Radioimmunodetection of amyloid deposits in patients with AL amyloidosis

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Introduction

Light chain–associated (AL) amyloidosis is a monoclonal plasma cell dyscrasia characterized by the pathologic deposition in vital tissues of fibrils formed from κ or λ immunoglobulin (Ig) light chain–related components.1-3 The relentless accumulation of such fibrillar material typically leads to progressive organ dysfunction and death within 18 to 36 months. In the case of cardiac involvement, the prognosis is even more ominous, with a survival time of 3 to 9 months; fewer than 5% of all AL amyloidosis patients live more than 10 years after diagnosis.4 Currently, therapeutic options are limited to diminishing light chain production with anti–plasma cell chemotherapy (eg, melphalan and/or corticosteroids) given in conventional amounts or high doses combined with autologous stem cell transplantation.4,5 This approach, which is based on the premise that reduction in synthesis of the amyloidogenic precursor will slow fibril formation, has extended length of life and, in some instances, resulted in improved organ function over time; nonetheless, the prognosis remains poor because of persistent amyloid burden.

To address this issue, we have focused on passive immunotherapy as a means to expedite removal of amyloid deposits and, through these research efforts, developed a murine (m) IgG1 anti–human light chain monoclonal antibody (mAb), designated 11-1F4, which recognized a conformational epitope present on amyloid fibrils, but not the soluble amyloidogenic precursor protein.6,11 Furthermore, when administered to mice bearing subcutaneous human AL amyloidomas, the antibody bound to the pathologic material and initiated an inflammatory response that led to elimination of the induced tumors.12 Notably, we also demonstrated that m11-1F4, after radiolabeling with the positron-emitting isotope I-124, imaged the xenograft, as evidenced by micro–positron emission tomography/computed tomography (PET/CT).13 These results have led to a Food and Drug Administration (FDA)–approved Phase I Exploratory investigational new drug (IND 100472) study to determine the safety and biodistribution of 124I-m11-1F4 in patients with AL amyloidosis. We now report the results of this trial that have involved, to date, 18 subjects in whom the radioiodinated antibody was well tolerated, elicited no human anti–mouse antibody (HAMA) response, and notably in 9 subjects, was taken up by organs deemed to contain amyloid.

Methods

Patients

All 18 patients entered on study (Table 1)14 were HAMA-negative and had AL amyloidosis based on accepted clinical and laboratory criteria,15 as well as (with 1 exception) the results of chemical analysis of amyloid extracted from tissue or fat biopsy specimens. Based on these findings, we posit that 124I-mAb m11-1F4 can be used to identify AL candidates for passive immunotherapy using the chimeric form of the antibody. This trial was registered at www.clinicaltrials.gov as NCT00807872. (Blood. 2010;116(13):2241-2244)

Production and radiolabeling of m11-1F4 mAb

Good medical practice-grade m11-1F4 (National Service Center No. 740550) and isotope I-124 were furnished by the National Cancer Institute–Frederick Cancer Research and Development Center’s Biologic Resource Branch and by IBA Molecular, respectively. The antibody was radioiodinated using Iodogen (Pierce) as an oxidant, purified by solid phase
Table 1. Summary of the patient population and PET/CT imaging and immunohistochemical results for the $^{124}$I-m11-1F4 study in patients with AL amyloidosis.

