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Sara L Warber
University of Michigan

Jack Edward Juni
Stage 2 Innovations

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Localization and Imaging with Radioiodine-labeled Monoclonal Antibodies in a Xenogeneic Tumor Model for Human B-Cell Lymphoma[1]

Donald J. Buchsbaum,[2] Joseph A. Sinkule, M. Susan Stites, Patricia A. Fodor, David E. Hanna, Stephen M. Ford, Sara L. Warber-Matich, Jack E. Juni, and Kenneth A. Foon[3]

Department of Radiation Oncology [D. J. B., J. A. S., M. S. S., D. E. H., S. M. F.], and Department of Internal Medicine [P. A. F., S. L. W-M., J. E. J., K. A. F.], University of Michigan, Ann Arbor, Michigan 48109

ABSTRACT

Two MoAbs directed towards human B-cell malignancies have been studied in a preclinical animal model to evaluate their potential for *in vivo* imaging and therapy of B-cell lymphomas. Anti-B1 reacts with virtually all immunoglobulin-bearing malignancies and non-T acute lymphoblastic leukemia. Anti-J5 reacts with the common acute lymphoblastic leukemia antigen found on non-T acute lymphoblastic leukemia and follicular lymphomas. Anti-T1 which recognizes the CD5 antigen on most T-cell leukemias and lymphomas was used as a control antibody. These monoclonal antibodies were radiolabeled with ^{125}I or ^{131}I by the ICl method. Namalwa (B-cell) and MOLT-4 (T-cell) tumors were grown s.c. in irradiated nude mice. The highest tissue concentration of ^{125}I-labeled anti-J5 in Namalwa-bearing mice was in blood and tumor. The tumor/blood ratio ranged from 0.7–1.2, with the highest ratio 4 days after injection. Pharmacokinetic analysis indicated that the $t_{1/2}\beta$ of anti-J5 from blood and other tissues ranged from 40–50 h, while the $t_{1/2}\beta$ for tumor averaged 65 h. The area under the curve of tumor was 2- to 5-fold higher than the area under the curve of liver, kidney, skin, and muscle. The peak tissue levels of ^{125}I-labeled anti-B1 in Namalwa-bearing mice were again in blood and tumor and 6 days following injection more than 5-fold greater activity was found in tumor compared to normal tissues other than blood. The tumor/blood ratio was 1.2 and 0.7 at 4 and 6 days after injection. ^{125}I-labeled anti-B1 showed minimal uptake in antigen-negative MOLT-4 tumors and ^{125}I-labeled anti-T1 showed little uptake in Namalwa tumors. Scintigraphic images were obtained following the injection of ^{131}I-labeled anti-J5 and anti-B1 in nude mice bearing Namalwa tumors. These results indicate that radiolabeled anti-J5 and anti-B1 show promise as diagnostic and possibly therapeutic agents for human B-cell lymphoma, although there may be a limitation to clinical utility due to cross-reactivity with some normal cells.

INTRODUCTION

MoAbs[4] have an important role in the classification of leukemia and lymphoma (1) but have had a more limited role in diagnostics and therapy. In order to develop good diagnostic and therapeutic reagents, it has generally been useful to have an animal model for preclinical testing. The study of human lymphomas has been limited because of the lack of reproducible experimental models. Techniques to develop human B- and T-cell leukemia models in athymic mice have recently been reported (2–4) and we have successfully used these techniques to grow T-cell as well as B-cell tumors s.c. in athymic mice.

A series of MoAbs directed towards human B-lymphocytes and B-cell malignancies have been reported (5–10). Over 80% of adult cases of non-Hodgkin's lymphoma have lymphocyte surface membrane characteristics of B-lymphocytes. These antibodies have been utilized to purge target cells in autologous bone marrow transplantation (11, 12), but only a limited number of clinical serotherapy studies have been reported (13–15). One of these antibodies, designated anti-B1, has a pan-B distribution reacting with virtually all immunoglobulin-bearing malignancies as well as many non-T acute lymphoblastic leukemias (6). This antibody reacts with greater than 85% of peripheral blood B-cells. The anti-B1 antibody also reacts with B-cells in normal lymphoid tissues and bone marrow (5). The anti-J5 antibody (10) reacts with a CALLA found on cells from most patients with non-T acute lymphoblastic leukemia and follicular lymphomas. This antibody also reacts with granulocytes and renal tubular and glomerular cells. It has previously been demonstrated, utilizing anti-T-cell antibodies in humans with a T-cell tumor where the antibody also reacts with normal human T-cells, that one can successfully image tumors as small as 0.5 cm (16, 17). The preclinical studies reported in this manuscript are designed to address the critical preliminary issues prior to embarking on a clinical trial with the anti-B1 and anti-J5 antibodies.

