Therapeutic Clearance of Amyloid by Antibodies to Serum Amyloid P Component

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ABSTRACT

BACKGROUND

The amyloid fibril deposits that cause systemic amyloidosis always contain the nonfibrillar normal plasma protein, serum amyloid P component (SAP). The drug (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC) efficiently depletes SAP from the plasma but leaves some SAP in amyloid deposits that can be specifically targeted by therapeutic IgG anti-SAP antibodies. In murine amyloid A type amyloidosis, the binding of these antibodies to the residual SAP in amyloid deposits activates complement and triggers the rapid clearance of amyloid by macrophage-derived multinucleated giant cells.

METHODS

We conducted an open-label, single-dose-escalation, phase 1 trial involving 15 patients with systemic amyloidosis. After first using CPHPC to deplete circulating SAP, we infused a fully humanized monoclonal IgG1 anti-SAP antibody. Patients with clinical evidence of cardiac involvement were not included for safety reasons. Organ function, inflammatory markers, and amyloid load were monitored.

RESULTS

There were no serious adverse events. Infusion reactions occurred in some of the initial recipients of larger doses of antibody; reactions were reduced by slowing the infusion rate for later patients. At 6 weeks, patients who had received a sufficient dose of antibody in relation to their amyloid load had decreased liver stiffness, as measured with the use of transient elastography. These patients also had improvements in liver function in association with a substantial reduction in hepatic amyloid load, as shown by means of SAP scintigraphy and measurement of extracellular volume by magnetic resonance imaging. A reduction in kidney amyloid load and shrinkage of an amyloid-laden lymph node were also observed.

CONCLUSIONS

Treatment with CPHPC followed by an anti-SAP antibody safely triggered clearance of amyloid deposits from the liver and some other tissues. (Funded by GlaxoSmithKline; ClinicalTrials.gov number, NCT01777243.)
In systemic amyloidosis, the extracellular deposition of normally soluble plasma proteins as insoluble amyloid fibrils damages the structure and function of tissues and organs. Current treatment consists of support or replacement of failing organs and measures to reduce the abundance of the amyloid fibril precursor protein. A sufficient reduction of precursor supply arrests the accumulation of amyloid and can reduce morbidity and mortality. However, amyloid regression is very slow and often does not occur at all, in contrast to the usually swift clearance of other extracellular debris and efficient tissue remodeling — for example, after trauma. At least 65% of cases of systemic amyloidosis in the United States and Western Europe are of the monoclonal immunoglobulin-light-chain (AL) type that is caused by B-cell or plasma-cell dyscrasia. The effectiveness of cytotoxic chemotherapy to suppress the pathogenic clone is often limited by dysfunction of amyloid-infiltrated organs, and 20% or more of patients with AL amyloidosis die within 6 months after diagnosis, before the delayed benefits of chemotherapy are realized. Furthermore, there are no effective therapies for most hereditary forms of systemic amyloidosis. New treatments are required to improve organ function by eliminating systemic amyloid deposits at the time of diagnosis.

We report on a clinical trial of such a treatment, which was designed to engage potent normal phagocytic clearance mechanisms. It involves the use of a small-molecule drug, (R)-1-[[6-[[R]-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC), to deplete circulating serum amyloid P component (SAP), followed by administration of a fully humanized monoclonal IgG1 anti-SAP antibody to activate macrophage destruction of the SAP-containing amyloid deposits in tissues.

Regardless of the protein precursor of amyloid fibrils in the different clinical types of systemic amyloidosis, all amyloid deposits also always contain abundant SAP, an invariant, normal, nonfibrillar plasma glycoprotein. SAP binds avidly but reversibly to all types of amyloid fibrils and is thus specifically concentrated in all amyloid deposits. The administration of CPHPC swiftly depletes almost all circulating SAP and removes some, but not all, SAP from systemic amyloid deposits. In mice with systemic amyloid A (AA) amyloidosis that are transgenic for human SAP, IgG anti–human SAP antibodies can be safely administered after treatment with CPHPC. These antibodies localize swiftly to the residual amyloid-bound human SAP and trigger clearance of the deposits. In this study, we sought to determine whether administration of a single dose of fully humanized monoclonal IgG1 anti-SAP antibody after CPHPC infusion would be nontoxic in patients with different types of systemic amyloidosis and would produce a clinically relevant reduction in amyloid load.

