Phase I Trial study of $^{131}$I-labeled chimeric 81C6 monoclonal antibody for the treatment of patients with non-Hodgkin’s lymphoma


* Both authors contributed equally to the completion of this study

1Department of Medicine, Division of Oncology and Stem Cell Transplantation, 2Department of Pathology, Division of Hematopathology, 3Department of Pathology, Division of Immunohistochemistry, and 4Department of Radiology, Division of Nuclear Medicine, Duke University Medical Center, Durham, North Carolina.

Correspondence: David A. Rizzieri, MD
Associate Professor of Medicine
Box 3961
Duke University Medical Center
Durham, NC 27710
Telephone: (919) 668-1040
Fax: (919) 668-1091
E-mail: rizzi003@mc.duke.edu

Word Count Abstract: 200
Manuscript: 4,363

Supported in part by the Lisa M. Stafford Young Investigator Award (DAR), and by the National Institutes of Health grant no. CA11898, CA70164 and S10 RR15697.

Abbreviated Title: Phase I $^{131}$I-ch81C6 radioimmunotherapy of NHL
Keywords: Radioimmunotherapy; Iodine-131; Dosimetry; Non-Hodgkin’s lymphoma; monoclonal antibody 81C6
ABSTRACT

We report a phase I study of pharmacokinetics, dosimetry, toxicity and response of $^{131}$I anti-tenascin chimeric 81C6 for the treatment of lymphoma. Nine patients received a dosimetric dose of 370 MBq (10mCi). Three patients received an administered activity of 1480 MBq (40mCi) and 2 developed hematological toxicity requiring stem cell infusion. Six patients received an administered activity of 1110 MBq (30mCi) and 2 developed toxicity requiring stem cell infusion. The clearance of whole-body activity was mono-exponential with a mean [range] effective half-life of 110 [90 – 136] hours and a mean effective whole-body residence time of 159 [130 – 196] hours. There was rapid uptake within the viscera; however, tumor uptake was slower. Activity in normal viscera decreased proportional to the whole body; however, tumor sites presented a slow clearance ($T_{1/2}$: 86 - 191 hours). The mean [range] absorbed dose to whole-body was 67 [51 – 89] cGy whereas the dose to tumor sites was 963 [363 – 1517] cGy. Despite lack of a ‘blocking’ antibody, 1 of 9 patients attained a complete remission and 1 a partial remission. These data demonstrate this radiopharmaceutical to be an encouraging agent for the treatment of lymphoma particularly if methods to protect the normal viscera are developed.
INTRODUCTION

Non-Hodgkin’s lymphomas (NHL) represent an array of lymphoproliferative disorders with indolent, as well as rapidly progressive, subtypes. While many patients with NHL respond to therapy initially, most relapse and often die with progressive disease within 10 years of diagnosis. New radio-immunotherapy modalities have been developed that target the cellular differentiation antigens CD20 and CD22 using naked or radiolabeled antibodies. Two anti-CD20 radiolabeled murine monoclonal antibodies have been approved in the United States for the treatment of relapsed follicular or transformed lymphoma (Zevalin, i.e. ⁹⁰Y-ibritumomab tiuxetan, IDEC-Y2B8, IDEC Pharmaceuticals, San Diego, CA; and Bexxar, ¹³¹I-tositumomab, Corixa Corp., Seattle, WA). Kaminski et al. summarized their experience with non-myeloablative activities of ¹³¹I-tositumomab where patients received a targeted whole body dose of 75 cGy. In these studies, the response rate was 71% with 31% achieving CR. Moreover, the response rate in low-grade or transformed NHL was 83% in contrast to 41% in intermediate-grade NHL. These studies reveal that targeting lymphoma with radiolabeled antibodies is efficacious, though there is significant room for improvement in complete remission rates and duration of responses, as well as expanding the types of lymphomas to be targeted with these agents. Our group has investigated the changes in the tumor stroma that accompany disease activity in NHL and have noted significant changes in the expression of the extracellular matrix protein tenasin-C, which is limited to the site of lymphoma. Furthermore, the increased stromal expression correlates with increased resistance and disease progression as well. Thus, tenasin-C expression, as in high-grade gliomas, correlates with the degree of angiogenesis and the protein is contained in the microvessel
membrane and in the membrane-bound reticular stromal network. In NHL it has been shown that tenascin-C expression becomes interwoven with tumor cells, where expression correlates with angiogenesis, vessel immaturity, and aggressiveness in B-NHL. The enhanced expression of tenascin in diffuse and aggressive lymphomas, has prompted the evaluation of radiolabeled therapy directed at this stromal component. Targeting the extra-cellular matrix in patients with diffuse, large cell lymphomas with radiolabeled monoclonal antibodies may provide a new therapeutic avenue for the treatment of patients who have failed treatments directed at differentiation antigens on tumor cells themselves.