MATERIALS AND METHODS

Monoclonal Antibodies. The murine MoAb, anti-J5, is an IgG2a antibody reactive with the M_r 100,000 glycoprotein CALLA (CD10), which is expressed on follicular lymphoma cells as well as most acute lymphoblastic leukemia cells (10). Murine MoAb anti-B1 is an IgG2a antibody reactive with a M_r 35,000 phosphorylated cell surface molecule (CD20), which is expressed on normal B-cells and most B-cell lymphomas and leukemias (5). MoAb anti-T1 is a murine IgG2a reactive with a M_r 65,000 antigen (CD5) present on normal human T-cells, and most T-cell leukemias and lymphomas (18). Anti-T1 was used as an irrelevant control antibody for this study. These antibodies were generously provided in purified form by Dr. Kenneth Kortright (Coulter Immunology, Hialeah, FL). Antibody concentration was determined using the Bio-Rad protein assay standardized with bovine γ-globulin (Bio-Rad, Richmond, CA).

Radiolabeling and Characterization of Labeled Antibodies. Iodination and characterization of anti-J5, anti-B1, and anti-T1 antibodies were performed as previously described (19). Purified antibodies were labeled with ^{125}I or ^{131}I (Amersham Corp., Arlington Heights, IL) by the ICl method of Contreras *et al.* (20). Aliquots of 200–500 μg of antibody were labeled with 1–5 mCi of radionuclide and 5 equivalents of ICl. Free iodine was removed by passage over a 1- x 5-cm Dowex 1-4 X resin column. Normal human serum albumin was used as a protective protein at a final concentration of 2%. Labeling efficiency was determined as the amount of radioactive iodine incorporated into the recovered product as compared to the amount of radioactive iodine added to the reaction mixture. Specific activity is expressed as a ratio of mCi of radionuclide attached per mg of antibody in the final product. Small portions of the radiolabeled MoAb preparations were injected into groups of animals.

HPLC was used to analyze the radiolabeled MoAbs. Radiochemical

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[2] To whom requests for reprints should be addressed, at Department of Radiation Oncology, University of Michigan, UH-Rm. B2C490/0010, 1500 E. Medical Center Drive, Ann Arbor, MI 48109-0010.

[3] Present address: Roswell Park Memorial Institute, Division of Clinical Immunology, 666 Elm Street, Buffalo, NY 14263.

[4] The abbreviations used are: MoAb, monoclonal antibody; AUC, area under the curve; CALLA, common acute lymphoblastic leukemia antigen; CTCL, cutaneous T-cell lymphoma; FCS, fetal calf serum; HPLC, high performance liquid chromatography; PBS, phosphate buffered saline; *T/NT*, tumor/non-tumor ratio; $C \times t$, concentration × time.

purity was determined by HPLC analysis using a high-pressure, gradient pumping system (Waters Model 1600; Waters Chromatography Division, Milford, MA), mobile phase of pH 7.4 PBS (1 ml/min), and a 7.8- x 300-mm Protein Pak SW 300 (Waters Chromatography Division, Milford, MA) size exclusion column connected to a multichannel UV/Vis HPLC detector (Waters Model 490) in series with an in-line radioactivity detector (Beckman Model 170; Beckman Instruments Irvine, CA). A DEAE (7.5 mm x 7.5 cm)-PW5 anion exchange column (Waters Chromatography Division) was also used to check antibody purity with a 30-min linear gradient starting in 20 mM Tris-HCl (pH 8.5) and running to 20 mM Tris-HCl/0.3 M NaCl (pH 7) at 1 ml/min. Gel electrophoresis under reducing and nonreducing conditions was also performed with the labeled antibodies to reaffirm purity and to determine the molecular weights of the products.