METHODS

PATIENTS
A total of 16 patients, 18 to 70 years of age, who were attending the U.K. National Amyloidosis Centre and had different types of biopsy-proven systemic amyloidosis were screened at Quintiles (a contract research organization), London. All the patients provided written informed consent. Patient 3 was withdrawn from the study before administration of the study drug, because of poor venous access. The demographic and clinical characteristics of the patients at baseline are shown in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. All the patients were ambulatory with good functional status, which enabled them to participate in this clinical study.

Patients were treated one at a time, with a minimum interval of 2 weeks between patients. All previous safety information was continuously reviewed throughout the study period. The estimated glomerular filtration rate was more than 60 ml per minute in the first four patients and was more than 30 ml per minute in the subsequent patients. For safety reasons, the regulatory protocol did not allow the inclusion of potentially childbearing women and patients with clinical evidence of cardiac involvement. The first six patients selected for treatment had small or moderate amyloid loads, as determined by means of 123I-labeled SAP (123I-SAP) scintigraphy (see below). Eight of the nine subsequent patients had substantial hepatic involvement and were selected because hepatic amyloid can be quantified with the use of several independent methods.

STUDY DESIGN
We conducted a single-center, open-label, single-dose-escalation study at Quintiles, London. The
study regimen consisted of intravenous infusion of CPHPC to deplete circulating SAP, followed by a single intravenous administration of a fully humanized monoclonal IgG1 anti-SAP antibody. CPHPC has previously been shown to have an acceptable side-effect profile in patients with systemic amyloidosis, as well as in patients with other conditions. CPHPC was typically administered at a dose of 20 mg per hour for 3 days, until the SAP concentration in serum decreased from the mean (±SD) values typically seen in patients with amyloidosis (30±8 mg per liter) to below 2.0 mg per liter. The first two patients received 5 mg of antibody, a minimal dose that was calculated to produce SAP–anti-SAP immune complexes that would be in antibody excess over the trace residual circulating SAP. The next three patients received 1 mg per kilogram of body weight, the following two patients received 3 mg per kilogram, and the next patient received 10 mg per kilogram. In the final seven patients, a specific dose per patient was based on the pretreatment SAP content of the amyloid load, which was estimated by means of 123I-SAP scintigraphy. Doses of 400 mg or more were infused over a period of 2 hours; Patients 14, 15, and 16 received two 300-mg infusions, each over a period of 2 hours, with a 2-hour interval between doses. After the antibody infusion, CPHPC treatment was continued as a subcutaneous injection, typically at a dose of 60 mg three times daily, to maintain SAP depletion until the circulating anti-SAP antibody concentration fell below 300 μg per liter, a process that took 4 to 16 days (Fig. S1 in the Supplementary Appendix).

The patients were monitored for 42 days after the antibody was administered before returning to their routine follow-up monitoring. The study began on June 1, 2013, and ended on September 15, 2014, when Patient 16 completed the study.

