Direct Intralymphatic Injection of Radiolabeled ¹¹¹In-T101 in Patients with Cutaneous T-Cell Lymphoma

James L. Mulshine, Jorge A. Carrasquillo, John N. Weinstein, Andrew M. Keenan, James C. Reynolds, Jean Herdt, Paul A. Bunn, Edward Sausville, Joyce Eddy, James D. Cotelingam, Patricia Perentesis, Carl Pinsky, and Steven M. Larson

Department of Nuclear Medicine and Radiology, National Institutes of Health, Bethesda, Maryland 20892; Department of Nuclear Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021; Department of Medical Oncology, University of Colorado Cancer Center and Health Service Center, Denver, Colorado 80262; Department of Pathology, Naval Hospital, Bethesda, Maryland 20814; and Biological Response Modifier Program, Frederick Cancer Research Center, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Frederick, Maryland 21701.

ABSTRACT

Direct intralymphatic administration of radiolabeled monoclonal antibody in targeting antigen-bearing lymphoma cells in regional lymph nodes of patients with cutaneous T-cell lymphoma was evaluated. Seven consecutive patients undergoing staging lymphangiography received intralymphatic infusions of ¹¹¹In-T101 to evaluate lymph node involvement. This procedure was accomplished without significant complication. The ¹¹¹In-T101 rapidly distributed throughout the regional lymphatic compartment and passed into the systemic circulation. Tumor-bearing sites in the inguinal-femoral lymph nodes retained from 0.42 to 4.8% of the injected dose of radiolabeled antibody. Three patients were upstaged to Stage IVA based on tumor involvement found after radiolymphoscintigraphy-directed biopsy of groin lymph nodes, selected because of intense radioactivity by gamma camera imaging. Compared with previously reported s.c. antibody administration, there was a marked reduction in the radioactive exposure of normal tissues at the injection sites in the lower extremities. Direct intralymphatic delivery of ¹¹¹In-T101 appears to be a feasible, efficient method for delivering therapeutic doses of radiolabeled antibody.

INTRODUCTION

CTCL is a distinctive subtype of lymphoma, possibly of retroviral etiology (1), which is rarely cured by existing therapies (2). T101 is a murine monoclonal antibody which binds to CD5, an M, 65,000 antigen, expressed on normal T-cells as well as on malignant T- and B-cells (3). Administration of the radiocugate ¹¹¹In-T101 i.v. for radioimmunoscintigraphy of both CTCL and chronic lymphocytic leukemia patients has resulted in reproducible imaging of tumor-involved sites (4-7). Biopsy of involved lymph nodes in patients with CTCL showed recovery of only a very small fraction of the injected dose (<0.02%/g), but this was approximately 10-100 times greater than previously reported delivery of radiolabeled monoclonal antibodies in solid tumors (4-8). Delivery of ¹¹¹In-T101 i.v. also resulted in >50% of the injected dose localizing in the liver and spleen.

In a therapeutic trial, administration of large doses of unlabeled T101 did not result in significant clinical benefit despite a transient lowering of circulating tumor cell number (9, 10). To enhance the therapeutic effect, Rosen et al. (11) coupled iodine-131 to T101 for administration to patients with refractory CTCL. The five patients with CTCL treated with ¹³¹I-T101 experienced brief objective and subjective improvements, but bone marrow toxicity was dose limiting.

One way to decrease nonspecific reticuloendothelial uptake, to improve the efficiency of lymph node imaging, and to reduce toxicity in therapeutic trials is to administer antibody s.c. for regional lymphatic uptake. Extensive animal studies have shown that s.c. delivery of monoclonal antibody results in highly efficient delivery to lymphoid target cells in the regional lymphatics with much less systemic uptake than is seen with i.v. injection (12-16).

Delivery of ¹¹¹In-T101 s.c., into the web spaces of the foot of patients with CTCL also resulted in efficient delivery of the labeled antibody to normal and tumor-involved lymph nodes along the draining regional lymphatic vessels (17, 18). Comparison of biopsy data from patients receiving ¹¹¹In-T101 by s.c. administration showed a much greater percentage of injected dose recovered from cancerous lymph nodes than previously reported with i.v. radiolabeled T101 administration (18). Since regional lymph nodes are thought to be an important proliferative compartment for malignant cells in CTCL (19, 20), this is a potentially important target for radiolabeled T101 therapy. After s.c. ¹¹¹In-T101 antibody administration, a large fraction of the injected radioactive dose remained at the injection sites, which cleared with a half-life of 14-29 h. This local retention resulted in 136-280 rads/mCi, or a total of 34-70 rads, to each foot per study (17, 18). This radiation dose exposure did not result in clinical toxicity. However, the α- and β-emitting isotopes required for therapeutic purposes would exceed limits of local tissue tolerance if delivered s.c. (21).

