with bone marrow infiltration by a lymphoplasmacytic monoclonal population with MYD88 L265P mutation and serum monoclonal IgM kappa at 1.14 g/l. The patient achieved a very good partial response (VGPR) after six cycles of R-CHOP regimen (every 21 days). Ten months later, he developed general weakness, weight loss, impaired memory, sphincter disorders, disturbed gait, and falls. Serum IgM kappa was measured at 2.7 g/l and CSF analysis revealed a WBC count of 26/mm3 with monoclonal CD20+ lymphocytes and an elevated protein level at 3.57 g/l (normal <0.45) (Fig. 1A,B). Brain and spinal cord MRI revealed periventricular edema and T2 hypersignal lesions with contrast enhancement at C2–C3 level consistent with the diagnosis of Bing–Neel syndrome (Fig. 1C). Six cycles of Rituximab (375 mg/m2) and high-dose methotrexate (3 g/m2) were administered every 21 days, with four intrathecal injections of liposomial cytarabine (3 g/m2) every 21 days. In the 12th cycle, ibrutinib was administered instead of liposomial cytarabine. We observed a rapid decrease of CSF WBC count and CSF protein level, but intrathecal IgM synthesis persisted, neurological symptoms incompletely improved and post-treatment MRI showed persistence of lesions consistent with a partial response. After 10 months follow-up, neurological symptoms exacerbated, spinal MRI revealed a new corpus callosum lesion, and CSF protein was at 1.11 g/l with intrathecal monoclonal IgM synthesis. After four cycles of high-dose aracytine (2 g/m2/day for 2 days), BNS was refractory. Following its recent FDA approval, we started ibrutinib at 420 mg/day. Within 8 days, we observed significant clinical improvement, promptly decreasing of CSF protein level until normalization persisting at 6 months, and remarkable improvement of MRI lesions lasted at 6 months (Fig. 1).

Patient 2. A 56-year-old woman was diagnosed with WM and achieved a PR after first-line treatment with chlorambucil (8 mg/m2/day, 10 consecutive days monthly, 12 cycles). Five years later, she presented bilateral and very intensive burning pain, mostly in extremities. Brain and spinal cord MRI and electromyography were normal. CSF analysis revealed 0.3 WBC count of 11/mm3 with monotypic CD20+ lymphocytes present in CSF. Brain MRI lesions lasted at 6 months (Fig. 1). After several cycles of corticosteroids, we started ibrutinib instead of the last cycle of high-dose aracytine (2 g/m2/day for 2 days), with a little effect on neurological symptoms. MRI showed persistence of lesions consistent with a partial response. After 10 months follow-up, neurological symptoms improved, spinal MRI revealed a new corpus callosum lesion, and CSF protein was 0.24 g/l. Intrathecal IgM synthesis persisted, neurological symptoms incompletely improved and post-treatment MRI showed persistence of lesions consistent with a partial response. After 6 months, ibrutinib was administered at 420 mg/day, with a little effect on neurological symptoms. MRI showed persistence of lesions consistent with a partial response.