**ASSESSMENTS**

The clinical status of the patients at baseline was documented by means of comprehensive hematologic testing of venous blood, biochemical testing of serum, and biochemical testing and microscopic examination of urine. The baseline amyloid distribution and load were determined with the use of whole-body 123I-SAP scintigraphy, and the load was categorized according to the intensity of 123I-SAP uptake in the organs and the residual blood-pool signal at 24 hours after tracer injection. The categories of amyloid load were as follows: small (definite organ uptake but substantial blood-pool signal), moderate (more intense organ localization and reduced blood-pool signal), and heavy (very strong organ localization with little or no blood-pool activity). Computed tomography (CT)–single-photon-emission CT was performed to quantify the organ retention of tracer at 24 hours as a proportion of the injected dose, and this proportion was used as an additional measure of changes in the amyloid load in individual patients. The expansion of the extracellular volume by systemic amyloid deposits was measured by means of equilibrium magnetic resonance imaging (MRI) of the liver, spleen, and heart. Liver stiffness is substantially increased by major hepatic amyloid deposits; stiffness was measured with the use of transient elastography (FibroScan, Echosens). Cardiac, renal, and immunologic functions were monitored for adverse effects, including consequences of immune-complex formation, during infusion of anti-SAP antibody and in the days immediately afterward, as well as on days 6, 14, 21, and 42. Elastography and equilibrium MRI were repeated on days 6, 14, and 42, and SAP scintigraphy was repeated on day 42, when SAP had again reached equilibrium between the plasma and amyloid compartments.

**STUDY OVERSIGHT**

The study protocol, available at NEJM.org, was approved by the Berkshire B Research Ethics Committee; all patients provided written informed consent. The study was funded by GlaxoSmithKline, employees of which, together with the authors, analyzed the data. All the authors had access to all the data and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the final protocol, and all the authors were involved in the interpretation of the data. The manuscript was written by the last and first authors, and all the authors participated in the review and editing of the manuscript; no one who is not an author contributed to the writing of the manuscript. An independent safety advisory committee reviewed the available data before the enrollment of patients with heavy amyloid loads and was available to review any serious adverse effects.
RESULTS

SAFETY AND SIDE-EFFECT PROFILE

No serious adverse events were observed. Three brief episodes of headache and one brief episode of nausea were reported during exposure to CPHPC. Most patients who were receiving more than 200 mg of anti-SAP antibody had transient self-limiting symptoms during and up to 12 hours after the infusion, including but not limited to a sensation of warmth, flush, headache, increases and decreases in heart rate and blood pressure, diarrhea or loose stools, nausea, and abdominal discomfort. We were able to mitigate these symptoms by slowing the infusion rate, splitting the dose, or adjusting patient positioning (or by a combination of these measures), and no subsequent notable clinical manifestations were reported. The adverse events and the principal hematologic and biochemical results are provided in Tables S2 and S3, respectively, in the Supplementary Appendix. There were no adverse changes in serum markers of renal or hepatic function (Table S3 in the Supplementary Appendix) and no new abnormalities of urine or urinary sediment. Although no abnormalities of ventricular wall thickness were detected in serial echocardiographic scans, some of the patients probably had some amyloid in the heart, because, as compared with the mean normal cardiac extracellular volume fraction of 0.27±0.03, Patient 11 had a substantially increased extracellular volume fraction of 0.37, and borderline increased values were seen in Patients 8 (0.31), 9 (0.32), 10 (0.33), 15 (0.34), and 16 (0.33). Nevertheless, there were no consistent or substantial changes in concentrations of troponin T (Table S4 in the Supplementary Appendix) or N-terminal pro–brain natriuretic peptide (NT-proBNP) (Table S5 in the Supplementary Appendix), and there were no clinically significant electrocardiographic changes, including in 24-hour Holter recordings, on the day of antibody infusion or on day 3. No clinically significant trends were observed in any of the other monitored measures.

PHARMACOKINETICS AND SAP DEPLETION

The pharmacokinetics of CPHPC were consistent with those in previous phase 1 studies.19 CPHPC rapidly depleted circulating SAP to less than 0.5 mg per liter in patients with a small or moderate amyloid load and to less than 2 mg per liter in patients with a large amyloid load.19 In patients with a small amyloid load, the plasma anti-SAP antibody concentration declined with a half-life of approximately 16 hours. In patients with a large amyloid load, most of the antibody disappeared from the plasma in the first 4 hours, a finding consistent with the rapid sequestration in amyloid observed in our experimental model, and was followed by a slow terminal clearance phase (Fig. S1 in the Supplementary Appendix).