In the present study, we infused radiolabeled monoclonal antibody directly into cannulated lymphatic vessels of the foot at the time of routine diagnostic lymphangiography. The technique of direct antibody injection in the lymphatics was pioneered by Order et al. (22) using heteroantibody preparations directed against tumor-associated antigens. Further work with regional delivery of radiolabeled antibody in patients with carcinoma was reported by DeLand et al. (23). The goal of this approach would be to preserve the efficiency of tumor tissue...
targeting reported with the s.c. lower extremity monoclonal antibody injections and to ameliorate the problem of retention of radiolabeled monoclonal antibody at the injection site.

MATERIALS AND METHODS

Patients. Seven patients with histologically confirmed CTCL were studied (Table 1). These investigations were done under a protocol approved by a National Cancer Institute-Institutional Review Board, and all patients gave informed consent. Patients being evaluated for this study were also being evaluated for an ongoing National Cancer Institute-Navy Medical Oncology Branch therapy protocol for treatment of CTCL that entailed an extensive staging evaluation of lymph nodes, peripheral blood, skin, and viscera for evidence of disseminated disease. The details and rationale for this staging approach have been previously published, and this evaluation includes standard lymphangiography (24-27). The median age was 45 years and 5 of the 7 patients were males. In all of the patients in this study the sites of documented tumor involvement were restricted to skin and lymph nodes. Three patients had T1 skin involvement (<10% of their total skin area was involved with lesions) suggestive of CTCL. Three patients had T2 skin involvement (>10% of their total skin clinically involved with CTCL plaques). The final patient had extensive CTCL tumors of the skin. Two patients had palpable inguinal lymphadenopathy, and the 5 remaining patients had no evidence of nodal involvement.

No patient had visceral involvement. Although one lymphangiogram was abnormal, it was classified for staging as not being diagnostic of lymphoma. All but one patient had clinical stage IA disease, considering the result of the routine staging evaluation.

Monoclonal Antibody Characteristics. The T101 monoclonal antibody has been the subject of numerous previous publications (3-11, 17, 18, 28). It is a murine IgG2a that recognizes the CD5 cell surface antigen, which is known to modulate (29). The monoclonal antibody 9.2.27 (IgG2a), which binds to a high molecular weight proteoglycan antigen expressed on melanoma but not CTCL cells, was used as an isotypically matched control antibody (30). Although not previously reported, studies with s.c. administered 111In-9.2.27 showed preferential binding to normal lymph nodes when compared to 123I-Bl-3 and IgG1 antiidiotypic monoclonal antibody (30). The 9.2.27 and the T101 used for these studies were prepared and labeled as previously reported (6, 31). As summarized in Table 2 quality control analysis revealed an incorporation of 92-99% of the indium-111 onto the antibody, and the final immunoreactivity ranged from 61-87% (mean, 76.8%) as determined in a cell-binding assay (4-6) (Table 2). The final antibody conjugate preparations were sterile and pyrogen free.

Study Methods. A total of six patients were studied on the day of the lymphangiography by intralymphatic injection of T101 in doses including 0.1, 0.5, and 1 mg (Table 2). Five patients received bilateral intralymphatic 111In-T101 antibody, with one-half of the injectate infused into the cannulated lymphatic of the dorsum of each foot. One patient, because of severe chronic obstructive pulmonary disease, received an ipsilateral contrast pedal lymphangiography with direct intralymphatic administration of 111In-T101 (500 μCi/5 ml) and a contralateral s.c. injection of the radiolabeled T101. The previously reported s.c. injection technique was used, and the radionuclide conjugate was injected into the web spaces of the foot using a 25-gauge needle (17).

The seventh patient underwent a lymphangiography study to evaluate the specificity of the imaging. First, he received bilateral intralymphatic injections of the isotypically matched control antibody, 111In-9.2.27. Twelve days later, he received bilateral injections of 111In-T101 in the web spaces of the feet. The direct intralymphatic injections were performed in the course of standard diagnostic lymphangiography. A small volume of Evans blue dye with 1% xylocaine was injected into the web spaces of the foot to facilitate localization of the lymphatic vessels. A lymphatic vessel was then cannulated with a 30-gauge lymphangiography needle. Patency was established by infusing normal saline with a Hobb's pressure-sensitive pump. For patients in whom adequate cannulation of the lymphatic vessel was in question, a small amount of radiodense lymphangiogram contrast agent (ethiodol) was injected to determine patency. For the direct intralymphatic antibody delivery, the total activity of the indium-111 injected was 500 μCi. The radioisotope was chelated to approximately 0.1 mg of T101, and additional unlabeled T101 was added when necessary to obtain the final antibody mass (up to 1 mg). The preparation was then diluted to a volume of 10 ml and divided in half. Five-ml portions were then infused intermittently into the cannulated lymphatic vessels by the pressure-sensitive pumps over approximately 30 min. Immediately after infusion of the radiolabeled T101, ethiodol lymphangiographic dye for the diagnostic lymphangiogram was injected, typically over a 2-h period.

