Phase I Study of the Pharmacokinetics of a Radioimmunoconjugate, $^{90}$Y-T101, in Patients with CD5-expressing Leukemia and Lymphoma

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ABSTRACT

Ten patients with advanced or refractory CD5-expressing hematologic neoplasms [two with chronic lymphocytic leukemia and eight with cutaneous T-cell lymphoma (CTCL)] were treated in a Phase I study with the radioimmunoconjugate $^{90}$Y-T101, which targets CD5+ lymphocytes. Prior imaging studies using $^{111}$In-T101 demonstrated uptake in involved lymph nodes and skin in patients with CTCL, and Phase I studies with unmodified T101 demonstrated transient responses. In this study, patients were treated with 5 or 10 mCi of $^{90}$Y chelated to T101 via isothiocyanatobenzyl diethylenetriamine pentaacetic acid, along with tracer doses of $^{111}$In-T101 for imaging. The biodistribution of the radioimmunoconjugate was determined by measuring $^{90}$Y and $^{111}$In in blood clearance, urine excretion, and accumulation in bone marrow and in involved skin lesions. The intravascular pharmacokinetics of $^{90}$Y were predicted by $^{111}$In-labeled T101. The greatest differences in biodistribution between $^{111}$In and $^{90}$Y were in the higher bone accumulation of $^{90}$Y and its lower urinary excretion. Imaging studies demonstrated targeting of skin lesions and involved lymph nodes in CTCL patients. The predominant toxicity was bone marrow suppression. Rapid antigenic modulation of CD5 on circulating T and B cells was observed. Recovery of T-cell populations occurred within 2–3 weeks; however, suppression of B-cell populations persisted after 5+ weeks. All CTCL patients developed human antimouse antibody after one cycle and thus were not retreated; one patient with chronic lymphocytic leukemia received a second cycle of therapy. Partial responses occurred in five patients, two with chronic lymphocytic leukemia and three with CTCL. The median response duration was 23 weeks. One CTCL patient who subsequently received electron beam irradiation to a residual lesion is disease-free after 6 years.

INTRODUCTION

T101 is a monoclonal belonging to the IgG2a subclass, which recognizes CD5, a surface antigen present on circulating normal T lymphocytes, a subset of B cells, as well as on nonsecretory CLL3 cells and CTCL cells (1). T101 recognizes the same antigen as the monoclonal antibodies anti-Leu-1 and OKT1. Previous imaging studies using $^{111}$In-T101 and $^{131}$I-T101 have demonstrated uptake of the antibody radioconjugates in malignant skin and lymph node infiltrates in CTCL patients and binding to and uptake by circulating CD5-positive neoplastic lymphocytes in CLL patients (2–4). Early Phase I studies using unmodified T101 at doses of 1–140 mg by slow i.v. infusion demonstrated rapid but transient responses in patients with CLL and CTCL with minimal toxicity (5).

The observation of clinical improvement in patients treated with unmodified antibody together with the excellent localization of $^{111}$In-T101 in sites of disease has prompted further clinical investigation of radioimmunoconjugates of T101. Rosen et al. (3) demonstrated clinical responses in CTCL patients treated with $^{131}$I-T101 in therapeutic doses of 100–150 mCi; however, the specificity of the therapeutic response was in question due to associated rapid dehalogenation of the radioconjugate (6).

We have conducted a Phase I trial using the radioimmunoconjugate $^{90}$Y-T101 in patients with CD5-expressing CTCL and CLL. $^{90}$Y is a high energy β-emitter without any associated γ emission on decay. It was anticipated that $^{90}$Y would behave similarly to $^{111}$In in remaining intracellular once internalized as part of an antigen-antibody complex. The high antigen density...
in circulating neoplastic cells rapidly decreases free isotope blood levels and minimizes nonspecific toxicity secondary to bone accumulation (7). Tracer doses of \(^{111}\text{In}-\text{TlOl}\) were coadministered for localization and biodistribution studies, and the uptake of \(^{111}\text{In}\) and \(^{90}\text{Y}\) was measured in biopsies of bone marrow and clinically involved skin to validate that \(^{111}\text{In}-\text{TlOl}\) distribution also reflects \(^{90}\text{Y}-\text{TlOl}\) distribution. Significant disease regression was documented in 5 of 10 patients treated at very low dose levels. We performed an extensive pharmacokinetic analysis of both \(^{111}\text{In}\)- and \(^{90}\text{Y}-\text{TlOl}\) and also used imaging results to evaluate the \(^{111}\text{In}/^{90}\text{Y}\) biodistribution. Although other radioimmunotherapy studies have been performed using \(^{90}\text{Y}\) and \(^{111}\text{In}\) for imaging, this study represents the most detailed pharmacokinetic analyses including biopsies to determine the similarities in biodistribution between \(^{111}\text{In}\) and \(^{90}\text{Y}\).