SYSTEMIC RESPONSES

There was no systemic response to the smallest doses of anti-SAP antibody; however, in patients who received more than 200 mg, there was generally a brief spike in serum interleukin-6 and interleukin-8 at 2 hours after the start of the antibody infusion. One patient had a modest increase in the concentration of tumor necrosis factor α. In patients who received doses greater than 200 mg, there was a brief acute-phase response of C-reactive protein and serum amyloid A protein, which peaked at approximately 24 hours (Table 1 and Fig. 1). In six of the nine patients who received more than 200 mg of antibody, there was also a transient increase, by as much as a factor of 2, in the peripheral-blood neutrophil count at 24 hours.

In all patients who received 1 mg or more of antibody per kilogram, with the exception of Patient 15, complement C3 concentration in serum decreased substantially within the first day and sometimes remained low for up to a week, which indicated that several grams of C3 were consumed (Table 1 and Fig. 1), but no new organ dysfunction was observed. There were also modest decreases in the concentrations of C4 and CH50 in some patients.

AMYLOID ELIMINATION AND CLINICAL BENEFIT

At 42 days after treatment with anti-SAP antibody, there was a significant — and in some cases substantial — decrease in liver stiffness in six of the eight patients with hepatic involvement who had received antibody doses greater than 200 mg (Table 1 and Fig. 1). Liver stiffness increased transiently at day 6 in some patients (Table S6 in the Supplementary Appendix), a finding consistent
Table 1. Responses of Patients with Systemic Amyloidosis to Anti–Serum Amyloid P Component (SAP) Antibody after Depletion of Plasma SAP with CPHPC.*