Upon completion of infusion, patients were scanned using a large field-of-view gamma camera equipped with a medium-energy collimator. Twenty % windows were centered over the 173- and 247-keV photopeaks of indium-111. Serial 10-min spot images of the feet, pelvis, abdomen, and chest were obtained on a nuclear medicine computer at intervals starting within 4 h of injection and continued up to 4 days thereafter. Anterior whole body images were also obtained 2-4 days after injection. The images were analyzed by drawing regions of interest over the liver, spleen, lymph nodes, and lymphatics of the lower extremities. The total counts administered were calculated from the region of interest analysis of a previously studied group of patients who received identical amounts of the labeled antibody via a s.c. injection (17, 18).

Decay-corrected data were used for calculating the uptake and clearance of radioactivity in lymph nodes and other organs. Activity in inguinal-femoral nodes, iliac nodes, and injection site on each side was expressed as a percentage of initial activity injected on that side. Activity in paraaortic nodes, liver, and spleen was expressed as a fraction of total initial administered activity, (i.e., amount of activity injected in both feet). Because of their relatively superficial location, counts in the lymphatics and inguinal-femoral and iliac nodes were not corrected for attenuation. Liver, spleen, and paraaortic nodes were assumed to be underestimated by a factor of 2 because of tissue attenuation based on the half-value layer of approximately 15 cm in water for the γ-photon
of indium-111 (17, 18). The activity in the lymphatics was expressed as a percentage of total activity in the whole body at the time of evaluation. While these calculations are inexact because of variable attenuation of radioactivity from the study subject, the quantitation is valid to permit approximate comparison with other published reports (17, 18, 31). Vital signs were monitored. Serial blood and plasma samples were obtained at 5 and 30 min; 1, 2, 24, 48, and 72 h; and later time points when feasible. Serial 24-h urine collections were performed up to 3 days.

Plasma volume was determined from nomograms using the patient’s body surface area. The total circulating plasma activity was calculated based on the concentration of indium-111 in plasma and the plasma volume. Liver and renal function were monitored at the time of the study and 1 week after infusion. Five of 7 patients underwent lymph node biopsy at 1–10 days after the labeled antibody administration. For each patient the surgeon was directed to biopsy a lymph node from a site of the inguinal chain which was marked and corresponded to the highest tracer uptake on the gamma camera images. The biopsy sample was weighed and counted in a gamma counter along with a standard, and the percentage of the injected dose/g was calculated. Since only one-half of the total injected dose was available for binding to these ipsilateral inguinal-femoral lymph nodes, the percentage of injected dose/g was doubled to allow relevant comparisons to other organs.

Patients were evaluated for development of a human anti-mouse antibody response. Baseline and serial post-antibody infusion sera were tested as previously described (5, 32). Briefly, 1 μl of patient serum was incubated with 0.5–1 ng (10,000 cpm) of iodine-125-labeled B72.3, an IgG1 anti-carcinoma monoclonal antibody. The total reaction volume was 110 μl and the incubation was for 20 h at 40°C. Then, 20 mg of formalin-fixed staphylococcal A cells (Immunoprecipitin; Bethesda Research Laboratories, Bethesda, MD) was added for 15 min at 40°C. The immunoglobulins were then precipitated by centrifugation at 3000 rpm. Human anti-murine antibody level was calculated as a percentage of the total counts in each assay tube that bound to protein A after correcting for nonspecific binding. To determine nonspecific binding, a control using normal serum was used. Generally, the activity bound by protein A in these tubes was the same as, or 1–2% lower than, the activity bound by protein A when no serum was added. The variance of the nonspecific tubes was used to determine the significance of a human anti-mouse antibody level, which had to be >3 SD above the average nonspecific binding value to be considered positive. The mean SD over the nonspecific tubes was used to determine the significance of a human anti-mouse antibody level, which had to be >3 SD above the average nonspecific binding value to be considered positive. The mean SD over the nonspecific tubes was used to determine the significance of a human anti-mouse antibody level, which had to be >3 SD above the average nonspecific binding value to be considered positive.

