Radioimmunodetection of amyloid deposits in patients with AL amyloidosis

Jonathan S. Wall,1 Stephen J. Kennel,1 Alan C. Stuckey,1 Misty J. Long,2 David W. Townsend,1,4 Gary T. Smith,2,5 Karen J. Wells,2 Yitong Fu,2 Michael G. Stabin,3 Deborah T. Weiss,1 and Alan Solomon1

1Departments of Medicine and 2Radiology, University of Tennessee Graduate School of Medicine, 1924 Alcoa Highway, Knoxville, TN, USA and the 3Department of Radiology and Radiological Sciences, Vanderbilt University, Nashville, TN, USA
4Current address: PET and SPECT Development Group, Singapore Bioimaging Consortium, 11 Biopolis Way, Singapore 138667; 5Department of Nuclear Medicine, Tennessee Valley Healthcare System, Department of Veterans Affairs, Nashville TN, USA

Running title: Radioimmunoimaging of AL amyloid

Corresponding Author: Jonathan S. Wall, Department of Medicine, Human Immunology and Cancer Program, University of Tennessee Graduate School of Medicine, 1924 Alcoa Highway Knoxville, TN 37920; phone: 865-305-5447; fax: 865-305-6865; e-mail: jwall@utmck.edu
Care of patients with AL amyloidosis currently is limited by the lack of objective means to document disease extent, as well as therapeutic options that expedite removal of pathologic deposits. To address these issues, we have initiated a Phase I Exploratory IND study to determine the biodistribution of the fibril-reactive, amyloidolytic murine IgG1 mAb 11-1F4 labeled with I-124. Individuals were infused with < 1 mg (~ 74 MBq) of GMP-grade antibody and imaged by PET/CT 48 and 120 hours later. Among 9 of 18 subjects, there was striking uptake of the reagent in liver, lymph nodes, bone marrow, intestine, or, unexpectedly, spleen (but not kidneys or heart). Generally, positive or negative results correlated with those obtained immunohistochemically using diagnostic tissue biopsies. Based on these findings, we posit that 124I-mAb m11-1F4 can be used to identify AL candidates for passive immunotherapy utilizing the chimeric form of the antibody. This trial is registered at http://www.clinicaltrials.gov as NCT 00807872.
Introduction

AL amyloidosis is a monoclonal plasma cell dyscrasia characterized by the pathologic deposition in vital tissues of fibrils comprised of κ or λ immunoglobulin light chain-related components.\textsuperscript{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 patients live more than 10 years after diagnosis.\textsuperscript{4} Currently, therapeutic options are limited to diminishing light-chain production with anti-plasma cell chemotherapy (e.g., melphalan and/or corticosteroids) given in conventional amounts or high doses combined with autologous stem cell transplantation.\textsuperscript{4-9} 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 due to 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 mAb, designated 11-1F4, which recognized a conformational epitope present on amyloid fibrils, but not the soluble amyloidogenic precursor protein.\textsuperscript{10,11} Further, 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.\textsuperscript{12} Notably, we also demonstrated that m11-1F4, after radiolabeling with the positron-emitting isotope I-124, imaged the xenograft, as evidenced by microPET/CT.\textsuperscript{13} These results have led to an FDA-approved Phase I Exploratory IND (100472) study to determine the safety and biodistribution of $^{124}$I-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 HAMA response, and notably in 9, was taken up by organs deemed to contain amyloid.

**Methods**

**Patients**

All 18 patients entered on study (Table 1) were HAMA negative and had AL amyloidosis based on accepted clinical and laboratory criteria, as well as (with 1 exception) the results of chemical analysis of amyloid extracted from tissue or fat biopsy specimens. All subjects provided written informed consent in accordance with the Declaration of Helsinki under a protocol approved by the FDA and the University of Tennessee Graduate School of Medicine’s Institutional Review Board.

**Production and radiolabeling of m11-1F4 mAb**

GMP-grade m11-1F4 (NSC# 740550) and I-124 were furnished by the NCI-Frederick Cancer Research and Development Center’s Biologic Resource Branch and IBA Molecular, respectively. The antibody was radioiodinated using Iodogen (Pierce) as an oxidant, purified by solid phase size-exclusion chromatography (PD-10 desalting column, GE Healthcare), and eluted with sterile PBS. The peak protein-bound radioactive fraction was diluted to 3 mL with PBS, passaged through a 0.22 μm pore-sized filter, and an aliquot removed to determine protein concentration, radiochemical purity, specific activity, stability, and immunoreactivity. The final product, which was tested for sterility and endotoxin content, was prepared for patient injection by further dilution to 30 mL and contained < 1 mg m11-1F4 labeled with ~ 2 mCi (74 MBq) I-
124, plus 5% HSA and 15 mg ascorbic acid. To inhibit thyroidal uptake of free radioiodide, 0.3 mL of SSKI was prescribed 24 hours prior to $^{124}$I-m11-1F4 administration (and then continued for an additional 9 days); the following day, patients were pre-medicated with 25 mg diphenhydramine/650 mg acetaminophen and infused over ~ 15 minutes with the reagent.