<table>
<thead>
<tr>
<th>Patient No.†</th>
<th>Amyloid Type</th>
<th>Amyloid Load</th>
<th>Body Weight</th>
<th>Anti-SAP Dose</th>
<th>C3 Depletion‡</th>
<th>Acute-Phase Response§</th>
<th>Liver ECV¶</th>
<th>Improved SAP Scan‖</th>
<th>Liver Stiffness**</th>
<th>Evidence of Change in Amyloid Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AA</td>
<td>Moderate in kidney and spleen; none in liver</td>
<td>93.5</td>
<td>5</td>
<td>No</td>
<td>No</td>
<td>0.20</td>
<td>0.24</td>
<td>6.4 (1.5)</td>
<td>5.9 (0.8)</td>
</tr>
<tr>
<td>2</td>
<td>AFib</td>
<td>Small in kidney and spleen; none in liver</td>
<td>100.8</td>
<td>5</td>
<td>No</td>
<td>No</td>
<td>0.28</td>
<td>0.25</td>
<td>6.1 (0.8)</td>
<td>3.8 (0.4)</td>
</tr>
<tr>
<td>3</td>
<td>AFib</td>
<td>Small in kidney and spleen; none in liver</td>
<td>81.0</td>
<td>80</td>
<td>12</td>
<td>No</td>
<td>0.36</td>
<td>0.33</td>
<td>4.7 (0.3)</td>
<td>6.6 (1.5)</td>
</tr>
<tr>
<td>4</td>
<td>AFib</td>
<td>Small in kidney and spleen; none in liver</td>
<td>78.3</td>
<td>78</td>
<td>16</td>
<td>No</td>
<td>0.34</td>
<td>0.29</td>
<td>6.7 (0.9)</td>
<td>5.1 (1.2)</td>
</tr>
<tr>
<td>5</td>
<td>AFib</td>
<td>Small in kidney, moderate in spleen; none in liver</td>
<td>82.3</td>
<td>82</td>
<td>17</td>
<td>Yes</td>
<td>0.26</td>
<td>0.25</td>
<td>4.1 (0.5)</td>
<td>7.8 (0.9)</td>
</tr>
<tr>
<td>6</td>
<td>AFib</td>
<td>Small in kidney, moderate in spleen; none in liver</td>
<td>50.8</td>
<td>152</td>
<td>No</td>
<td>No</td>
<td>0.45</td>
<td>0.50</td>
<td>10.4 (0.9)</td>
<td>9.8 (4.1)</td>
</tr>
<tr>
<td>7</td>
<td>AL</td>
<td>Large in liver and spleen</td>
<td>81.9</td>
<td>246</td>
<td>20</td>
<td>Yes</td>
<td>0.37</td>
<td>0.33</td>
<td>14.4 (9.1)</td>
<td>8.9 (2.6)</td>
</tr>
<tr>
<td>8</td>
<td>AL</td>
<td>Large in liver, moderate in spleen, small in bone marrow and kidney</td>
<td>63.7</td>
<td>637</td>
<td>46</td>
<td>Yes</td>
<td>0.48</td>
<td>0.42</td>
<td>24.2 (4.0)</td>
<td>11.9 (0.8)</td>
</tr>
<tr>
<td>9</td>
<td>AApoAI</td>
<td>Large in liver and spleen</td>
<td>90.6</td>
<td>400</td>
<td>11</td>
<td>Yes</td>
<td>0.58</td>
<td>0.61</td>
<td>46.5 (14.3)</td>
<td>25.7 (6.2)</td>
</tr>
<tr>
<td>10</td>
<td>AL</td>
<td>Large in liver and spleen</td>
<td>54.0</td>
<td>650</td>
<td>21</td>
<td>Yes</td>
<td>0.54</td>
<td>0.53</td>
<td>27.0 (6.2)</td>
<td>15.7 (1.7)</td>
</tr>
<tr>
<td>11</td>
<td>AL</td>
<td>Large in liver and spleen; moderate in bone marrow</td>
<td>49.5</td>
<td>650</td>
<td>57</td>
<td>Yes</td>
<td>0.30</td>
<td>0.36</td>
<td>2.5 (0.6)</td>
<td>5.6 (0.6)</td>
</tr>
<tr>
<td>12</td>
<td>AA</td>
<td>Large in spleen, small in kidney and adrenals; none in liver</td>
<td>63.7</td>
<td>650</td>
<td>25</td>
<td>Yes</td>
<td>0.36</td>
<td>0.29</td>
<td>5.7 (0.6)</td>
<td>2.8 (0.1)</td>
</tr>
<tr>
<td>13</td>
<td>AL</td>
<td>Large in liver; moderate in spleen; small in kidney and bone marrow; amyloidotic lymph node present</td>
<td>115.2</td>
<td>600</td>
<td>29</td>
<td>Yes</td>
<td>0.35</td>
<td>0.34</td>
<td>8.9 (0.9)</td>
<td>4.4 (0.7)</td>
</tr>
<tr>
<td>14</td>
<td>AL</td>
<td>Large in liver; moderate in spleen</td>
<td>61.0</td>
<td>600</td>
<td>No</td>
<td>Yes</td>
<td>0.42</td>
<td>0.38</td>
<td>4.9 (1.2)</td>
<td>5.2 (0.8)</td>
</tr>
<tr>
<td>15</td>
<td>AL</td>
<td>Large in liver, large in spleen</td>
<td>78.9</td>
<td>600</td>
<td>32</td>
<td>Yes</td>
<td>0.45</td>
<td>0.43</td>
<td>27.7 (1.2)</td>
<td>27.0 (2.0)</td>
</tr>
</tbody>
</table>
Clearance of Amyloid by Antibodies to SAP

with the expected macrophage invasion of the opsonized amyloid deposits and the effect of inflammatory cell infiltration on hepatic elastography.20

In the three patients in whom major clearance of liver amyloid was shown with the use of other methods, equilibrium MRI confirmed that hepatic extracellular volume was reduced toward normal (Table 1). No significant changes in ECV were observed in the heart or spleen.