Cell Lines. Namalwa (21) and Raji (22) are malignant B-lymphoblastoid cell lines derived from patients with Burkitt's lymphoma. CEM (23) and MOLT-4 (24) are T-lymphoblastoid cell lines derived from patients with T-cell acute lymphoblastic leukemia. The Namalwa and MOLT-4 cell lines were kindly provided by Dr. Robert Dillman (Scripps Clinic and Research Foundation, La Jolla, CA). The Raji and CEM cell lines were obtained from the American Type Culture Collection (Rockville, MD). Cell lines were grown in suspension culture and maintained at 37°C in a humidified atmosphere with 5% CO_2. Cells were passaged every 2–3 days in RPMI 1640 medium (GIBCO Laboratories, Grand Island, NY) supplemented with 10% heat inactivated (56°C, 30 min) FCS, L-glutamine, and penicillin/streptomycin antibiotics. Cells to be injected into mice were taken from culture during the log phase of growth. The human fibrosarcoma cell line HT-1080 was obtained from the American Type Culture Collection. HT-1080 cells were grown in monolayer culture in Earle's minimal essential medium containing 10% FCS, 1% nonessential amino acids, 1% L-glutamine, and 1% penicillin/streptomycin.

Indirect Immunofluorescence. Reactivity of the anti-J5, anti-B1, and anti-T1 antibodies with Namalwa, Raji, CEM, and MOLT-4 leukemia cell lines was determined by flow cytometry analysis. Leukemia cells were counted and adjusted to 5×10^5–2×10^7 cells/ml in Dulbecco's PBS containing 2.5% (v:v) human serum antibody which was heat inactivated at 56°C for 30 min (GIBCO, Grand Island, NY), and 0.1% (w:v) glucose. One hundred μl of cell suspension were added per well of a V-bottomed 96-well plate (Costar, Cambridge, MA). Saturating amounts of antibody (10 μg/ml) were added to cells and incubated at 4°C for 30 min. Cells were washed once with PBS solution, resuspended in 100 μl of a 1:40 dilution of goat anti-mouse IgG + IgM conjugated with fluorescein isothiocyanate (Tago, Burlingame, CA), and incubated at 4°C for 30 min. After washing, cells were analyzed for immunofluorescence using a Coulter EPICS C cell sorter (Coulter Corp., Hialeah, FL). Cells were gated using forward and 90° light scatter to exclude dead cells and debris and analyzed with a 488-nm argon laser.

Live Cell Radioimmunoassay. The immunoreactivity of radiolabeled anti-J5, anti-B1, and anti-T1 antibody preparations was measured using an *in vitro* live cell binding assay. Namalwa, Raji, CEM, or MOLT-4 leukemia cells were suspended in RPMI 1640 medium containing 5% heat inactivated FCS at a concentration of 2×10^7 cells/ml. Three-tenths ml of the cell suspension (6×10^6 cells) was placed into 12- x 75-mm borosilicate glass test tubes (VWR Scientific, San Francisco, CA), and a volume of radiolabeled antibody containing 0.04 μCi of radioactivity was added to duplicate tubes. Tubes were incubated at 37°C for 1 h with shaking, and then counted in a well-type gamma counter (Auto-Gamma spectrometer, Model 5650; Packard Instruments, Downers Grove, IL) to determine the total radioactivity added. After 3 washes with 1 ml RPMI 1640 medium containing 5% FCS, the tubes were recounted to determine the percentage of radiolabeled antibody bound to the cells. The percentage binding was calculated by dividing the amount of radioactivity bound after washing by the amount added to the tubes. In sequential binding studies, the unbound antibody in the supernatant was then transferred to fresh cells in a tube and the assay repeated. This procedure was repeated a second time.

Mice. Athymic nude CD-1 female mice, 4–5 weeks old, were obtained from Charles River, Inc. (Wilmington, MA) or Taconic Farms, Inc. (Germantown, NY). Mice were kept under sterile conditions in a laminar flow room in cages with filter bonnets and were fed sterilized mouse diet and sterilized tap water.