**MATERIALS AND METHODS**

**Patients.** Adults with CTCL or CLL who had failed prior therapy and whose tumor cells expressed CD5 were eligible. CD5 expression was determined on circulating neoplastic cells by flow cytometry or on skin biopsies by immunohistochemical analysis using T1Ol and Leu-1 and peroxidase-labeled antiamouse IgG. Patients were eligible if 25% or more of the tumor cells stained for T1Ol. All patients had bilateral iliac crest bone marrow biopsies to assess tumor involvement and adequacy of bone marrow reserve, pulmonary function testing with a forced expiratory volume at 1 s and vital capacity >65% of predicted value and pO\(_2\) >65%, no cardiovascular disease, or if cardiac history was present, a gated cardiac blood pool ejection fraction of >35%. Hematologic parameters included WBC > 2000/mm\(^3\), platelets > 100,000/mm\(^3\), bilirubin < 1.5 mg/dl, and creatinine < 2 mg/dl. Patients were required to have a Karnofsky performance status of at least 60%, measurable disease, a life expectancy of at least 3 months, and no HAMAs as determined by an HPLC method (8). The protocol was approved by the National Cancer Institute Institutional Review Board, and all patients gave their written informed consent to participate. A total of 10 patients were enrolled; 9 patients were treated once and 1 patient received two courses of therapy. Patients were accrued from July 1989 to October 1991, and the last follow-up date was April 1998. The trial was discontinued before maximum tolerated dose was reached due to unavailability of the antibody.

**Antibody Preparation.** T1Ol was provided by Hybritech Incorporated (La Jolla, CA) and was prepared from the ascites of BALB/c mice injected with the hybridoma. The antibody was purified by 18% sodium sulfate precipitation and subsequent fractionation on a DEAE-Sepharose column (Pharmacia Fine Chemicals, Piscataway, NJ). The purified antibody was conjugated to the chelating agent, isothiocyanatobenzyl-DTPA (9). The antibody was labeled with \(^{90}\text{Y}\), purified through a sizing column, shipped overnight on ice, and used within 24 h of preparation. The specific activity of the final product was 40 mCi/mg. Prior to patient injection, the material was checked for endotoxin by standard limulus lysate assay and protein-bound radioactivity by ITLC. In all cases, >97% (97.8 ± 0.6%, mean ± SD) of the \(^{90}\text{Y}\) was bound to T1Ol. The \(^{111}\text{In}\)-labeled T1Ol (\(^{111}\text{In}-\text{TlOl}\)) was prepared on site by incubating the chelate-conjugated T1Ol with buffered indium chloride. The reaction was quenched with excess DTPA to bind any ionic \(^{111}\text{In}\). This resulted in >75% (87.8 ± 8.4%) protein-bound \(^{111}\text{In}\), and no further purification was performed. The preparation for the last patient consisted of the same conjugated T1Ol but differed from the others in that both \(^{111}\text{In}\) and \(^{90}\text{Y}\) labeling were performed on site, followed by purification through a size exclusion HPLC.

**Immunoreactivity.** The immunoreactivity of the \(^{111}\text{In}-\text{TlOl}\) and \(^{90}\text{Y}-\text{TlOl}\) was determined by a cell-binding assay. Serial dilutions of the antibody were incubated with a CD5-positive T-cell line, and the immunoreactivity was expressed as the maximum percentage of radioactivity bound to the cells at antigen excess (10). The \(^{90}\text{Y}-\text{TlOl}\) was 63 ± 10% immunoreactive and the \(^{111}\text{In}-\text{TlOl}\) was 58 ± 12% immunoreactive when corrected for the protein-bound fraction determined from ITLC.

**Antibody Administration.** All patients received co-injections of a mixture of \(^{111}\text{In}-\text{TlOl}\) and \(^{90}\text{Y}-\text{TlOl}\) and unlabeled (DTPA-conjugated) T1Ol to achieve the total protein mass desired. Each patient received approximately 1.0 mg of T1Ol labeled with a mean of 5 mCi of \(^{111}\text{In}\) (4.95 ± 0.17 mCi). The first three patients received 0.5 mg of T1Ol with 10 mCi of \(^{90}\text{Y}\) formulated to a total dose of 2 mg with unlabeled T1Ol. Because grade 4 bone marrow toxicity was observed in one patient, the next six patients received 5 mCi of \(^{90}\text{Y}-\text{TlOl}\) with the total dose of T1Ol increased to 10 mg. The 10-mg dose was selected because prior studies suggested lower bone marrow concentration when 1 mg versus 10 or 50 mg were utilized (11).