At 45 days after the start of CPHPC treatment and 42 days after anti-SAP antibody treatment, when SAP distribution had fully re-equilibrated between the soluble and amyloid-bound compartments, whole-body 123I-SAP scintigraphy scans revealed substantial reductions in hepatic amyloid load in four patients (Table 1 and Fig. 1). In Patient 13, who had macroglobulinemia in clonal relapse throughout the study and in whom AL amyloidosis had been diagnosed by biopsy of a greatly enlarged amyloid-laden cervical lymph node immediately from approximately 5 cm in diameter, the mass shrank from approximately 1 cm at day 42. In Patient 12, who had no hepatic amyloid, renal function and proteinuria were unchanged at day 42, but SAP scintigraphy revealed a modest reduction in renal amyloid load.

Patients who had clinical evidence of cardiac involvement or hepatic failure but who were not included in the study, including Patients 8 and 17, had markedly reduced hepatic amyloid load by 45 days after the start of CPHPC treatment. In Patient 8, who had undergone a hepatic biopsy at day 1 and was the only patient to have a reduction in the 123I-SAP scintigram at day 42, liver stiffness was measured with the use of transient elastography (FibroScan [Echosens]) and is expressed as the median (interquartile range) in kilopascals for 10 to 13 replicate measurements in each patient on each occasion; values were measured immediately before antibody administration (upper limit of normal) and at day 42 after antibody administration (lower limit of normal). The median value in healthy persons is 5.3 kPa, and 90% have a value of less than 7.0 kPa.

** Liver stiffness was measured with the use of transient elastography (FibroScan [Echosens]) and is expressed as the median (interquartile range) of liver stiffness, equilibrium MRI confirmed that hepatic extracellular volume was reduced toward normal (Table 1). No significant changes in ECV were observed in the heart or spleen.
Figure 1. Response to Administration of CPHPC and Anti–Serum Amyloid P Component (SAP) Antibody.

Panels A and B show serum concentrations of complement C3, C-reactive protein (CRP), and serum amyloid A protein (SAA) in Patients 8 and 13. Patient 8 had symptoms of an upper respiratory tract infection on day 3, associated with the modest increase in acute-phase protein values on day 5. In Patient 8, the serum C3 concentration was not available on day 5, but it was 1.145 g per liter on day 6. Panels C and D show whole-body anterior SAP scintigraphy scans immediately before antibody infusion (left) and at day 42 after antibody infusion (right). After treatment, both patients had a marked reduction in hepatic amyloid load, which was detected in a visual comparison of the scans taken before and after treatment and confirmed by means of CT–single-photon-emission CT quantification of the retention of 123I-SAP tracer (measured as a proportion of the injected dose). The “hot spot” in the antecubital fossa of Patient 13 in the pretreatment scan is a tiny fraction of the injected dose of 123I-SAP that extravasated during the tracer injection. CPHPC denotes (R)-1-[6-[[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid.
DISCUSSION

After the depletion of SAP in plasma by CPHPC, administration of a single intravenous dose of up to 650 mg of humanized monoclonal IgG1 anti-SAP antibody in patients with systemic amyloidosis produced only transient low-grade infusional side effects. We observed no adverse effects that were attributable to the formation of soluble SAP–anti-SAP immune complexes; to antibody binding to the normal, non–amyloid-related SAP in the tissues;21,22; or to cardiotoxicity.