This is the first intravenous use of a radiolabeled antibody directed towards a stromal target, tenascin-C, in patients with lymphoma. Following the standard approach approved by the Food and Drug Administration for development of new radiolabeled antibodies, we report the pharmacokinetics, dosimetry, toxicity and response in nine patients who did not receive a cold or ‘blocking’ antibody prior to a fixed radiolabeled dose of therapy. The preferential uptake in the tumor and enhanced expression of tenascin in intermediate and high-grade lymphomas, demonstrates the encouraging potential of this approach. Even in this phase I trial with no ‘blocking antibody’ of normal organs anti-lymphoma effects were observed. Our results provide the foundation for future trials to improve therapy by blocking non-specific binding and binding to tenascin-C expressed in normal organs such as the marrow and liver.
PATIENTS, MATERIALS, AND METHODS

Patient Eligibility

Patients with relapsed or refractory non-Hodgkin’s lymphoma who had failed at least one prior regimen and were not considered eligible for other standard approaches with curative intent were included on this trial. Patients were 18 years of age or older with a minimum white blood cell count of $4 \times 10^9 \text{ L}^{-1}$, an absolute neutrophil count greater than $1 \times 10^9 \text{ L}^{-1}$, platelet count greater than $100 \times 10^9 \text{ L}^{-1}$ and hematocrit greater than 30% pre-therapy. Aspartate aminotransferase and alanine aminotransferase, alkaline phosphatase and total bilirubin must have been less than 2 times the upper limit of normal and patients with greater than 25% of their liver involved with disease were not eligible. Estimated creatinine clearance was greater than 60 mg ml$^{-1}$ for all patients and they may not have had any active obstructive hydronephrosis. Cancer and Leukemia Group B performance status of $\leq 3$ was required with life expectancy of at least 3 months. Patients must have been HIV negative, have no active viral (A, B, or C) or autoimmune hepatitis by screening or history, may not have more than 25% of the marrow involved with lymphoma (on bilateral examination), and not have undergone prior high dose chemotherapy requiring bone marrow or hematopoietic stem cell support. Furthermore, patients may not have previously received radiation to a maximum dose to any organ, and no more of 25% of the skeleton may have been previously radiated.

Prior to initiation of therapy, patients had hematopoietic stem cells collected as a ‘backup’ in case of prolonged aplasia. Blood work and radiographic studies were carried out to assess patients’ disease state and organ function within two weeks and four weeks of study entry, respectively.
Antibody preparation and iodination

The production of human/mouse chimeric 81C6 (ch81C6) was carried out by combining the murine 81C6 variable region genes with those for the human IgG2 constant regions, and the resulting chimeric mAb was characterized as described elsewhere.\textsuperscript{14} The ch81C6 mAb was grown in a Mini-Max hollow fiber bioreactor with CD Hybridoma media with no serum or protein additives. Purification was by affinity chromatography over a Sepharose-staphylococcal protein-A column followed by polyethylyamine (PEI) ion exchange chromatography. The preparation of each clinical batch followed the Food and Drug Administration Manufacture and Testing Guidelines,\textsuperscript{15} where all procedures were under GNP conditions. Radiolabeling was performed by a modified Iodo-Gen procedure.\textsuperscript{16} All preparations had an immunoreactivity of more than 75\%, with more than 95\% of the label eluting as immunoglobulin G on size-exclusion high-pressure liquid chromatography.

Antibody Administration

After obtaining informed consent, patients receive thyroid suppression with SSKI 2 drops orally and Cytomel 75 µg orally, daily beginning 24-48 hours prior to the first dose of therapy and continuing for one month following initiation of therapy. The patients received premedications including diphenhydramine 50 mg IV and acetaminophen 650 po approximately 30 minutes prior to radiolabeled antibody administration, which was delivered intravenously through a peripheral or central venous catheter, with vital signs monitored every 15 minutes for one hour post infusion.
Following the Food and Drug Administration guidelines for development of new radiolabeled antibodies, patients were given a fixed dose of radiolabel and not dosed by total body dosimetry in this phase I trial. Patients were given an initial dosimetric dose of 370 MBq [10 mCi] of $^{131}$I-labeled ch81C6 mAb on a constant amount of 10 mg of monoclonal antibody. Because of slight variations in yield and transfer of radiolabeled protein to injection syringe, the administered amount was ± 2 mg of the antibody. The amount of administered radiation activity may vary ± 10% for the same reason. Patients had gamma camera imaging shortly after infusion and on five of the ensuing 7 days. Pharmacokinetic measurements were performed 4-6 hours post infusion and on 5 of the next 7 days for each patient, as well. Following this, a therapeutic dose with an escalating dose of $^{131}$I-labeled chimeric 81C6 monoclonal antibody was delivered to the patient. Patients were observed daily after infusion for 3 days and then a minimum of weekly until recovery from all toxicity due to the therapy. At the completion of recovery from side effects of the radiolabeled antibody, patients had restaging examinations performed, as well as human anti-mouse antibody (HAMA) assessments.

Quantitative Imaging, pharmacokinetics and dosimetry

Blood samples, serial whole body gamma camera images, and single photon emission computed tomography (SPECT) were evaluated in order to estimate absorbed doses to normal organs and identifiable tumor sites. Blood samples were used to measure the activity concentration within the blood pool as a function of time and they were obtained immediately after administration, and approximately 0.5, 1, 5, and 24 hr thereafter. A trichloroacetic acid precipitation was used to assess the protein-associated
fraction of $^{131}$I in the blood samples. The activity concentration of iodine in blood samples was measured in a calibrated scintillation counter using 2 ml aliquots. The percentage of injected dose in the blood was calculated by comparison with standards prepared from the same administered dose. In 3 patients, marrow or nodal core samples were also obtained for direct measurement of activity, allowing a specific activity concentration scale to be developed.