**HAMA.** A size exclusion HPLC-based assay was used to detect evidence of a human immune response to T1Ol. Iodinated T1Ol was incubated with patient serum and then assayed on an HPLC equipped with a TSK 2000 column. Immune complexes were separated from intact IgG, and quantitation was done by integrating the free and complexed peaks. When complexes were detected, specificity was determined by competition assays with unlabeled T1Ol or an excess of unlabeled IgG (8).

**Flow Cytometric Analysis.** Flow cytometry was performed using a whole blood lysis technique, which included two prewashes in PBS to eliminate any nonbound monoclonal antibody in the plasma. The cells were then resuspended to the original blood volume, and 100 ul of cell suspension was incubated with the specific antibody or antibodies for 30 min at 4°C. The incubation mix was treated with FACS Lyse (Becton Dickinson, San Jose, CA) according to manufacturer’s instructions, washed twice in PBS, and fixed in 0.1% paraformaldehyde. A minimum of 10,000 events was acquired on each sample using a FACScan (Becton Dickinson) equipped with an air-cooled laser. The photomultiplier tubes for side angle scatter, FL1 (515 nm), and FL2 (585 nm) were operated using logarithmic amplification. Instrument calibration was performed daily using Calibrite beads (Becton Dickinson) according to manufacturer’s instructions, and the data were analyzed using a Consort 32 computer system using Lysis II software. The cells were evaluated for CD5 expression using unconjugated T1Ol followed by a wash step and incubation with goat antiamouse FITC (Caltag, Burlingame, CA). Cells incubated with an unconjugated plasmacytoma of the same subclass served as the control for the T1Ol. The directly conjugated monoclonal antibodies were obtained from Becton Dickinson and included CD3 for T cells,
CD4 and CD8 for T-cell subsets, CD20 for B cells, and CD45/CD14 as gating reagents. The cells were collected in a modified lymphocyte gate, and additional gating was done on the basis of forward and side angle scatter. This was confirmed using the combination of CD45 and CD14 monoclonal antibodies. The percentage of positive cells was determined using the subclass control for positive-negative discrimination. The number of cells staining for each marker was determined using the total lymphocyte count determined with a hematology instrument and the percentage of cells staining positive by flow cytometry. The T101 staining pattern was followed by monitoring the geometric mean channel fluorescence (FL1). Studies were planned to be performed prior to therapy, 1, 2, 3, 4, and 5 days, and 1, 2, 3, and 5 weeks posttherapy.

**Imaging.** Analog scintillation camera images as well as digital images were recorded with a General Electric 500 gamma camera. Anterior and posterior whole body images and four spot views of the anterior and posterior chest and abdomen were acquired with a medium energy collimator using a 20% window centered around the 173-keV and 247-keV X-ray peaks of $^{111}$In. Typical spot views contained 300,000 to 1,000,000 counts, whereas the whole body views were acquired over 1060/s/side.

**Pharmacokinetics.** In addition to gamma scans, blood samples were taken following the infusion, typically at the end of infusion, 30 and 60 min, and 2, 6, and 12 h after infusion, and then daily for 1 week. The retention of $^{90}$Y and $^{111}$In in blood and plasma were determined by sampling both blood and plasma, with sequential counting in a $\gamma$ and then in a beta counter. Because Cerenkov counting is sensitive to quench and geometry, all samples were processed in a similar and reproducible manner. Samples of 0.1 ml of blood, plasma, and urine were first treated with 0.5 ml of SDS at 56°C for 15 min, followed by cooling for 10 min at 5°C. The samples were then bleached with 0.4 ml of 30% hydrogen peroxide. The counts in the samples were referred back to a standard of the injected dose. To mimic the quench observed in the patient samples, separate standards were prepared by mixing with 0.1 ml of the patient’s baseline blood or serum. These standards underwent the same processing and resulted in quench similar to the patient samples. All samples were then brought up to 11 ml with distilled water. Beta counting of Cerenkov radiation was performed using a 0–200 keV range (A4530D; Packard, Downes Grove, IL). The samples were also counted in a gamma counter using a 100–500 keV window. The counts were corrected for cross-talk and decay-corrected accounting for the time differences between counting in the gamma and beta counters.