In Table 3 data obtained from the in vitro analysis of lymph node biopsies are summarized. The five lymph nodes evaluated had a mean weight of 2.9 g (range, 0.8–6.2 g). The percentages of total injected dose/g of tissue were 0.22 and 0.42% for the two histologically benign nodes (LN-2) and ranged from 0.28 to 4.4% for the three histologically malignant nodes (LN-3). The total percentage of injected dose counted in the resected lymph nodes ranged from 0.384 to 4.8. A mean of 0.87% of injected dose was recovered from the two biopsied lymph nodes with only mild dermopathic changes and a mean of 2.89% of injected dose was recovered from the three pathologically involved lymph nodes. Fig. 1 shows representative scan findings with region of interest analysis of the whole body scanning of patient 4. It shows that 16.6%, 13.5, and 3.8% of total injected radioactivity were retained in the right groin, left groin, and paraaortic lymph nodes, respectively.

In four patients, the inguinal lymph node biopsies were performed in the area of most intense radioactivity after infusion of the radiolabeled antibody. None of these sites were suspected clinically to be involved with tumor. Three of these four scan-directed biopsies revealed tumor involvement (extensive dermopathic changes with large clusters of convoluted cells or a totally effaced lymph node) (27). The fifth biopsied patient was the one who first received radiolabeled 9.2.27. The node biopsy was performed on a clinically suspicious node, but no pathological evidence of tumor was seen. This biopsy was performed on a lymph node region thought to be part of the most intense area of radioactivity after the s.c. injection of radiolabeled T101. Although a 1.8-g lymph node was removed, follow-up scanning revealed little decrease in the original radioactivity of the targeted nodal cluster (in contrast to what was observed for all of the other post-biopsy scans). At completion of pathological staging, including the results of the scan-directed lymph node biopsies, four patients were considered to have disseminated (IVA) disease (19). Three of the five patients who had a T101-directed lymph node biopsy were upstaged from stage I to stage IV, in the absence of any other clinical tests demonstrating advanced disease.

Table 4 summarizes the region of interest analysis from whole body scans. Intralymphatic delivery of labeled T101 in five patients is compared with intralymphatic delivery of 111In-9.2.27 in the control patient. A greater mean percentage of radioactivity was counted in lymph nodes after infusion of T101 conjugate than after infusion of 9.2.27 conjugate (31 versus 43%). The activity in the lymphatics was expressed as a percentage of total activity in the whole body at the time of evaluation. While these calculations are inexact because of variable attenuation of radioactivity from the study subject, the quantitation is valid to permit approximate comparison with other published reports (17, 18, 31). Vital signs were monitored. Serial blood and plasma samples were obtained at 5 and 30 min; 1, 2, 24, 48, and 72 h; and later time points when feasible. Serial 24-h urine collections were performed up to 3 days.

RESULTS

All three concentrations of T101 used in this study resulted in good visualization of lower trunk and paraaortic lymph nodes, even at the earliest imaging time points (17, 18). A representative scintigraphic image, obtained 48 h after infusion of the monoclonal antibody, is shown in Fig. 1. At the time of the initial imaging inguinal, iliac, and paraaortic nodes could be visualized. Liver and spleen were also visualized, although to a lesser degree (liver and spleen not included in the field of view in Fig. 1). In all patients, there was irregular uptake along the length of the lower extremity lymphatic channel after intralymphatic delivery of radiolabeled antibody (Fig. 2) (ranging from 6 to 26% of the retained dose/extent, as determined from the whole body scan). Radionuclide uptake in the lymphatic channels decreased >50% within 48 h (Fig. 2, right). Residual activity at 4 h in the injection site ranged from 0.2 to 14.2% (3.8 ± 4.7%, mean ± SD) of the ipsilateral injected dose as determined by a large-field region of interest quantitation, which counted the entire foot.

In Table 3 data obtained from the in vitro analysis of lymph nodes are compared with that obtained from the in vivo analysis of lymph node uptake with radiolabeled 9.2.27. The uptake in the lymph nodes was calculated as a percentage of total counts in each assay tube that bound to protein A after correcting for nonspecific binding. To determine nonspecific binding, a control using normal serum was used. Generally, the activity bound by protein A in these tubes was the same as, or 1–2% lower than, the activity bound by protein A when no serum was added. The variance of the nonspecific tubes was used to determine the significance of a human anti-mouse antibody level, which had to be >3 SD above the average nonspecific binding value to be considered positive. The mean SD over the nonspecific tubes was used to determine the significance of a human anti-mouse antibody level, which had to be >3 SD above the average nonspecific binding value to be considered positive. The mean SD over the nonspecific tubes was used to determine the significance of a human anti-mouse antibody level, which had to be >3 SD above the average nonspecific binding value to be considered positive.
Fig. 1. Representative immunoscintigram (right) and lymphangiogram (left). Patient 3 underwent imaging approximately 48 h after intralymphatic delivery of $^{111}$In-T101 (0.5 mg, 500 μCi). Half of the amount was delivered into the pedal lymphatic chain of each foot. The anterior view of the pelvis shows focal accumulation of indium-111 in the inguinal lymph nodes (arrows) and to a lesser extent in the next nodes in the chain, the iliacs (arrowheads) and paraaortics (open arrows). There is also some accumulation in the lymphatic channels (curved arrows). The corresponding lymphangiogram (left) shows normal-appearing lymph nodes. Biopsy of a left inguinal lymph node (with the greatest uptake of indium-111) showed lymphoma.