Planar whole body images were acquired immediately after infusion, between 2 and 4 hours, 24 hours, and between day 4 and 7. All images were acquired on a dual-head gamma camera system equipped with high-energy general-purpose collimators, set at a 15% energy window and centered on the $^{131}$I photo-peak of 364 keV. Moreover, quantitative SPECT imaging was performed using high-energy collimation in order to assess the three dimensional distribution of $^{131}$I-labeled ch81C6 in liver, spleen, lung, kidney, thyroid and bone marrow of the spine. The analysis of whole-body images was performed using the gamma camera system software to generate regions of interest (ROI). A set of ROIs for the whole body, liver, spleen, lungs, kidneys, thyroid, discernable tumor sites, and background was defined for each patient and used on all sequential images in the anterior and posterior views to obtain count rates, and the geometric mean was obtained for each ROI. This analysis yielded relative clearance curves for the whole body and organs. Organ and tumor dosimetry was calculated based on standard quantitative SPECT methods for $^{131}$I and patient-specific quantitative SPECT-based dosimetry as described elsewhere.
Bone Marrow and Lymph Node Needle Biopsy Samples

Core needle bone marrow and, when accessible, lymph node biopsy samples were obtained within 48 hours after the dosimetric activity of 370 MBq [10mCi] was administered. These were approximately 20 mg in mass. Biopsy samples were evaluated for tumor infiltration and morphology and also they were used to assess the activity concentration and distribution at the small-scale level. Activity measurements in tumor needle biopsy samples were compared to activities computed from quantitative SPECT scans. Because of the high uptake of \( ^{131}I \)-labeled ch81C6 mAb in bone, bone marrow biopsy samples were used as a reference for bone surface and red marrow dosimetry. All bone marrow biopsies were obtained from the posterior iliac crest of patients. Bone marrow dosimetry was carried out using histological images stained for tenascin-C that reflect the morphology of bone and activity distribution of tenascin-C (see Figure 1). The Monte Carlo transport code EGS4-PRESTA was adapted to assess the small-scale dose coefficients (S-values expressed in cGy-g MBq\(^{-1}\) h\(^{-1}\)) in bone marrow, trabecular bone and bone surfaces using histological images. The absorbed dose \( D_T \) to target \( T \) (bone marrow or bone surfaces) from sources in \( R \) (represented by tenascin-C expression) is given as

\[
D_T = S(T \leftarrow R)\tilde{a},
\]

where \( D_T \) is expressed in cGy, \( \tilde{a} \) is the estimated cumulated activity concentration in the bone biopsy sample expressed in MBq-h g\(^{-1}\), and \( S(T \leftarrow R) \) is the small-scale S-value to target \( T \) from sources in \( R \) expressed in cGy g MBq\(^{-1}\) h\(^{-1}\).

Toxicity and Efficacy

Hematological toxicity was assessed based on the National Cancer Institute common toxicity criteria (CTC version 3.0; Common Toxicity Criteria, Cancer Therapy
Evaluation Program, National Cancer Institute, Bethesda, MD). Toxicity grades were obtained for platelets, neutrophils, and leukocytes. Patients were evaluated upon recovery of side effects (approximately 6-12 weeks post infusion) with physical exam, laboratory analyses and restaging radiographs to assess for degree of response as a secondary end point of this Phase I trial. Previously published criteria for response were used.24 Human antimouse antibody (HAMA) titers were obtained after $^{131}$I-ch81C6 therapy.

The primary end point of this trial was to estimate the maximum tolerated dose in terms of toxicity of $^{131}$I-labeled chimeric 81C6 anti-tenascin antibody delivered intravenously to patients with progressive non-Hodgkin’s lymphoma. National Cancer Institute Toxicity Criteria, Version 2.0 was followed, with documentation of all toxicities encountered. Dose limiting toxicity was defined as a Grade III or higher non-hematopoietic toxicity, or prolonged cytopenia. Prolonged cytopenia was defined as hematopoietic toxicity consisting of more than 10 days of an ANC < $0.5 \times 10^9$ cells L$^{-1}$ or the requirement of platelet transfusions for more than 14 days to maintain a count > $10 \times 10^9$ platelets L$^{-1}$, or the need for red cell transfusion to maintain a hematocrit greater than 25%, unless due to the underlying disease documented by the treating physician. The maximum tolerated dose was the dose of radiolabeled antibody that produced 0/3 or $\leq 2/6$ patients experiencing a dose-limiting toxicity at a specific dose. Dose escalation or de-escalation followed the requirements of the Food and Drug Administration (FDA) for development of new radiolabeled antibodies and was based on total administered activity (millicurries) and not dosimetry. Our Institutional Review Board and the Food and Drug Administration approved the protocol, and a signed informed consent was obtained prior to initiation of therapy.
RESULTS

Imaging

Whole body gamma camera images showed mainly blood pool activity immediately after administration, which decreased over time but was clearly present up to 200 hours after administration (see Figure 2). There was rapid uptake of $^{131}$I-labeled ch81C6 mAb in bone marrow on all patients because of the presence of tenascin-C in the extracellular matrix of bone marrow and tumor involvement.\textsuperscript{25,26} Patients with large tumor masses, as shown in Figures 2 and 3, showed tumor retention of $^{131}$I-labeled ch81C6 with an effective half-life close to the physical half-life of $^{131}$I demonstrating the long term retention of ch81C6 mAb; however, in small masses of tumor as determined by CT, tumor contrast was very low because of the prolonged blood activity, and tumor retention was difficult to determine.