This counting technique yielded a crossover of $^{111}$In into the $^{90}$Y window of <1% and a crossover of $^{90}$Y into the $^{111}$In window of <5%. The percentage of injected activity retained in blood and plasma volume was then estimated using the patient’s height and weight to estimate the volumes. Because the infusion time was short compared with the disposition half-life ($T_{1/2}$), the intravascular data were treated similarly to an intravenous bolus. The percentage of the injected dose per ml was fitted to a biexponential curve to obtain both the alpha and beta phase $T_{1/2}$, using a least squares fit algorithm (SigmaPlot; Jandel Scientific, Duarte, CA). Conventional pharmacokinetic parameters were then derived (12). The AUCs for the blood or plasma curves were calculated in two steps. First the AUC from the end of infusion to 168 h was obtained by trapezoidal integration of the decay-corrected blood and plasma data, and then the terminal AUC was estimated using the terminal clearance rate to extrapolate from the activity retained at 168 h. These data were expressed on %ID × h/ml to allow comparison between doses. Using these data we then estimated additional pharmacokinetic parameters including $V_{ss}$, $V_{ies}$, and maximum retained dose (12). Whole-body retention of $^{111}$In was determined by counting the patient immediately after the end of the infusion and daily using a sodium iodide counter. Urine was collected serially for a 96-h period.

Bone marrow biopsies were taken at 7 days after infusion in eight patients and on days 6 and 8 in one patient each. Individual core biopsies were obtained from each iliac crest. Both samples were weighed on an analytical balance, placed in PBS, and counted in both the gamma and beta counters to allow an estimate of sampling-site variability of radioactivity concentration. One core was then sent for routine pathology, and the other was further processed in 8 of the 10 patients. The biopsy core to be processed was put in a conical tube with 10 ml of PBS for 1 h, and the core was broken with a jagged-edge glass rod. The core was centrifuged for 10 min at 640 × g, and the supernatant was removed and counted (saline fraction). The pelleted core was broken with a jagged-edge glass rod and then mixed with 0.5 ml of 10% SDS for 30 min at 56°C in an attempt to remove any cell-bound activity. After the sample cooled, 0.4 ml of 30% hydrogen peroxide was added and incubated at 56°C for 1 h to bleach the sample. Ten ml of distilled water were added, and the sample was again centrifuged for 10 min. The supernatant was separated for counting (SDS fraction). Perchloric acid (0.2 ml) was then added to the remaining bone chips and incubated at 56°C until the chips dissolved. This sample was then treated with hydrogen peroxide, as described above. After cooling, the sample was transferred to a counting vial with 10 ml of distilled water (bone fraction). All samples were then counted on both the beta and gamma counters, as described above, and the %ID/g was determined on the basis of the protein-bound injected activity (i.e., corrected for ITLC).

**Response and Toxicity.** Responses were assessed at the end of each cycle of therapy and monthly thereafter. A CR was defined as the complete resolution of all clinically evident disease confirmed pathologically by appropriate biopsies and lasting at least 1 month. A PR was at least a 50% reduction in the sum of all measurable and evaluable disease lasting at least 1 month. Stable disease was a minor improvement in disease insufficient to meet the criteria for PR. Progressive disease was defined as a 25% or greater increase in the sum of measurable and evaluable lesions or the development of new extracutaneous lesions. Progression-free survival was calculated on the patients who achieved a PR or CR from the first day of therapy until progressive disease was documented.

Toxicity was scored according to the National Cancer Institute’s Common Toxicity Criteria (13). Complete blood counts were obtained immediately after the infusion, at 1, 2, 6, and 12 h after the infusion, and weekly thereafter until recovery from nadir.

**Statistics.** When the differences between $^{111}$In and $^{90}$Y were compared, paired $t$ tests were performed.
RESULTS

Disease Characteristics of Patients. Ten patients were entered onto the study, one with B-CLL, one with T-CLL, and eight with CTCL. Patient characteristics are shown in Table 1. All patients had refractory disease. Both CLL patients and seven of eight patients with CTCL had failed cytotoxic chemotherapy regimens. Bone marrow involvement was noted in one patient with CLL and one with CTCL. Circulating neoplastic cells were present in the B-CLL patient (50,000/ul), in the T-CLL patient (46,000/ul), and in two of the CTCL patients, one with <2000 Sezary cells/ul and one with >4000/ul.

Biodistribution Data. Serial gamma imaging obtained after antibody administration demonstrated rapid clearance of $^{111}$In-T101 from the blood (Fig. 1) and prominent uptake into the liver, spleen and bone marrow (Fig. 2A). Imaging of known nodal-involved sites was not seen in the initial 2-h images but was consistently present at 24 h in patients with CTCL. Not all lymph node regions were visualized, suggesting that there may have been some selectivity for involved nodes. There was no nodal uptake in the patient with B-CLL. The patient with T-CLL had some lymph node visualization in the early images (2 h) but much better visualization at 24 h (Fig. 2B). Uptake in clinically involved skin sites was noted in six of the eight patients with CTCL (Fig. 3A). One of the two patients without skin uptake had very minimal patch-stage skin disease, and the other had nodal involvement but no visible skin disease at the time of therapy. The patient with circulating Sezary cells had diffuse infiltrative erythroderma, and imaging studies showed diffuse
Fig. 2 A. Whole body images of patient 6 obtained the next day after infusion showed excellent localization in clinically involved nodal sites as well as prominent skin uptake. B, spot images from patient 1, who had T-CLL, were obtained near the end of infusion and at \sim 24 \text{ h} \text{ afterwards}. The images show prominent uptake in multiple nodal sites in a markedly enlarged spleen and in liver and bone marrow. No skin uptake is noted. Although lymph node uptake is present in the early images, it was more prominent in the delayed images. C, spot images from patient 5, who had B-CLL and received 5 mCi of $^{111}$In-T101 and a total of 2 mg. The images show increased tracer uptake in an enlarged spleen, liver, and in the bone marrow. No accumulation is noted in nodes in the axilla, and the inguinal area that were palpable on physical exam.