PET/CT images were obtained using a Siemens Biograph or mCT instrument that consisted of a low-dose CT from mid-thigh to crown, followed by a series of seven 5-minute PET acquisitions covering the same region. After on-line correction for random scatter, prompt gamma emission, and attenuation (CT-based correction algorithm), the data were reconstructed by means of a point spread function-based iterative algorithm (Siemens, TrueX) with an image matrix of 168 × 168 that provided an ~8 mm full-width half-maximum (FWHM) resolution. Maximum intensity projection images (MIP) were generated using Siemens Inveon Research Workplace software.

**Ex vivo reactivity of m11-1F4 mAb**

Six-µm-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 µg/mL of mAb 11-1F4, followed by biotinylated goat anti-mouse IgG, and then the avidin-biotin complex solution (ABC, Vector Labs). 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 post-infusion. The radiolabeled antibody plasma T\(_{1/2}\) was ~ 25 hours which was longer than that seen in mice\(^{13}\) but consistent with clearance of murine IgG in humans i.e., ~ 30 hours.\(^{17}\) The calculated effective radiation dose (0.4 mSv/MBq) proved acceptable to the FDA. By 48 hours, ~ 70% of radioactivity in the blood pool had cleared (although, in the case of 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 post-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; e.g., liver, lymph nodes, bone marrow, and intestine, as well as spleen which may represent another source of the amyloidogenic precursor protein.\(^{18}\) In contrast, those with cardiac or renal amyloid had no demonstrable uptake in these sites. In 3 of the 5 individuals 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.

Since the immunoreactivity of m11-1F4 was not affected by labeling with I-124, we investigated if the in vivo results could be related to those derived immunohistochemically using diagnostic tissue biopsies available from 14 of the 18 cases. In these studies, which utilized the unmodified antibody, the reagent immunostained (Figure 1) the deposits in 10, of which 7 had positive PET/CT scans, but only 3 of the 6 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.\textsuperscript{10,11} Thus, the inability of the radiolabeled antibody to bind certain AL deposits, both in vivo and in vitro, may reflect a structural alteration in this cryptic epitope or its inaccessibility, as seen in the cases of renal amyloid where (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. In the 3 cases where 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 also is incapable of imaging cardiac amyloid,\textsuperscript{19} presumably due to vascular factors.

Given these results, we posit that $^{124}$I-m11-1F4 radioimmunooimaging could be used to predict which AL patients would be candidates for passive immunotherapy using the chimeric version\textsuperscript{20} 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 $T_{1/2}$. This novel approach, namely passive immunotherapy, would offer an additional therapeutic option for individuals with this invariably fatal disorder.\textsuperscript{21}

**Authorship**

Contributions: J.S.W., S.J.K., D.W.T., and A.S. designed the study; S.J.K. radioiodinated the mAb and S.J.K. and J.S.W. performed quality control assays for the product; A.C.S. and M.J.L. were responsible for the PET/CT imaging and data processing; G.T.S., K.J.W., and Y.F. reviewed the PET/CT images; M.G.S. provided the dosimetry data; J.S.W., D.T.W., and A.S. wrote the paper.
Conflict of interest disclosure: J.S.W. and A.S. have intellectual property rights for the use of mAb 11-1F4 in the diagnosis and treatment of patients with amyloidosis; D.W.T. is a consultant for Siemens.

Acknowledgements

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References


Table 1

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<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Affected organ*</th>
<th>AL isotype</th>
<th>Free κ/λ (mg/L)†</th>
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Table 1. Summary of the study population and results from PET/CT imaging of $^{125}$I-m11-1F4 mAb in patients with AL amyloidosis

* Affected tissue: K, kidney; H, heart; L, liver; LN, lymph node; Lu, lung; F, fat only; BM, bone marrow; T, tongue, I, intestine, R, rectum; GB, gall bladder.
† Free κ/λ: Determined by an ELISA-based assay (normal values: κ, 4.2-13.0 mg/L; λ, 16.4-127.3 mg/L).14
Figure Legend

Figure 1. Radioimmunoimaging and immunohistochemical detection of AL amyloid. Three patients with systemic AL amyloidosis (AL 2, 11, 12) received an intravenous infusion of ~ 2 mCi (1 mg) $^{124}$I-labeled m11-1F4. (A) Fused coronal and sagittal PET/CT images acquired 5-days post infusion using the high-resolution Siemens Biograph 16 (AL 2) or mCT (AL 11, 12) instruments. (B) Maximum intensity projection (MIP) 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 (upper) or immunohistochemical (IHC) studies using, as primary reagent, mAb 11-1F4 (middle), or, as a negative control, the antibody pre-incubated with a 22-mer peptide containing the conformational fibril-related epitope recognized by mAb 11-1F4 (lower). Photomicrographs were acquired with a Leica DM 500 light microscope equipped with cross-polarizing filters; digital images were obtained using a cooled CCD camera and dedicated SPOT software (original magnification, ×160).
Figure 1

PET/CT

AL 2

Coronal

Sagittal

PET MIP

HC/IHC

CR

11-1F4

11-1F4 + peptide

AL 11

CR

11-1F4

11-1F4 + peptide

AL 12

CR

11-1F4

11-1F4 + peptide
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