Pharmacokinetics and Dosimetry

The whole body activity, as determined by whole body scintigraphy, of $^{131}$I-labeled ch81C6 mAb decreased mono-exponentially in all nine patients with an average [range] effective half-life of 110 [90 – 136] hours. The corresponding average [range] effective residence time of $^{131}$I-labeled ch81C6 in the whole body was 159 [130 – 196] hours. The average [range] whole body dose among all nine patients was 67 [51 –89] cGy. The pharmacokinetics of $^{131}$I-labeled ch81C6 in blood was bi-exponential, with an alpha component (fast clearance) of 1.9 [0.18 – 4.4] hours and a fraction 0.65 [0.36 –
0.94], and a beta component (slow clearance) of 70 [45 – 91] hours and a fraction of 0.35 [0.06 – 0.64].

The dose-limiting organ was the bone marrow where hematological toxicity was observed in two of three patients treated at the 1480 MBq [40mCi] level. The administered dose was then reduced to 1110 MBq [30 mCi] where only two out of six patients developed hematological toxicity. The activity concentration measured in bone marrow biopsy samples was 31 kBq g⁻¹ with an activity fraction in bone surfaces (AFBS) of 35% for patient No 1, 44 kBq g⁻¹ with an AFBS of 51% for patient No 2, and 76 kBq g⁻¹ with an AFBS of 49% for patient No. 3. Using bone marrow histological samples stained for tenascin-C, we estimated the S-values to red marrow and bone surfaces and corresponding absorbed doses. The range of absorbed doses to red marrow and bone surfaces among all patients varied between 232 and 745 cGy, and 397 and 1135 cGy, respectively. There were few treated people in order to evaluate for a potential causal relationship between absorbed dose and hematological toxicity. Absorbed doses to other organs are summarized in Table 1. The organ that received the largest absorbed dose, as expected was the thyroid.

Tumor sites were selected according to discernable radiolabeled uptake from both whole-body planar images and SPECT images. However, many tumor sites that were identified by CT were not discernable in either whole-body images or SPECT because of minimal tumor mass or overlap with normal viscera such as the liver or spleen; therefore, there was a clear heterogeneity in absorbed doses to tumor sites. As an example, Figure 2 and 3 shows the persistence of radiolabeled antibody in the nodal disease of patients # 1 and 2, respectively, even after 169 hours following infusion where the half-life of the
clearance phase of the radiolabeled antibody was higher than 150 hours, which was close to that of the physical half-life of $^{131}$I, demonstrating good antibody retention. Figure 4 shows similar clearance rates for selected tumor sites in other patients. Among all nine patients, the average absorbed dose to selected tumor sites was 953 [363 – 1517] cGy and the average absorbed dose per administered activity was 0.60 [0.25 – 0.82] cGy MBq$^{-1}$.

**Toxicity**

Patients initially received a therapeutic dose of 1480 MBq [40mCi]. However, because of hematopoietic toxicity, this was de-escalated in subsequent patients to 1110 MBq [30 mCi] (Table #1). There was no significant non-hematologic toxicity encountered in this study. In the first cohort of patients who received 1480 MBq for the therapeutic dose, the dose per kilogram of patient weight ranged between 18 and 30 MBq kg$^{-1}$. Two of the three patients in this cohort had grade 4 white cell, neutrophil and platelet toxicity. The time to recover from white cells being under $1 \times 10^9$ L$^{-1}$ (grade 4) to $> 3 \times 10^9$ L$^{-1}$ (grade 1) or better was 20 and 12 days for these two patients, respectively. Severe platelet suppression was noted with recovery from $< 25 \times 10^9$ L$^{-1}$ (grade 4) to grade 1 or better taking 20 and 30 days for these 2 patients. Both of these patients had stem cells infused to assist recovery. These two patients had moderate hemoglobin toxicity, though the 1 patient in this cohort without a dose limiting toxicity had grade 3 hemoglobin toxicity (65-80 g L$^{-1}$). The subsequent 6 patients were treated with 1110 MBq, corresponding to dose per kilogram of patient weight that ranged between 15 and 20 MBq kg$^{-1}$. All but one of these 6 patients experienced at least grade 3 white cell suppression, with a median of 41 days (range 14-49 days) to recover to a white cell count
>3 \times 10^9 \text{ L}^{-1} \text{ in the patients in this cohort, though only 3 of these patients had an absolute neutrophil count < 0.5 \times 10^9 \text{ L}^{-1} \text{ with a median time to recover neutrophils of 3.5 days (range 0-21) in this cohort. All patients in this cohort also had grade 3 or 4 thrombocytopenia with a median days to recover to grade 1 or better of 31 days (range 22-70). The median number of days of grade 4 thrombocytopenia in this cohort was 14 days (range 0-40). Patients # 7 and # 9 had prolonged thrombocytopenia, however they were showing recovering within 2 weeks of onset of the white count decreasing under 2 \times 10^9 \text{ L}^{-1} \text{ and were not considered to have a DLT. Two patients in this group had stem cells infused to assist recovery (#6 and #8). Only one patient in this cohort had severe hemoglobin toxicity as well. Interestingly, the patient with the highest administered activity per unit mass (patient #5) in this cohort did not have any severe or prolonged cytopenias. These data demonstrate that the only organ with toxicity from this therapy was the bone marrow.}