<table>
<thead>
<tr>
<th>AL patient no.</th>
<th>Age, y/sex</th>
<th>Affected organ*</th>
<th>AL isotype</th>
<th>Free $\mathrm{w}/\lambda$, mg/L†</th>
<th>PET‡</th>
<th>IHC (tissue)‡</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>60/M</td>
<td>K</td>
<td>$\kappa$</td>
<td>40/54</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>66/F</td>
<td>LN</td>
<td>$\lambda$</td>
<td>4/125</td>
<td>LN (+LN)</td>
<td>NA</td>
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<tr>
<td>3</td>
<td>79/F</td>
<td>K</td>
<td>$\lambda$</td>
<td>13/119</td>
<td>0</td>
<td>(K)</td>
</tr>
<tr>
<td>4</td>
<td>75/F</td>
<td>Lu</td>
<td>$\lambda$</td>
<td>142/97</td>
<td>0 (+Lu)</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>63/M</td>
<td>K</td>
<td>$\lambda$</td>
<td>8/287</td>
<td>0</td>
<td>(K)</td>
</tr>
<tr>
<td>6</td>
<td>74/F</td>
<td>F</td>
<td>$\lambda$</td>
<td>6/264</td>
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<td>NA</td>
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<tr>
<td>7</td>
<td>74/F</td>
<td>H</td>
<td>$\kappa$</td>
<td>252/14</td>
<td>0</td>
<td>(+F)</td>
</tr>
<tr>
<td>8</td>
<td>73/F</td>
<td>K</td>
<td>$\lambda$</td>
<td>7/195</td>
<td>I</td>
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</tr>
<tr>
<td>9</td>
<td>54/F</td>
<td>H, I</td>
<td>$\kappa$</td>
<td>256/2</td>
<td>L, S</td>
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</tr>
<tr>
<td>10</td>
<td>48/M</td>
<td>T</td>
<td>$\lambda$</td>
<td>2/643</td>
<td>0</td>
<td>0 (T)</td>
</tr>
<tr>
<td>11</td>
<td>62/M</td>
<td>L, BM</td>
<td>$\lambda$</td>
<td>58/141</td>
<td>L, S, BM (+BM)</td>
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</tr>
<tr>
<td>12</td>
<td>57/M</td>
<td>H, L</td>
<td>$\kappa$</td>
<td>67/26</td>
<td>L, S</td>
<td>(+GB)</td>
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<tr>
<td>13</td>
<td>52/F</td>
<td>K</td>
<td>$\lambda$</td>
<td>6/52</td>
<td>L, S</td>
<td>0 (K)</td>
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<tr>
<td>14</td>
<td>57/M</td>
<td>K, L, LN</td>
<td>$\lambda$</td>
<td>1/8</td>
<td>L, S, BM</td>
<td>? (LN)</td>
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<tr>
<td>15</td>
<td>51/M</td>
<td>H, K</td>
<td>$\lambda$</td>
<td>7/204</td>
<td>S</td>
<td>0 (K)</td>
</tr>
<tr>
<td>16</td>
<td>66/M</td>
<td>I</td>
<td>$\lambda$</td>
<td>1/15</td>
<td>0</td>
<td>? (R)</td>
</tr>
<tr>
<td>17</td>
<td>49/M</td>
<td>K</td>
<td>$\lambda$</td>
<td>12/4871</td>
<td>S, BM</td>
<td>(+BM)</td>
</tr>
<tr>
<td>18</td>
<td>57/F</td>
<td>K</td>
<td>$\lambda$</td>
<td>4/31</td>
<td>0</td>
<td>? (K)</td>
</tr>
</tbody>
</table>

*For affected organ, K indicates kidney; H, heart; L, liver; LN, lymph node; Lu, lung; F, fat only; BM, bone marrow; T, tongue; and I, intestine.
†Free $\mathrm{w}/\lambda$ determined by an enzyme-linked immunosorbent assay (normal range: $<4.2-13.0$ mg/L; $\lambda$, 16.4-127.3 mg/L).‡
‡For PET imaging, 0 indicates no uptake. For immunohistochemistry (IHC), 0 indicates negative immunostain; $\kappa$, strongly positive immunostain; $\lambda$, weakly positive immunostain; BM, bone marrow; I, intestine; S, spleen; GB, gallbladder; L, liver; LN, lymph node; Lu, lung; F, fat only; K, kidney; R, rectum; and NA, tissue not available.

Ex vivo reactivity of m11-1F4 mAb

Six-$\mu$m thick sections of formalin-fixed, paraffin-embedded tissue were subjected to antigen retrieval using Citra Plus or Glyca (BioGenex) and incubated at 4°C overnight with 1 $\mu$g/mL mAb m11-1F4, followed by biotinylated goat anti–mouse IgG, and then the avidin-biotin complex solution (ABC; Vector Laboratories). Consecutive tissue sections also were stained with Congo red. Slides were examined by light and polarizing microscopy.