Fig. 2. Clearance from the injection site after s.c. and intralymphatic injection. Images of the lower extremity were obtained on patient 5 at approximately 2 h following direct intralymphatic administration of $^{111}$In-T101 in the left foot (DILA) and s.c. administration of an equal dose in the right foot (s.c.) (right). Left, clearance of radioactivity from the foot after intralymphatic and s.c. injection. The cumulative radiation exposure to the foot was greater after s.c. administration.

In one patient, the intralymphatic and s.c. injection routes were compared directly. Fig. 4 shows the difference in dose retention for the intralymphatically delivered and s.c. T101. Initially, direct intralymphatic administration was associated with higher concentrations of radioactivity in the regional lymphatics, absence of uptake in distant nodal sites, and minimal accumulation in soft tissue tumor in the left breast. In contrast, axillary nodes were seen after radiolabeled T101 administration. Clearance of $^{111}$In-9.2.27 from the injection site and serum kinetics after intralymphatic administration were similar to those of $^{111}$In-T101. This pattern of 9.2.27 biodistribution after intralymphatic delivery is similar to that seen in two other CTCL patients who received $^{111}$In-9.2.27 after s.c. pedal injections (18), and the pattern of $^{111}$In-T101 biodistribution is similar to that previously reported for >0.1 mg of s.c. $^{111}$In-T101 (18).

In one patient, the intralymphatic and s.c. injection routes were compared directly. Fig. 4 shows the difference in dose retention for the intralymphatically delivered and s.c. T101. Initially, direct intralymphatic administration was associated with higher concentrations of radioactivity in the regional lymphatics, absence of uptake in distant nodal sites, and minimal accumulation in soft tissue tumor in the left breast. In contrast, axillary nodes were seen after radiolabeled T101 administration. Clearance of $^{111}$In-9.2.27 from the injection site and serum kinetics after intralymphatic administration were similar to those of $^{111}$In-T101. This pattern of 9.2.27 biodistribution after intralymphatic delivery is similar to that seen in two other CTCL patients who received $^{111}$In-9.2.27 after s.c. pedal injections (18), and the pattern of $^{111}$In-T101 biodistribution is similar to that previously reported for >0.1 mg of s.c. $^{111}$In-T101 (18).

In one patient, the intralymphatic and s.c. injection routes were compared directly. Fig. 4 shows the difference in dose retention for the intralymphatically delivered and s.c. T101. Initially, direct intralymphatic administration was associated with higher concentrations of radioactivity in the regional lymphatics, absence of uptake in distant nodal sites, and minimal accumulation in soft tissue tumor in the left breast. In contrast, axillary nodes were seen after radiolabeled T101 administration. Clearance of $^{111}$In-9.2.27 from the injection site and serum kinetics after intralymphatic administration were similar to those of $^{111}$In-T101. This pattern of 9.2.27 biodistribution after intralymphatic delivery is similar to that seen in two other CTCL patients who received $^{111}$In-9.2.27 after s.c. pedal injections (18), and the pattern of $^{111}$In-T101 biodistribution is similar to that previously reported for >0.1 mg of s.c. $^{111}$In-T101 (18).
INTRALYMPHATIC INJECTION OF $^{111}$In-T101

Table 3 Summary of outcome of scan-directed inguinal lymph node biopsy after intralymphatic administration of $^{111}$In-T101

<table>
<thead>
<tr>
<th>Patient</th>
<th>Weight of lymph node (g)</th>
<th>Injected dose/g of tissue</th>
<th>Total % of injected dose</th>
<th>Lymph node status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>0.22</td>
<td>0.980</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>0.8</td>
<td>4.4</td>
<td>3.47</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>1.5</td>
<td>0.28</td>
<td>0.42</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>6.2</td>
<td>0.78</td>
<td>4.8</td>
<td>Positive</td>
</tr>
<tr>
<td>7*</td>
<td>1.8</td>
<td>0.42</td>
<td>0.76</td>
<td>Negative</td>
</tr>
</tbody>
</table>

* Data was multiplied by 2 to reflect that only one-half of the total injected dose could target the lymph nodes.
* Received s.c. injection of $^{111}$In-T101.