**Response**

The patients treated on this trial had relapsed disease without other curative options with a median of 3 prior regimens (range 2-5) and 11 prior cycles (range 7-14). Seven of the nine had failed at least one course of rituximab therapy. Patients with either low grade or high grade NHL were included. Two had small lymphocytic NHL, 1 had mucosa-associated lymphoma, 1 follicular NHL, 2 had transformed NHL, and 3 had diffuse large cell NHL. Despite the heavy pre-treatment and refractory nature of most of the patients as well as no ‘blocking’ antibody being used, response in this phase I study
was noted with 1 complete remission, 1 partial remission, and the others had stable disease lasting from 2 to higher than 8 months (see Table 3).

**HAMA**

Six patients had HAMA testing performed from 33-166 days following injection. None of these patients developed HAMA. One patient had a low titer HAMA noted before therapy, presumably due to her known prior exposure to a murine antibody. This patient had a negative HAMA upon re-testing 33 days following the therapeutic dose.

**DISCUSSION**

Available radiolabeled agents approved for follicular (low grade) or transformed lymphoma are directed towards antigens expressed on the cell surface. The CD20 target is often not as highly expressed in the other types of more aggressive diffuse lymphomas or small lymphocytic disease. Further, recent work has identified the tumor stroma as an important supporting network relating to the growth and progression of many types of malignancies. \(^ {27-29}\) Recently developed therapies directed at affecting stromal elements, such as vasculogenesis, have been shown to be effective in some tumor types as well. \(^ {30}\) It has been reported that stromal changes, such as increased vasculogenesis and expression of extracellular matrix proteins such as tenascin, are increased in lymphoma. \(^ {13,31}\) Our group has extended these observations by noting that the change in the stromal components of microvessel density and tenascin expression are limited to the sites of disease in the patient. In addition, the expression of these stromal elements changes over time in correlation with the activity of disease (Rizzieri et al., in submission). These data
imply that targeting tumor stroma, rather than the tumor cells directly, may be an advantageous route for therapy. In addition, we and others have shown that the expression of tenascin increases with aggressiveness of NHL subtype. Our group has reported use of chimeric 81C6 radiolabeled antibody to tenascin with instillation directly into tumor cavities post resection in those with brain tumors, resulting in improved progression free survival. This phase I trial combines this recognition of the importance of the stromal compartment in promoting progression of lymphoma with intravenous delivery of this same radiolabeled antibody.

We have shown in this report that anti-tenascin antibody, delivered without any ‘blocking’ antibodies to prevent binding to the liver, spleen, marrow and nonspecific sites, can be safely delivered to patients intravenously. The prolonged half life of the chimeric antibody used in this study is encouraging for the effective half life of the agent in attacking tumor, however it may also remain problematic in terms of damaging normal viscera and the significant hematologic side effects remain dose limiting. Future studies will focus on minimizing the binding in the marrow, allowing increased delivery to the tumor and expansion of the use of this agent to many patients with diffuse disease. Strategies to address this take advantage of the fact that dosimetric studies reveal a distribution consistent with the known low expression of tenascin in normal visceral organs of the lungs, liver and bone marrow, as well as increased concentration in tumor tissue. Further, pharmacokinetic studies reveal rapid uptake in the liver and marrow, and a slower, but enhanced, uptake in selected tumors sites over normal organs. We have previously infused a murine anti-tenascin antibody linked to I into patients with escalating doses of antibody (10-100mg anti-tenascin) linked to the radiolabel. In doing
this, we noted a significant decrease in normal viscera uptake of the radiolabel, suggesting a similar approach with the human/mouse chimeric antibody we now employ may be efficacious. Following these data, future trials of this agent will deliver the unlabeled chimeric antibody intravenously for the prolonged blocking effect of normal viscera prior to the radiolabeled antibody. Alternatively, if this is not effective one could deliver the longer lived chimeric antibody as unlabeled for the blocking potential and use the shorter lived murine antibody for the therapeutic dosing to affect a more rapid clearance. Even without a blocking antibody, with the large differential between tumor and normal visceral organ uptake documented in this study, there was approximately 953 cGy of radiation delivered to the site of the tumor. We anticipate that by first blocking the areas of binding to normal visceral organs, and then proceeding with the therapeutic radiolabeled antibody dose the therapeutic window of this agent may significantly improve by decreasing the marrow uptake and corresponding toxicity and augmenting the uptake of radiolabeled antibody to tumor sites.