Skin uptake. Extensive bone marrow uptake was noted in both the B-CLL and the CTCL patients, with histopathologic evidence of bone marrow involvement as well as in two other CTCL patients with histopathologically uninvolved marrows. In addition to uptake in nodal sites and bone marrow, uptake in liver and spleen was consistently observed, and mild uptake in bowel was seen in some patients receiving the 10-mg dose (Fig. 2, B and C).

Two $^{90}$Y dose levels (5 or 10 mCi) were administered under this protocol along with either 2 or 10 mg of unconjugated T101 antibody (Table 2). The first three patients were dosed with 10 mCi of $^{90}$Y and 2 mg of T101. Significant bone marrow toxicity was noted in patient 3; therefore, the $^{90}$Y dose was decreased to 5 mCi for the next six patients and the dose of cold T101 was increased to 10 mg in patients with CTCL. The last CTCL patient received 10 mCi of $^{90}$Y-T101 with 10 mg of T101.

A skin biopsy was performed in seven patients with CTCL (Table 3) at 5 to 8 days postinfusion (mean, 6.7 days). The concentration of $^{111}$In, corrected for the protein-bound fraction,
ranged from 0.00066 to 0.0032 %ID/g, and for \(^{90}\)Y, the concentration from 0.00081 to 0.0039 %ID/g. This represented a mean of 1.5 ± 0.5-fold higher \(^{90}\)Y uptake than \(^{111}\)In uptake (\(P = 0.012\)). No visible skin uptake was seen in patients with CLL (Fig. 2, B and C). There was no correlation between total dose of antibody administered or \(^{90}\)Y-T101 dose and skin uptake in the CTCL patients.

The localization of radioactivity in bone marrow was measured in all patients. The %ID/g in the unprocessed bone marrow ranged from 0.0008 to 0.0136 %ID/g (0.0039 ± 0.0035 %ID/g, mean ± SD) for \(^{111}\)In and from 0.00109 to 0.0115 %ID/g (0.005542 ± 0.00488 %ID/g, mean ± SD) for \(^{90}\)Y. The activity of \(^{111}\)In recovered in the treated bone was 98 ± 5% of that of the nonprocessed bone marrow, indicating that very little loss occurs as the result of processing. The true amount of \(^{90}\)Y in the unprocessed marrow was underestimated due to geometry of the sample and quench. The recovery of \(^{90}\)Y from treated bone was 133 ± 17% of that of the unprocessed bone marrow and was significantly higher (\(P = 0.0027\)), indicating a greater efficiency of counting for \(^{90}\)Y when the bone marrow was processed. The accumulation of \(^{111}\)In in the processed bone was always lower than that of \(^{90}\)Y (Fig. 3) with 0.0046 ± 0.0045 %ID/g versus 0.00758 ± 0.00432 %ID/g, respectively (\(P = 0.0078\)). Overall there was 1.97 ± 0.75-fold more \(^{90}\)Y than \(^{111}\)In in the bone. Although the number of biopsies was small, there was no correlation between histopathologic evidence of bone marrow involvement by tumor and bone marrow radioisotope uptake. The distribution of activity in the saline fraction represented mainly plasma, that in the SDS fraction was mainly in the cells, and that in the perchloric acid represented bone-bound activity. A comparison of the uptake between the nonprocessed bone marrow from the right and left iliac crest showed a difference of 64 ± 20% from the highest to the lowest side for \(^{111}\)In and 69 ± 23% for \(^{90}\)Y, indicating variability due to sampling.

