Brief report

Radioimmunodetection of amyloid deposits in patients with AL amyloidosis

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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. Patients were infused with less than 1 mg (~ 74 MBq) of GMP-grade antibody and imaged by PET/CT scan 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 biopsy specimens. Based on these findings, we posit that ^{124}I-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)

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.[4-9] 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 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.[10,11] 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 ^{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 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[16] 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 Institutional Review Board.

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

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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

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

*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 κ/λ determined by an enzyme-linked immunosorbent assay (normal range: κ, 4.2-13.0 mg/L; λ, 16.4-127.3 mg/L).[14]

‡For PET imaging, 0 indicates no uptake. For immunohistochemistry (IHC), 0 indicates negative immunostain; +, strongly positive immunostain; ?, 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.

size-exclusion chromatography (PD-10 desalting column; GE Healthcare), and eluted with sterile phosphate-buffered saline. The peak protein–bound radioactive fraction was diluted to 3 mL with phosphate-buffered saline, 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 less than 1 mg of m11-1F4 labeled with approximately 2 mCi (74 MBq) of I-124, plus 5% human serum albumin and 15 mg of ascorbic acid. To inhibit thyroidal uptake of free radioiodide, 0.3 mL of super saturated potassium iodide was prescribed 24 hours before ^{124}I-m11-1F4 administration (and then continued for an additional 9 days); the next day, patients were premedicated with 25 mg of diphenhydramine/650 mg of acetaminophen and infused over approximately 15 minutes with the reagent.

PET/CT images were obtained using a Siemens Biograph mCT instrument that consisted of a low-dose CT from midthigh to crown, followed by a series of seven 5-minute PET acquisition scans covering the same region. After online correction for random scatter, prompt gamma emission, and attenuation (CT-based correction algorithm), these data were reconstructed by a point spread function-based iterative algorithm (TrueX; Siemens) with an image matrix of 168 × 168 that provided an approximately 8-mm full-width half-maximum resolution. Maximum intensity projection images were generated using Siemens Inveon Research Workplace software (Version 3.0).

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 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 $t_{½}$ was approximately 25 hours, which was longer than that seen in mice,[13] but consistent with clearance of mIgG in humans (ie, ~ 30 hours).[17] 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).[18] 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 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.[10,11] Thus, the inability of the radiolabeled antibody to bind certain AL deposits, both in vivo and in vitro, may reflect a

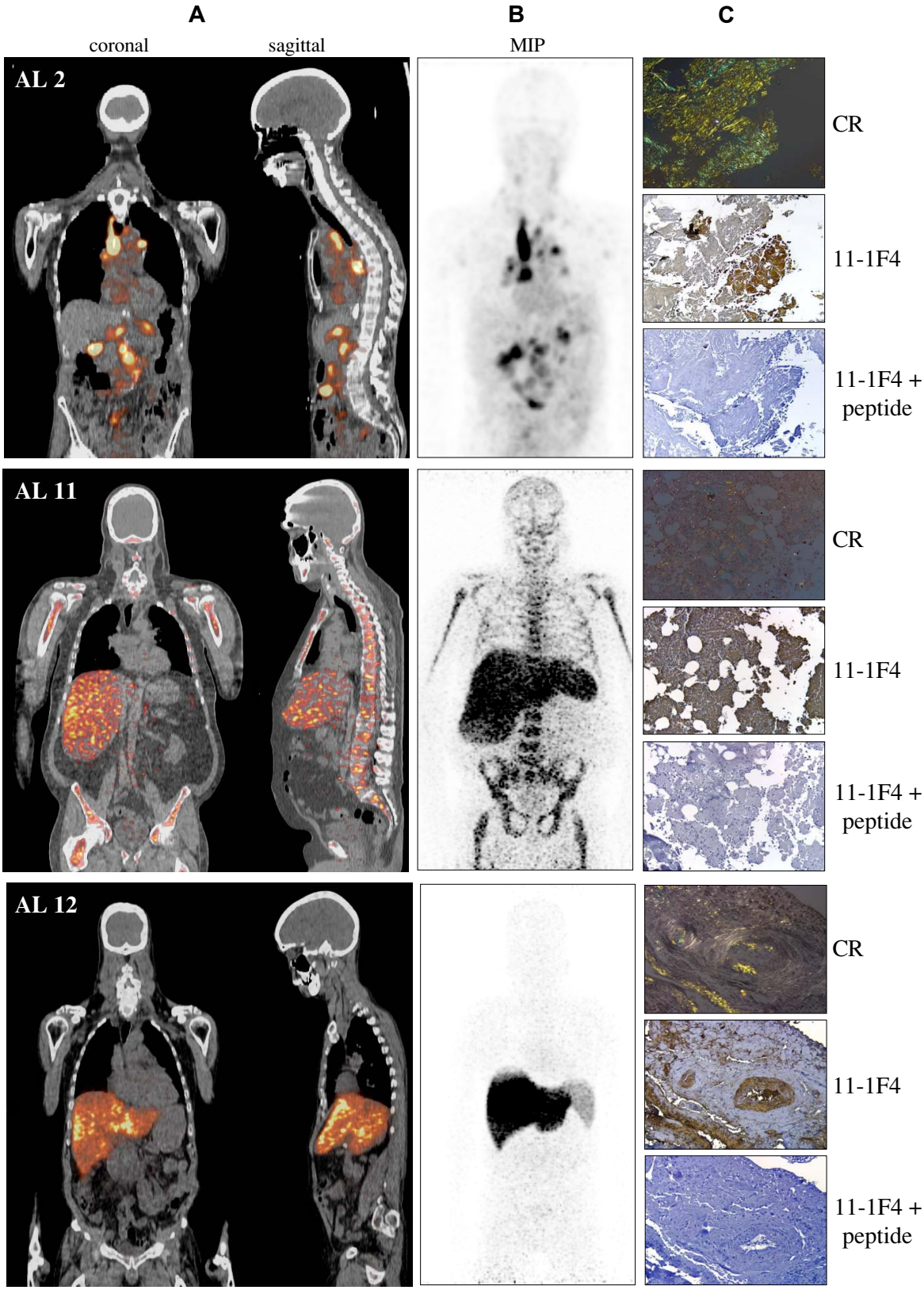

Figure 1. Radioimmunoimaging and immunohistochemical detection of AL amyloid. Three patients with systemic AL amyloidosis (AL 2, AL 11, AL 12) received an intravenous infusion of approximately 2 mCi (1 mg) of ^{124}I-labeled m11-1F4. (A) Fused coronal and sagittal PET/CT images acquired 5-days after 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 also is incapable of imaging cardiac amyloid,[19] presumably because of vascular factors.

Given these results, we posit that ^{124}I-m11-1F4 radioimmunoimaging could be used to predict which AL patients would be candidates for passive immunotherapy using the chimeric version[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 $t_{½}$. This novel approach, namely passive immunotherapy, would offer an additional therapeutic option for patients with this invariably fatal disorder.[21]

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Authorship

Contribution: J.S.W., S.J.K., D.W.T., and A.S. designed the study; S.J.K. radioiodinated the mAb and J.S.W. and S.J.K. 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; and 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. The remaining authors declare no competing financial interests.

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