The mean whole-body effective half-life of 131I-labeled ch81C6 mAb of 110 hours was similar to that of 131I-rituximab of 88 hours and longer than that of 131I-tositumomab of 56 hours. Blood kinetics of 131I-labeled ch81C6 were described by a bi-exponential clearance with a slow-clearance (beta component) half-life of 70 hours, which was lower than that of the whole-body of 110 hours and 36% longer, indicating the large uptake (or sink) observed in spleen, liver and bone marrow where tenascin-C is ubiquitously expressed. Radioactivity was excreted exclusively through the kidneys where approximately 47% of the injected activity was found in urine.
Tumor uptake was well defined in whole-body images where maximum uptake was observed between 24 and 48 hours. Clearance of $^{131}\text{I}$-labeled ch81C6 from tumor sites was slow reflecting closely the physical decay constant of $^{131}\text{I}$ and demonstrating the good tumor retention of ch81C6 mAb. Absorbed doses to pre-selected tumor sites, as determined using quantitative imaging, ranged between 353 and 1517 cGy with an average of 953 cGy, which are within the range of absorbed doses estimated for $^{131}\text{I}$-tositumomab between 37 and 1760 cGy.\textsuperscript{35} Furthermore, the estimated average [range] absorbed dose to selected tumors sites per unit administered activity among all patients was $0.60 \ [0.25 - 0.82]$ cGy MBq\textsuperscript{-1}, which was higher than that obtained from $^{131}\text{I}$-tositumomab.\textsuperscript{35} Comparison of dose factors shows that an absorbed dose of 25 Gy to normal organs other than the thyroid gland, and bone marrow would not be exceeded. However, a pre-therapeutic determination of the whole-body half life and residence time of $^{131}\text{I}$-labeled ch81C6 is necessary for each patient in order to determine the absorbed dose to these organs and tumor sites in future studies.

This study confirms the safety and potential therapeutic utility of targeting the tumor stroma with $^{131}\text{I}$-labeled anti-tenasin chimeric 81C6 monoclonal antibody. The high doses to red marrow did not always result in hematological toxicity in these few patients, though further study is needed to evaluate the true relationship with dose delivered, patient weight, dosimetric calculations and toxicity. The complete and partial remission noted in some patients treated on this phase I study is encouraging and supports further attempts to enhance localization of the radiolabel to the tumor while decreasing normal visceral uptake through the use of unlabeled antibody.
Anti-stromal radioimmunotherapy has not yet been fully evaluated for patients with lymphoma. Future trials will evaluate methods to minimize normal visceral and nonspecific binding and re-escalation of the radiolabeled dose to maximize tumor exposure. Given the known increased expression of tenascin-C in other tumor types, such as lung, breast and gastrointestinal tumors, the potential applicability of anti-tenascin chimeric 81C6 monoclonal antibody may have a broader therapeutic applicability as well.36-40

ACKNOWLEDGEMENTS

The authors would like to thank the referring physicians and nurses for the care of the patients.

*Supplemental Material is available online with final print publication
REFERENCES


15. Food and Drug Administration: Director OoBRaR Center for Drugs and Biologics: Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use. Bethesda, MD, Food and Drug Administration, 1987


Table 1. Patient characteristics, treatment parameters, whole-body pharmacokinetics and whole-body and organ dosimetry for NHL patients receiving $^{131}$I-chimeric 81C6