Table 4 Mean percentage of organ retention of radioactivity after intralymphatic injection

Data from anterior whole body scans 24–96 h after radiolabeled antibody administration. Corrected for isotope decay and expressed as percentage of total injected dose.

<table>
<thead>
<tr>
<th>Lymph nodes</th>
<th>T101 (N=5)</th>
<th>9.2.27 (N=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31 (23-48)*</td>
<td>19</td>
</tr>
<tr>
<td>Lower extremities</td>
<td>32 (14-47)</td>
<td>45</td>
</tr>
<tr>
<td>Liver</td>
<td>18 (8-38)</td>
<td>16</td>
</tr>
<tr>
<td>Spleen</td>
<td>7 (3-16)</td>
<td>19</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, range.

Fig. 3 Intralymphatic control antibody versus s.c. T101 48 h after injection. Patient 7 underwent an initial intralymphatic injection of $^{111}$In-9.2.27 control anti-melanoma antibody (top). Twelve days later the same patient was given an equal dose of $^{111}$In-T101 s.c. (bottom). The $^{111}$In-T101 shows greater and more discrete localization in the inguinal-femoral lymph nodes than does the $^{111}$In-9.2.27 (at comparable early time points). In addition the T101 images show a higher concentration in the involved skin tumors of the left breast (closed arrows) and localization in the left axillary node (arrowhead). Radioactivity in the lymphatics persists following intralymphatic injection of 9.2.27 but not of T101 (open arrows). Both preparations show uptake of radioactivity in the liver and spleen.

Fig. 4. Comparison of intralymphatic and s.c. delivery. Top, patient 5 received concurrent injections of $^{111}$In-T101 via the intralymphatic route in the left foot (open arrows) and the s.c. route in the right foot. Images obtained approximately 2 h postinfusion show faster cross-over delivery to right inguinal-femoral nodes from the left intralymphatic injection (closed arrows). Bottom, images obtained at 72 h demonstrate that the right inguinal-femoral nodes (closed arrows) have a higher concentration than the left (open arrows), indicating that s.c. delivery, although slower, was also capable of efficient delivery. In addition, the delayed views show targeting to distal lymphatic sites including axillary, hilar, supraclavicular, and cervical nodes (closed arrowheads).

Fig. 5. Blood kinetics of $^{111}$In-T101. The kinetics of $^{111}$In-T101 in the blood is plotted following intralymphatic (ILAG) administration. For the sake of comparison blood clearance data from a previous study of s.c. $^{111}$In-T101 was included (18). Points, means; bars, ±1 SD.

The maximum dose in the blood pool peaked at 12% (range, 2.5–12%) with a median of 24 h (3–96 h). As shown in Fig. 5, in 6 of the patients blood levels of $^{111}$In-T101 after 5 h were always <6% of injected dose. At 1 h 24–54% of the radioactivity recovered in blood was bound to circulating cells and not free in the plasma. The analysis of liver-spleen uptake demonstrates that radiolabeled antibody gains access to the systemic circulation even at the earliest imaging times, with persistence until 96 h. Access of the radioconjugate to the liver is presumably through systemic delivery. Urinary excretion of radioactivity was <7% of the injected dose/24 h, with a cumulative urinary excretion of 12–32% of the injected activity by 72 h.
INTERTYLMONIC INJECTION OF $^{111}$In-T101

Table 5 Sites of increased radioactivity on immunoscintigraphic imaging with intralymphatically administered $^{111}$In-T101

<table>
<thead>
<tr>
<th>Regional lymph nodes</th>
<th>Patient</th>
<th>Cervical*</th>
<th>Supraclavicular*</th>
<th>Axillary*</th>
<th>Hilar*</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Thyroid*</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Skin*</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>Skin, breast, hand*</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td></td>
</tr>
</tbody>
</table>

*No tissue evaluation of these sites was performed.

† After thyroid uptake was noted on scan, although clinically euthyroid, the patient was evaluated. Thyroid-stimulating hormone was evaluated, and needle aspiration revealed lymphocytic thyroiditis.

‡ Grossly involved with mycosis at site of scan positivity.

§ Grossly involved with mycosis in the locations of scan positivity.