The blood and plasma clearance varied from patient to patient (Fig. 1, and Table 4). Nevertheless, the differences between \(^{111}\)In and \(^{90}\)Y retention in blood and plasma were very small, and overall there were no statistical differences between the pharmacokinetic parameters obtained from \(^{111}\)In versus \(^{90}\)Y in blood or in plasma (\(P > 0.05\), paired \(t\) test). A comparison of the \(^{111}\)In versus \(^{90}\)Y radioactivity remaining in the blood at the end of infusion showed no statistical differences, with 66.7 ± 27.8 %ID/g for \(^{111}\)In-T101 and 68.7 ± 26.7 %ID/g for \(^{90}\)Y-T101 remaining in the blood (\(P = 0.356\), paired \(t\) test). Similarly, the amount of \(^{111}\)In and \(^{90}\)Y remaining in the blood at 24 h was 24.3 ± 15.8 %ID/g for \(^{111}\)In-T101 and 25.6 ± 14.6 %ID/g for \(^{90}\)Y-T101 (\(P = 0.488\), paired \(t\) test). In addition, the corresponding radioactivity remaining in the plasma at the end of infusion was 65.4 ± 36.8 %ID/g for \(^{111}\)In-T101 and 68.5 ± 36.9 %ID/g for \(^{90}\)Y-T101 (\(P = 0.118\), paired \(t\) test). A larger amount of \(^{111}\)In than \(^{90}\)Y was excreted in the urine at all time points examined (Table 5).

Antigenic Modulation on Peripheral Lymphocyte Populations. Flow cytometric analysis demonstrated a rapid drop in T and B cells within 24 h after T101 therapy was initiated (Table 6). The mean drop in CD3+ cells was 62.1%. In five of seven patients, this persisted for 3 weeks. The CD5 fluorescence intensity was less in the individual remaining T cells, suggesting antigenic modulation related to antibody exposure. A decrease in B cells was also observed, with a mean drop of 77.5% at 24 h. However, the number of circulating B cells declined slightly more during the 2–3 weeks posttherapy, reaching a mean decrease of 83.4% at 3 weeks posttherapy. The depression in B cells persisted at 5 weeks posttherapy in all patients studied (seven of seven).

The expression of T101 in all lymphocyte populations was altered in all patients with cutaneous lymphoma but not in the one patient with T-CLL. The alteration consisted of a decrease in mean fluorescence of 90.6% in the seven patients studied. The mean channel of all patients prior to therapy ranged from 406 to 699, whereas 24 h after therapy the range was 21–74. Conversely, the mean CD3+ channel fluorescence on all lymphocytes from the patient with T-CLL increased from 620 to 1148 after therapy, and this increase persisted for 5 weeks. Because pharmacokinetic studies showed rapid elimination of radioisotope at short time points, it may be that the large number of circulating CD5+ tumor cells adsorbed most of the infused antibody, thereby diminishing the effect of antigenic modulation on the normal lymphocytes.

**Toxicity.** Grade 3 or 4 hematologic toxicity occurred in two patients with CTCL and one with CLL, as shown in Table 2. One of these was a CTCL patient with extensive tumor stage disease, heavily pretreated with combination chemotherapy, who received the initial dosing of 10 mCi and developed grade 4 thrombocytopenia, neutropenia, and sepsis on day 34 after therapy. The blood counts did not recover until day 54. Although the patient’s day 7 bone marrow demonstrated significant \(^{90}\)Y uptake, the prolonged bone marrow suppression was likely secondary to poor marrow reserve from prior chemotherapy as well as from accumulation of \(^{90}\)Y. Another CTCL patient (patient 9) who had been heavily pretreated with cytotoxic...
after one cycle of therapy, including both CLL patients and PR able for response and toxicity. Five of 10 patients (50%) had a decrease in pharmacokinetics. Re-treated with a second dose of antibody without any alterations in hematologic toxicity. The median time to nadir was 4.5 weeks of ⁹⁰Y and two of four dosed at 10 mCi experienced grade 3 or 5 cytopeia consistent with CLL. Three of six patients dosed at 5 mCi of ⁹⁰Y developed grade 3 neutropenia on day 58 and recovered by day 60. Pretreatment bone marrow biopsy showed diffuse replacement by atypical lymphocytes consistent with CLL. Three of six patients dosed at 5 mCi of ⁹⁰Y developed grade 3 neutropenia on day 58 and recovered by day 60. Pretreatment bone marrow biopsy was notable for hypocellularity, with normal maturation of all lineages and no evidence of involvement by CTCL. Patient 8 with B-CLL who was also treated with 5 mCi of ⁹⁰Y developed grade 3 neutropenia on day 58 and recovered by day 60. Pretreatment bone marrow biopsy showed diffuse replacement by atypical lymphocytes consistent with CLL. Three of six patients dosed at 5 mCi of ⁹⁰Y and two of four dosed at 10 mCi experienced grade 3 or 4 hematologic toxicity. The median time to nadir was 4.5 weeks for all patients (range, 1–13 weeks), and median time to hematologic recovery from nadir was 2 weeks (range <1–4 weeks).