In patients with substantial hepatic involvement, the infused anti-SAP antibody disappeared rapidly from the circulation, a finding consistent with its binding to the readily accessible and abundant amyloid-associated SAP in the hepatic deposits. Indeed, after treatment with anti-SAP antibody, a reduction in amyloid load was most sensitively detected as decreased liver stiffness. Major clearance of hepatic amyloid was also revealed by means of SAP scintigraphy1,8,23 in some patients and was independently confirmed when equilibrium MRI showed a reduction in liver extracellular volume toward normal in these patients.14 The liver tolerates abundant amyloid deposition before the results of liver-function tests become abnormal,23 but some improved values were seen after treatment with anti-SAP antibody. A modest but definite reduction in renal SAP scintigraphy signal was also seen in Patient 12, and there was impressive shrinkage of the amyloid-laden cervical lymph node in Patient 13. The amyloid regression in Patients 9, 13, and 14 was particularly notable, because the respective amyloidogenic precursors were still being produced in these patients (Table S1 in the Supplementary Appendix), and the patients would have been expected to continue to have accumulation of amyloid.

SAP scintigraphy sensitively and specifically detects and quantifies visceral amyloid deposits9,23 but is less sensitive to modest changes in very heavily amyloidotic organs. Therefore, it may not have revealed some regression in patients with massive amyloid loads. In contrast, equilibrium MRI is relatively insensitive and is not amyloid-specific, but it directly measures the extracellular volume expansion produced by visceral amyloid.14 The median baseline values of liver and spleen extracellular volume were lower in patients in whom SAP scanning revealed amyloid regression (0.36 and 0.38, respectively; normal values, 0.29 and 0.34) than in patients with unchanged SAP scans (0.50 and 0.62, respectively), which confirmed that the latter group of patients had greater initial amyloid loads.

The binding of anti-SAP antibody to amyloid in the mouse model activates complement and attracts massive infiltration of macrophages that fuse into multinucleated giant cells that engulf and destroy the deposits.5 The giant cells have abundant surface membrane ruffles6 and are thus specialized for phagocytosis of large C3-opsonized objects. Here, we observed major depletion of serum C3 and more modest depletion of C4 and reduction in CH50, findings that are consistent with engagement of the same mechanism as in our mouse model and that suggest classic complement pathway activation, as required for amyloid clearance in mice,5 and alternative pathway amplification.

Depletion of serum C3 occurred in all but one recipient of 246 mg or more of antibody, but in the patients with the highest amyloid loads, the doses were probably insufficient, in relation to the number or availability of target SAP molecules, to opsonize the amyloid adequately or engage the macrophages effectively. Indeed, the highest dose of anti-SAP antibody — 13.1 mg per kilogram — was much smaller than the dose of 200 mg per kilogram required for maximal effect in the mouse AA amyloidosis model.5,24 The clearance of amyloid and improved liver function observed so far, therefore, suggest that larger or repeated doses of anti-SAP antibody may be more effective. SAP is present in all human amyloid deposits of all types, and anti-SAP treatment after treatment with CPHPC should therefore be applicable in all forms of systemic amyloidosis.

Patients with a new diagnosis of systemic amyloidosis require swift and safe elimination of amyloid deposits to maintain or restore vital organ function, so that measures to reduce production of amyloid fibril precursor protein and thus arrest amyloid accumulation can be effective. The main determinants of outcome are the severity of cardiac and renal involvement; it is encouraging that in the mouse model, we have observed anti-SAP–mediated reduction in cardiac amyloid deposits with no ill effects,25 as well as formation of multinucleated giant cells around renal amyloid. Although patients with clinical signs of cardiac amyloidosis were not included in the present study, measurements of
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extracellular volume suggested that some patients may have had small asymptomatic cardiac amyloid deposits. However, all these patients also had heavy liver and spleen amyloid loads, and most of the single dose of antibody would inevitably have localized swiftly to those organs, which would explain the lack of change in cardiac measures. In the next trial phase, patients with clinically significant renal and cardiac amyloidosis will be included and will receive larger and, if necessary, repeated doses of anti-SAP antibody, with the aim of achieving effective exposure in tissues that do not have the highly permeable sinusoidal endothelium of the liver and spleen.

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REFERENCES