Table 6 Human anti-mouse antibody response after intralymphatic infusion of $^{111}$In-T101 for seven patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>First positive assay</th>
<th>Highest assay titer</th>
<th>Mouse immunoglobulin-specific immune complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37 (40)*</td>
<td>37 (40)</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>3.4 (2)</td>
<td>33 (23)</td>
<td>98</td>
</tr>
<tr>
<td>3</td>
<td>Negative*</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Negative</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6.5 (6)</td>
<td>11.2 (10)</td>
<td>100</td>
</tr>
<tr>
<td>7*</td>
<td>8.3 (2)</td>
<td>8.3 (2)</td>
<td>100</td>
</tr>
</tbody>
</table>

* Titer (weeks).

† Patients with negative human anti-mouse antibody titers were followed at least 3 months (range, 13–49 weeks) after they received the mouse monoclonal antibody and those patients had an average of four negative human anti-mouse antibody assays during that time.

As summarized in Table 5, there was a variety of other systemic sites with discrete radioactive uptake. Additional nodal sites were identified in all patients, presumably by overflow from the lymphatics into the blood stream and/or by recirculation of labeled cells. In three patients, nonnodal tissue showed intense radioactivity outside of the regional drainage of the lower extremity. In two patients these nonnodal areas were grossly infiltrated with lymphoma. The final patient had increased uptake in the thyroid, leading to further evaluation. This included the discovery of an elevated thyroid-stimulating hormone level and a needle aspirate read as lymphatic infiltration consistent with thyroiditis. The patient was clinically euthyroid. The correlation between increased radioactivity on scan and clinical evidence of soft tissue involvement with mycosis fungoides was excellent.

The human anti-mouse antibodies analysis revealed that 4 of 7 patients tested by the staphylococcal protein assay and 6 of those tested by high-performance liquid chromatography developed significant titers of human anti-mouse antibodies as early as 2 weeks after the imaging procedure (Table 6).

The injections were accomplished with minimal morbidity and no mortality. Moderate to severe pain was associated with intralymphatic infusion of monoclonal antibodies, but this resolved quickly when the rate of the infusion was decreased. The patients tolerated the infusions without allergic complications. The single exception was an apparent chemical lymphangitis of the lower leg in one patient. This resolved over the course of 12 h after administration of diphenhydramine. Lymphangitis is a recognized complication of routine lymphangiography, so it is not clear whether it was related to the antibody infusion. There were no local tissue reactions or infections in the feet of patients injected with antibody.

DISCUSSION

Seven patients were treated with $^{111}$In-radiolabeled T101 monoclonal antibody during the course of contrast lymphangiography. We observed efficient delivery of labeled antibody to regional lymph nodes. The quality of images with intralymphatic $^{111}$In-T101 was very similar to that reported for s.c. administration of this antibody. Both routes of administration resulted in rapid and efficient delivery to regional lymph nodes, but intralymphatic delivery resulted in a much more rapid clearance from the injection site. Injection s.c. showed approximately 80% of the dose at the injection site 4 h (18) after injection, whereas direct intralymphatic injection showed only an average of 3.8% of injected dose at the initial imaging time (<4 h). This faster clearance resulted in an average of 13 rads of absorbed dose in the foot, compared with the previously published range of 34–70 rads after s.c. administration of $^{111}$In-T101 (18). The concentrations measured in biopsied nodes were similar to those seen after s.c. administration of T101, and they were 10–100 times higher than those observed after i.v. administration of the radiolabeled antibody (6, 18). Injections s.c. showed approximately 80% of injected radioactivity in the injection site at 4 h (18), whereas intralymphatic infusion resulted in only 3.8% of the injected dose localized to the foot 4 h postinjection. Lymph node biopsy specimens with only mild dermatoxic changes contained lower mean percentages of injected dose than did cancerous lymph nodes. A clear resolution of this point is difficult as no definitive histological marker of a malignant T-cell exists.

A large number of lymph node biopsies in CTCL patients would be required to establish whether the reported increased expression of CD5 in malignant T-cells (compared with normal T-cells) was sufficiently pronounced to permit a diagnostic or therapeutic advantage.

In contrast to the experience with the s.c. administration of $^{111}$In-T101, no dose dependence of targeting was observed for the limited range of antibody concentrations tested. All three concentrations of T101 resulted in rapid delivery of radioactivity to systemic sites at the earliest imaging time point (18). This observation may be explained by the larger effective dose administered via the intralymphatic route, since less of the injected dose was retained at the injection site.