Four of five patients who received 10 mg of cold T101 experienced transient allergic-like manifestations, consisting of chest tightness in three, hives in two, hypotension in one, and chills and fever in one. None of the four patients who received 2 ng of cold T101 had a hypersensitivity-type reaction. All reactions were immediate and were controlled by slowing of the infusion and administration of benadryl.

**HAMA Production.** All CTCL patients produced HAMAs after the first course of therapy, precluding further administration of the antibody. In contrast, neither of the CLL patients produced HAMAs, and the patient with T-CLL was re-treated with a second dose of antibody without any alterations in pharmacokinetics.

**Clinical Response.** All 10 treated patients were evaluable for response and toxicity. Five of 10 patients (50%) had a PR after one cycle of therapy, including both CLL patients and 3 of 8 CTCL patients (Table 7). The B-CLL patient had a marked decrease in splenomegaly after therapy, and the T-CLL patient had a decrease in spleen size as well as disappearance of palpable nodes and a decrease in the number of circulating CLL cells. This patient was initially treated at a dose of 10 mCi of ⁹⁰Y but then received a second cycle of therapy at 5 mCi after it was determined that he did not have significant titers of HAMA; he continued to demonstrate clinical improvement after the second dose of antibody. Two of the CTCL responders had improvement in cutaneous plaques and tumors, and one of these who had circulating atypical lymphocytes (Sezary cells) had a decrease in the absolute number of circulating cells from 1400 to 574 by day 9. Another CTCL responder with minimal skin disease but significant adenopathy had a >50% decrease in the size of his nodes. One CTCL patient with diffuse patch and plaque disease had a minor improvement in skin disease, which did not meet the criteria for a PR, and the remaining four patients had no response. None of the other responders were retreated due to elevated HAMA titers. The median duration of response for the five partial responders was 23 weeks (range, 16–33 weeks). One of the CTCL patients (patient 4) had irradiation of a nonresponding tumor on his extremity after completing one cycle of therapy with ⁹⁰Y-T101 and has had no disease recurrence at a follow-up of 6 years.

**DISCUSSION**

Because our prior imaging trials using ¹³¹I-T101 had demonstrated excellent localization of the T101 radioimmunoconjugate to skin and involved lymph nodes in patients with CTCL, we sought to further explore the therapeutic utility of this antibody conjugated to a high energy emitting isotope, ⁹⁰Y, in patients with CD5-expressing hematopoietic malignancies. Because this was one of the first clinical uses of ⁹⁰Y immunoconjugates, we report the effect of this treatment not only on the tumor sites but also on the most vulnerable normal tissue compartment, the bone marrow. In this Phase I study of a small cohort of refractory CTCL and CLL patients, 5 of 10 demonstrated a meaningful clinical response to therapeutic doses of ⁹⁰Y-T101. Toxicities were modest, and the major dose-limiting toxicity was bone marrow suppression, although dose-limiting toxicity was not achieved. The response durations observed here compare favorably with those reported in other radioimmunoconjugate studies and other newer therapies in CTCL (13–18).

The clinical efficacy of radioimmunoconjugates depends on several factors, including the stability of the conjugation.
immunoreactivity of the monoclonal, the distribution of the antigen on the target cells, metabolic fate of the antigen-antibody-radioisotope complex and biodistribution of the radioimmunoconjugate to tumor sites. The design of \(^{90}\)Y-Tl01 has evolved from prior studies demonstrating the favorable characteristics of CD5 as a targeting ligand and the unique biological features of certain hematologic malignancies, which lend to the successful clinical implementation of this approach (1, 2, 11). Although CD5 antigen is expressed on normal lymphocytes, a significantly greater receptor density has been demonstrated on neoplastic cells, and high levels of expression are fairly uniform among patients with CTCL and CLL. Antigenic modulation or initially high clinical response rate.

Neither the total quantity of antibody infused nor the total \(^{90}\)Y dose administered appear to affect the concentration of radioactivity in biopsied skin or tumor, as we had observed previously with \(^{111}\)In-T101 (2). Furthermore, the biodistribution and tumor targeting seen with \(^{111}\)In-T101 in this study was comparable to that reported with \(^{111}\)In in our prior studies and was superior to that reported by Rosen et al. (3), who used \(^{131}\)I-Tl01 in CTCL patients. In that study, there was no clear delineation of skin lesions by gamma scintigraphy, but rather a diffuse uptake by liver, spleen, palpable and nonpalpable nodes thyroid gland, and the gastrointestinal tract. These imaging findings are consistent with dehalogenation of the \(^{131}\)I conjugate. In contrast, little release from tumor or catabolic sites was seen with the \(^{111}\)In conjugate used for imaging in the current study.