Results and discussion

The AL fibril reactivity of the m11-1F4 mAb was not affected by radioiodination and, in all cases, the administered preparations were sterile, had negligible endotoxin content, were well tolerated, and elicited no HAMA response in serum specimens obtained 60 days later. For dosimetry purposes, the first 3 patients were scanned 3, 5, 48, 72, 120, and 168 hours after infusion. The radiolabeled antibody plasma $t_{1/2}$ was approximately 25 hours, which was longer than that seen in mice, but consistent with clearance of mlgG in humans (ie, ~30 hours). The calculated effective radiation dose (0.4 mSv/MBq) proved acceptable to the FDA. By 48 hours, approximately 70% of radioactivity in the blood pool had cleared (although, in the case of patient AL 2, amyloid-associated binding of mAb 11-1F4 in the mediastinal lymph nodes persisted for 168 hours, with a mean activity of 2.1 MBq/mL). In subsequent studies, subjects were imaged only on days 2 and 5 after infusion.

The results from the 18 patients are provided in Table 1. In 9, the PET/CT scans revealed uptake of the radiolabeled antibody in areas deemed to contain amyloid (eg, liver, lymph nodes, bone marrow, and intestine, as well as spleen, which may represent another source of the amyloidogenic precursor protein). In contrast, those with cardiac or renal amyloid had no demonstrable uptake in these sites. In 3 of 5 subjects with positive liver imaging, the serum alkaline phosphatase concentrations were abnormally high. There was no evident correlation between the radioimmunoimaging data and disease duration or therapy.

Because the immunoreactivity of m11-1F4 was not affected by labeling with I-124, we investigated whether the in vivo results could be related to those derived immunohistochemically using diagnostic tissue biopsy specimens available from 14 of the 18 cases. In these studies, which used the unmodified antibody, the reagent immunostained (Figure 1) the deposits in 18 cases. In these studies, which used the unmodified antibody, the reagent immunostained (Figure 1) the deposits in 10 specimens, of which 6 had positive PET/CT scans, and in 4 of 6 specimens that did not image.

We previously had shown through peptide mapping that the specificity of mAb 11-1F4 depends upon a conformational epitope present on light chain fibrils that is not exposed on the native protein. Thus, the inability of the radiolabeled antibody to bind certain AL deposits, both in vivo and in vitro, may reflect a...
Figure 1. Radioimmunoimaging and immunohistochemical detection of AL amyloid. Three patients with systemic AL amyloidosis (AL 2, AL 11, AL 12) received an intravenous infusion of approximately 2 mCi (1 mg) of 124I-labeled m11-1F4. (A) Fused coronal and sagittal PET/CT images acquired 5-days after infusion using the high-resolution Siemens Biograph 16 (patient AL 2) or molecular CT (patients AL 11, AL 12) instruments. (B) Maximum intensity projection PET images. (C) Polarizing and light microscopy. Consecutive tissue sections from each patient (AL 2, lymph node; AL 11, bone marrow, and AL 12, liver) were subjected to histochemical (HC) staining with Congo red (CR, top) or immunohistochemical (IHC) studies using, as primary reagent, mAb 11-1F4 (middle), or, as a negative control, the antibody preincubated with a 22-mer peptide containing the conformational fibril-related epitope recognized by mAb 11-1F4 (bottom). Photomicrographs were acquired with a Leica DM 500 light microscope equipped with cross-polarizing filters. Digital images were obtained using a cooled charged coupled device camera and dedicated SPOT software (Version 3.5.2) at an original magnification × 160.
structural alteration in this cryptic epitope or its inaccessibility, as seen in the cases of renal amyloid in which (in contrast to other tissues) this material was immunostained weakly, if at all, by the reagent. Alternatively, the concentration of the immune target per unit volume may have been too low and therefore undetectable by PET imaging. In the 3 cases in which the amyloid was immunostained by mAb 11-1F4 but was not imaged, it is possible that a higher dose of radiotracer would have yielded a positive result. Of note, although radiolabeled serum amyloid P component can be used to visualize renal deposits, it is also incapable of imaging cardiac amyloid, presumably because of vascular factors.

Given these results, we posit that 124I-m11-1F4 radioimmunomaging could be used to predict which AL patients would be candidates for passive immunotherapy using the chimeric version of this mAb, which currently is under production for an eventual Phase I clinical trial. Notably, the modified amyloid-reactive antibody, in contrast to the murine form, advantageously, could be administered repeatedly and has a considerably longer t½. This novel approach, namely passive immunotherapy, would offer an additional therapeutic option for patients with this invariably fatal disorder.

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References


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