<table>
<thead>
<tr>
<th>PT</th>
<th>G</th>
<th>WT (kg)</th>
<th>DA (MBq)</th>
<th>TA. (MBq)</th>
<th>SA (MBq kg$^{-1}$)</th>
<th>WB Eff. Half life (h)</th>
<th>WB Res. Time (h)</th>
<th>Absorbed dose‡ (cGy)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>WB Thyroid RM BS Blood Lungs Kidney Liver Spleen Tumor*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>101</td>
<td>370</td>
<td>1480</td>
<td>18.3</td>
<td>102</td>
<td>147</td>
<td>58</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>61</td>
<td>370</td>
<td>1480</td>
<td>30.3</td>
<td>109</td>
<td>158</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>79</td>
<td>370</td>
<td>1480</td>
<td>23.4</td>
<td>95</td>
<td>137</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>74</td>
<td>370</td>
<td>1110</td>
<td>20.0</td>
<td>125</td>
<td>180</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>59</td>
<td>370</td>
<td>1110</td>
<td>25.1</td>
<td>136</td>
<td>196</td>
<td>89</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>70</td>
<td>370</td>
<td>1110</td>
<td>21.1</td>
<td>90</td>
<td>130</td>
<td>51</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>86</td>
<td>370</td>
<td>1110</td>
<td>17.2</td>
<td>114</td>
<td>165</td>
<td>54</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>81</td>
<td>370</td>
<td>1110</td>
<td>18.3</td>
<td>121</td>
<td>175</td>
<td>69</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>97</td>
<td>370</td>
<td>1110</td>
<td>15.3</td>
<td>101</td>
<td>146</td>
<td>53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>WB</th>
<th>Thyroid</th>
<th>RM</th>
<th>BS</th>
<th>Blood</th>
<th>Lungs</th>
<th>Kidney</th>
<th>Liver</th>
<th>Spleen</th>
<th>Tumor*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>3009</td>
<td>329</td>
<td>508</td>
<td>141</td>
<td>223</td>
<td>213</td>
<td>206</td>
<td>567</td>
<td>1517</td>
</tr>
<tr>
<td>2</td>
<td>87</td>
<td>2155</td>
<td>505</td>
<td>888</td>
<td>247</td>
<td>297</td>
<td>241</td>
<td>306</td>
<td>650</td>
<td>1258</td>
</tr>
<tr>
<td>3</td>
<td>76</td>
<td>2375</td>
<td>745</td>
<td>1135</td>
<td>299</td>
<td>264</td>
<td>255</td>
<td>137</td>
<td>450</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>67</td>
<td>2419</td>
<td>329</td>
<td>566</td>
<td>192</td>
<td>307</td>
<td>429</td>
<td>195</td>
<td>350</td>
<td>811</td>
</tr>
<tr>
<td>5</td>
<td>89</td>
<td>2056</td>
<td>350</td>
<td>512</td>
<td>240</td>
<td>232</td>
<td>108</td>
<td>133</td>
<td>330</td>
<td>735</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>1096</td>
<td>370</td>
<td>558</td>
<td>137</td>
<td>312</td>
<td>242</td>
<td>149</td>
<td>474</td>
<td>1198</td>
</tr>
<tr>
<td>7</td>
<td>54</td>
<td>1416</td>
<td>345</td>
<td>543</td>
<td>177</td>
<td>426</td>
<td>319</td>
<td>158</td>
<td>362</td>
<td>682</td>
</tr>
<tr>
<td>8</td>
<td>69</td>
<td>2347</td>
<td>232</td>
<td>397</td>
<td>168</td>
<td>220</td>
<td>247</td>
<td>213</td>
<td>370</td>
<td>1060</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>1820</td>
<td>380</td>
<td>585</td>
<td>143</td>
<td>310</td>
<td>131</td>
<td>96</td>
<td>344</td>
<td>363</td>
</tr>
</tbody>
</table>

| Avg (cGy MBq$^{-1}$) | 0.04 | 1.29 | 0.25 | 0.51 | 0.12 | 0.18 | 0.15 | 0.11 | 0.27 | 0.60 |
| Min (cGy MBq$^{-1}$) | 0.03 | 0.74 | 0.16 | 0.34 | 0.08 | 0.12 | 0.07 | 0.06 | 0.22 | 0.25 |
| Max (cGy MBq$^{-1}$) | 0.06 | 1.63 | 0.40 | 0.77 | 0.16 | 0.29 | 0.29 | 0.17 | 0.35 | 0.82 |

PT: Patient; G: Gender; DA: Dosimetric activity; TA: Therapeutic activity; SA: Specific activity; WB: Whole Body; RM: Red marrow; BS: Bone surfaces. ‡ Absorbed doses were calculated based on total administered activity (DA+TA). *Estimated absorbed dose to selected discernable radiographic sites.
Table 2. Hematological and organ toxicity in patients with NHL treated with $^{131}$I-labeled ch81C6 mAb.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adm. dose (MBq)</td>
<td>1480</td>
<td>1480</td>
<td>1480</td>
<td>1110</td>
<td>1110</td>
<td>1110</td>
<td>1110</td>
<td>1110</td>
<td>1110</td>
</tr>
<tr>
<td>Adm. dose (MBq/kg)</td>
<td>14.6</td>
<td>24.4</td>
<td>18.7</td>
<td>14.9</td>
<td>18.7</td>
<td>15.9</td>
<td>12.9</td>
<td>13.7</td>
<td>11.5</td>
</tr>
<tr>
<td>White cell range $\times 10^9$ L$^{-1}$ and days for recovery to ≤ grade 1</td>
<td>5.8-12.2 (0 days)</td>
<td>4.7-5 (20 days)</td>
<td>4.3-4 (12 days)</td>
<td>4.7-5 (21 days)</td>
<td>4.1-2.0 (41 days)</td>
<td>9.9 (41 days)</td>
<td>3.2-1.0 (49 days)</td>
<td>6.6-1.0 (14 days)</td>
<td>5.8-1.1 (49 days)</td>
</tr>
<tr>
<td>Days ANC &lt; 0.5 $\times 10^9$ L$^{-1}$</td>
<td>0</td>
<td>20</td>
<td>4</td>
<td>7</td>
<td>0</td>
<td>21</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Platelet decrease ($\times 10^9$ L$^{-1}$) and days for recovery to ≤ grade 1</td>
<td>234-12 (35 days)</td>
<td>216-10 (20 days)</td>
<td>154-5 (30 days)</td>
<td>372-7 (28 days)</td>
<td>164-47 (22 days)</td>
<td>186-6 (70 Days)</td>
<td>113-8 (28 Days)</td>
<td>333-8 (33 Days)</td>
<td>190-8 (40 Days)</td>
</tr>
<tr>
<td>Days Platelets &lt; 25 $\times 10^9$ L$^{-1}$</td>
<td>27</td>
<td>13</td>
<td>18</td>
<td>12</td>
<td>0</td>
<td>40</td>
<td>13</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Hemoglobin decrease (g L$^{-1}$) and number of units (u) red cell transfusions delivered</td>
<td>110-67 (2u PRBC)</td>
<td>100-80 (2u PRBC)</td>
<td>107-90 (0u PRBC)</td>
<td>107-93 (0u PRBC)</td>
<td>103-93 (4u PRBC)</td>
<td>137-63 (0u PRBC)</td>
<td>120-87 (0u PRBC)</td>
<td>107-80 (0u PRBC)</td>
<td>123-90 (0u PRBC)</td>
</tr>
<tr>
<td>Required SC re-infusion</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Hepatic</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Neural</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Renal</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Cardiac</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