Comparison with the indium-111 isotypic control antibody 9.2.27 showed that mobilization of an irrelevant antibody out of the extremity is also rapid and efficient after direct intralymphatic injection. Localization of T101 in lymphatic tissue exceeded that of 9.2.27, indicating that at least a portion of the T101 targeting resulted from specific antigen binding. Quantitation of antigen-specific node uptake may be underestimated by comparing T101 with 9.2.27 since subsequent studies have shown a preferential concentration of 9.2.27 in lymph nodes when compared with that of a nonspecific immunoglobulin. Radioactivity accumulates in liver and spleen after injection of both $^{111}$In-T101 and $^{111}$In-9.2.27. This accumulation is related in part to the technique of $^{111}$In-diethylenetriamine pentaacetic acid conjugation (33) and in part to other, undefined factors (34).

This study did not include evaluation of the nature of reticuloendothelial uptake, so the radioactivity in the liver could be a free isotope, free radiolabeled antibody, radiolabeled antigen-antibody complexes, or radiolabeled antibody bound to T65-positive cells. However, in organs other than liver and spleen the differences in biodistribution of the two antibodies after...
reaching the systemic circulation [in both this and a previous study (18)] suggest that an element of T101 localization is due to antibody specificity.

This was a nonselected group of patients requiring lymphangiography as part of their initial staging for entry into a therapy protocol for CTCL. Even after a very extensive conventional staging workup, biopsies guided by 

111In-T101 led to upstaging of three of five patients. In addition to the biopsies-proven site, there were multiple other hot spots on the scans from this group of clinically early-stage patients. These T101 images may reflect very extensive CD5-positive cell trafficking as well as a significant systemic dissemination, even with early disease. If 

111In-T101 imaging helps define more accurately the full extent of disease, available treatments (especially potentially curative total body electron beam therapy) may be applied with a better therapeutic index to appropriately staged patients.

The use of 

111In-T101 as a diagnostic staging tool in T-cell lymphoma patients may be limited by the frequent incidence of human anti-mouse antibody formation. In this report of 5 of 7 patients developed serological evidence of an immune response to the mouse antibody. These findings are in the range reported for other trials with monoclonal antibodies (35, 36). A variety of prospects to decrease the frequency of human anti-mouse antibody response exist, including improved formulation of the antibody to exclude antibody aggregates, techniques to increase tolerance of the host to the mouse protein including the use of immunosuppressive agents, and development of “humanized” or human-derived antibodies.

We are currently conducting a clinical trial to evaluate the therapeutic benefit of 

99mTc-T101 conjugates of T101, given via either the i.v. or intralymphatic route, to patients with cutaneous T-cell lymphoma. Delivery of tumoricidal radioactivity to regional lymph nodes in selected cases may be useful adjunct to systemic therapy, especially if tumor cell trafficking through the lymphatic compartment is a major part of this disease process. The critical organ limiting the highest dose of radiolabeled monoclonal antibody would be dependent on the route of administration. Bone marrow stem cell toxicity, as with the 

131I-T101 experience (11), presumably will limit systemic therapy. This myelotoxicity may occur at doses that do not have tumoricidal activity in nodal sites of involvement. Endothelial cells and other normal cell populations in the lymphatic system may limit the dose for direct intralymphatic injection, but injection site toxicity would more severely restrict the maximal dose that could be delivered via s.c. injection. The comparison of lower extremity clearance between s.c. and direct intralymphatic injection favors the latter as the preferred route for delivery of a therapeutic dose of radiolabeled antibody. The therapeutic index for such an infusion could be further improved with a more precisely calibrated pressure-sensitive pump to maintain lower pressure during antibody infusion to minimize extravasation from the lymphatic channels. Retention of labeled antibody in lymphatic channels might perhaps be further diminished if cold antibody were infused prior to labeled antibody. Information from the ongoing Phase I 99mTc-T101 trial may resolve some of these issues.

One additionally speculative possibility deserves brief mention. Since T101 is directed at a pan T-cell antigen present in normal nodes, it may be possible to deliver larger doses of radiation selectively via the lymphatics to treat other, non-T-cell malignancies which localize in regional lymph nodes. One can envision settings in which diseases which extend to the regional nodes (e.g., with early-stage melanoma of the extremities, testicular cancer, or breast cancer) might be better candidates for such an approach than cutaneous T-cell lymphoma.

ACKNOWLEDGEMENTS

We would like to thank Drs. D. Ihe, B. Kramer, and A. Treest for their thoughtful review of the manuscript, and Marilyn Fourcacut and Gail Gray for their expert help in preparing the manuscript.

REFERENCES


Direct Intralymphatic Injection of Radiolabeled $^{111}$In-T101 in Patients with Cutaneous T-Cell Lymphoma

James L. Mulshine, Jorge A. Carrasquillo, John N. Weinstein, et al.


Updated version  Access the most recent version of this article at: [http://cancerres.aacrjournals.org/content/51/2/688](http://cancerres.aacrjournals.org/content/51/2/688)

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.