The clinical response rate observed in this study is similar to that reported by Rosen et al. (3) using \(^{131}\)I-T101. In that study, two of five evaluable patients had a PR, with response duration ranging from 3 to 12 weeks. Three of five patients were re-treated with a second dose of radiolabeled antibody after plasmapheresis was performed to remove HAMAs, and two demonstrated a second significant disease response. We report longer response durations, ranging from 16 to 33 weeks, with one patient in durable response for more than 6 years after irradiation of a residual lesion. Due to the small study size, it cannot be established whether the longer response durations observed here compared to the \(^{131}\)I immunon conjugate represent improved biodistribution and targeting of tumor lesions and disease sites or may be due to prolonged exposure to the \(^{90}\)Y-conjugated isotope due to trafficking of intracellularly bound isotope.

The two predominant toxicities observed in this study were...
TlOl therapy was expected because prior studies have demonstrated grade 3 thrombocytopenia in one of three patients receiving 10 mCi of 90Y-anti-Tac and grade 4 neutropenia in one of three at the 15 mCi dose. In our study, there was no correlation between the drop in WBC count and dose; however, there was a correlation between dose and percentage drop in platelet count ($P = 0.0065$). The differences in the degree of hematologic toxicity observed among patients may be related to differences in bone marrow accumulation of 90Y or to differences in baseline hematopoietic reserve among patients, perhaps related to prior chemotherapy.

The rapid decline in circulating T cells observed following TlOl therapy was expected because prior studies have demonstrated rapid lymphocyte clearance following in vivo administration of unconjugated T101 antibody. This appeared to involve both the CD4 and CD8 subpopulations, with relatively equal distribution (data not shown). However, the suppression of normal T cells lasted 2–3 weeks, compared to days in prior studies using naked antibody, suggesting a significant cytotoxic effect of the radioconjugate, perhaps in bone marrow.

The profound and persistent decrease in B cells using this antibody conjugate was unexpected. The small subset of circulating mature CD5+ B cells were likely targeted by the conjugate. Because most circulating mature B cells do not express CD5, the prolonged decrease in CD20 populations seen here may reflect targeting of a CD5+ normal bone marrow precursor compartment, either directly by the antibody or indirectly through the bone-seeking effects of the radionuclide.

The marked decrease in T101 fluorescent staining was consistent among the cutaneous lymphoma patients ($n = 6$) and parallels the decrease in overall T-cell populations. This suggests that the normal T cells that predominated in the former group were subject to significant antigenic modulation of CD5 in the presence of the antibody. In contrast, the leukemic cells from the T-CLL patient showed an increase in immunofluorescent intensity for CD5, suggesting that antigenic modulation did not occur in the tumor cells. These results are similar to those of Dillman et al. (5), who demonstrated rapid clearance of CLL cells with an anti-CD5 antibody but no antigenic modulation on the cells. However, the effects of the antibody on B cells was similar to that observed in the other patients.

Although our results showed significant differences in biodistribution between 111In and 90Y, these differences do not prevent us from obtaining useful information regarding 90Y when we used 111In as a surrogate. Our studies showed that no significant differences in clearance were seen in the circulation between the 111In- and 90Y-radiolabeled T101. This suggested that the differences in retention between both isotopes in the chelate of the circulating antibody were very small. Nevertheless, some differences were seen at the tissue level. We documented significant differences between the two isotopes in involved skin, with a mean of 1.58-fold greater retention of 90Y than 111In. This would suggest that, in tumors the 111In distribution image represents a lower limit of the accumulated activity. Although there is no reason to suspect that accumulation in nodal sites would be different than in skin, this was not directly examined because of the greater difficulties in obtaining nodal biopsies. In bone marrow, the target organ for toxicity, the accumulation of 90Y also was higher than that of 111In. Similar differences between 111In and 90Y distribution in bone have been observed with other chelates because the strength of association of the chelate for both isotopes is different (19). When the conjugated antibody is metabolized, the rate of release for the isotope may vary in different tissues. The free isotope may also have distinct biodistribution. The differences between 111In and 90Y in bone marrow were expected because 90Y is rapidly incorporated into bone matrix (20).

The 111In biodistribution studies for the CTCL and CLL patients showed some similarities in terms of high splenic, liver, and bone marrow uptake and frequent lymph node accumulation. The greatest difference was the lack of skin uptake in noninvolved CLL skin. Although comparisons between the CTCL and CLL patients need to be made cautiously in terms of the small study size, skin infiltration is thought to be a more characteristic feature. Another difference between the two populations was in the rate of antibody localization. As seen in one previous patient with T-CLL (unreported result) the targeting of involved lymph nodes occurred promptly (within 2 h), at a time when no accumulation was seen in lymph node areas of CTCL patients, suggesting a difference in T-cell trafficking between the two diseases. Further evaluation of this observation is warranted to determine the degree to which trafficking affects the biodistribution and therapeutic efficacy of these and other radioimmunoconjugates.

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