SC: Stem cell; WBC: White Blood Cells; PRBC: Packed Red Blood Cells

National Cancer Institute toxicity grading scale for hematologic parameters in the table:

White cell toxicity grade 2: $2-3 \times 10^9$ L$^{-1}$; grade 3: $1-2 \times 10^9$ L$^{-1}$; grade 4: $<1 \times 10^9$ L$^{-1}$ (growth factor support was NOT used in this trial).

Platelet toxicity grade 2: 50-75 $\times 10^9$ L$^{-1}$; grade 3: 25-50 $\times 10^9$ L$^{-1}$; grade 4: $<25 \times 10^9$ L$^{-1}$. Hemoglobin toxicity grade 2: 80-100 g L$^{-1}$; grade 3: 65-80 g L$^{-1}$; grade 4: $<65$ g L$^{-1}$. 
Table 3. Clinical response to $^{131}$I-labeled chimeric 81C6 mAb.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Disease</th>
<th>Prior regimens</th>
<th>Prior cycles</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CLL/SLL</td>
<td>3</td>
<td>13</td>
<td>SD</td>
</tr>
<tr>
<td>2</td>
<td>DLCL</td>
<td>4</td>
<td>11</td>
<td>SD</td>
</tr>
<tr>
<td>3</td>
<td>Transformed NHL</td>
<td>2</td>
<td>7</td>
<td>SD</td>
</tr>
<tr>
<td>4</td>
<td>MALT lymphoma</td>
<td>2</td>
<td>7</td>
<td>CRu (6 months)</td>
</tr>
<tr>
<td>5</td>
<td>Transformed NHL</td>
<td>4</td>
<td>14</td>
<td>SD</td>
</tr>
<tr>
<td>6</td>
<td>Follicular</td>
<td>4</td>
<td>18</td>
<td>SD</td>
</tr>
<tr>
<td>7</td>
<td>DLCL</td>
<td>2</td>
<td>8</td>
<td>SD</td>
</tr>
<tr>
<td>8</td>
<td>DLCL</td>
<td>5</td>
<td>14</td>
<td>SD</td>
</tr>
<tr>
<td>9</td>
<td>CLL/SLL</td>
<td>3</td>
<td>8</td>
<td>PR (continues &gt; 8 months)</td>
</tr>
</tbody>
</table>

CLL: Chronic lymphocytic leukemia, SLL: Small lymphocytic lymphoma; DLCL: Diffuse large cell lymphoma; MALT: Mucosa associated lymphoid tissue lymphoma.
Figure 1. Histological samples of bone marrow biopsy samples from patients with NHL stained for tenascin-C expression. a) Tenascin-C expression along bone surfaces in trabecular bone. b) Tenascin-C expression around Haversian canals and tumor saturated bone marrow, and c) Tenascin-C expression on a singular tumor nodule within bone marrow stroma.
Figure 2. a) Whole body images of a patient obtained at different time points after infusion of 370 MBq of $^{131}$I-labeled chimeric 81C6 mAb. Note the prolonged uptake in the nodal disease in the neck. b) CT scan depicting the nodal disease that accumulated the radiolabeled antibody. A biopsy sample was obtained from this node 44 hours after dosimetric infusion of 370 MBq and the measured activity concentration was 70 kBq g$^{-1}$. 
Figure 3. a) Serial anterior whole body images of patient No. 2 showing increased accumulation of $^{131}$I-labeled 81C6 mAb in a large mediastal lesion. b) CT image showing the location of the lesion affecting the left lung. c) Supplemental image #1: High energy SPECT reconstruction of the chest obtained after therapy with 1480 MBq of $^{131}$I-labeled chimeric 81C6 showing uptake in the spleen, liver, spinal bone marrow and lesion (available on-line). A biopsy sample was obtained from this node 17 hours after a dosimetric infusion of 370 MBq. The measured activity concentration was 37 kBq g$^{-1}$. 
Figure 4. Pharmacokinetics of $^{131}$I-labeled ch81C6 mAb of selected tumor sites from several patients. The initial tumor uptake reached a maximum between 40 and 100 h among different tumor sites where the half-life of the clearance phase varied between 86 and 191 h. The estimated average biological clearance half-life of the mAb in these tumors was estimated at 410 h demonstrating the long-term retention of the antibody in tumor sites. The range of absorbed doses varied between 363 and 1517 cGy. The measured activity concentrations from tumor biopsy samples were 70 and 36-kBq-g$^{-1}$ for patients 1 (♀) and 2 (♂), respectively.
Phase I Trial study of $^{\text{131}}\text{I}$-labeled chimeric 81C6 monoclonal antibody for the treatment of patients with non-Hodgkin's lymphoma