Establishing Namalwa or MOLT-4 Tumors in Nude Mice. The procedure to establish human B-cell tumors in athymic nude mice was similar to that described by Leonard *et al.* (4). For irradiation, mice were placed in a plastic holder and then exposed to 200 rads ^{60}Co X-irradiation once a week for 3 consecutive weeks. One week following the final dose, animals were given injections s.c. in the flank with a mixture of either 1.2×10^7 Namalwa or 10^7 MOLT-4 cells, and 5×10^6 HT-1080 fibrosarcoma cells in 0.5 ml RPMI 1640 medium containing 10% FCS. HT-1080 cells were X-irradiated with 6000 rads from a ^{60}Co irradiator within 1 h of injection. Tumors began to appear within 2–4 weeks after injection.

Biodistribution and Pharmacokinetic Analysis. *In vivo* tissue distribution and pharmacokinetic modeling were conducted in nude mice bearing Namalwa or CEM tumors following i.p. administration of 0.5 μCi ^{125}I-labeled anti-J5, anti-B1, or anti-T1 antibodies. Groups of at least 5 animals were sacrificed and dissected at 12 and 24 h and then at 2, 4, 5, 6, and 7 days after i.p. injection. Tissues and organs were immediately removed, rinsed with saline, blotted dry, weighed, and placed in counting tubes. The samples of blood, tumor, spleen, liver, heart, lung, kidney, muscle, skin, small intestine, stomach, and femur were counted in a well-type gamma counter (Packard Instruments Model 5650). All animals received potassium iodide in their drinking water starting 2 days prior to administration of radiolabeled antibodies. Results of labeled antibody biodistribution are typically expressed as a percentage of injected dose per g tissue and as tissue/blood ratios of the concentration (cpm per g) in the tissues relative to the blood at selected time points. For pharmacokinetic analysis, tissue concentrations (cpm per g) of the groups of mice at each time point were averaged. The mean concentration *versus* time ($C \times t$) data were analyzed by least squares nonlinear regression to calculate the coefficients and rate constants after fitting these data to the appropriate one- or 2-compartment open model. Concentration (C) data were weighted as $1/(C)^2$. Pharmacokinetic parameters (AUC, mean residence time, blood clearance, tissue, or organ half-life, etc.) were generated using the program RSTRIP (MicroMath, Inc., Salt Lake City, UT).

Imaging Studies. Mice with Namalwa or CEM tumors were given injections i.p. of 30–300 μCi of ^{131}I-labeled anti-J5, anti-B1, or anti-T1 antibody. Scintigraphic imaging was performed at 2, 4, 8, and 11 days after antibody administration. Mice were anesthetized with sodium pentobarbitol, and imaging was then performed using a General Electric 400 ACT gamma camera equipped with a 4-mm pinhole collimator. Analog and digital images were acquired from the dorsal view with the collimator positioned approximately 7 cm from the animal. Images were acquired for approximately 50,000 counts each resulting in imaging times of 600–1,500 s. Digital images were normalized using a General Electric STAR computer to produce visually similar levels of activity in the central torso. Tumor and whole body regions of interest were identified on each mouse image. Total counts per region were used to calculate the T/NT for each time point. Calculations were performed using the formula

$$\frac{T}{NT} = \left(\frac{\text{tumor counts}}{\text{whole body counts} - \text{tumor counts}}\right) \times 100\%.$$

Mice were dissected following the final images and their tissues were counted in a well-type gamma counter. Animals received potassium iodide in their drinking water prior to and following ^{131}I-labeled antibody administration to reduce thyroid uptake.

RESULTS

Radiolabeling of Monoclonal Antibodies and Characterization of the Products. When 200- to 500-μg aliquots of anti-J5, anti-B1, and anti-T1 were labeled with 1–5 mCi of ^{125}I using 5 equivalents of ICl, the efficiency of incorporation of ^{125}I ranged from 16.5–73.3%, and the radiolabeled antibodies had specific activities ranging from 0.19–1.68 mCi/mg as shown in Table 1. When 500 μg aliquots of anti-J5, anti-B1, and anti-T1 were

Table 1 *Radiolabeling of monoclonal antibodies anti-J5, anti-B1, and anti-T1 with ^{125}I and ^{131}I*

Antibody	Radionuclide	Amount labeled (mg)	Efficiency (%)	Specific activity (mCi/mg)
J5	^{125}I	0.2	16.5	0.19
J5	^{125}I	0.25	20.3	1.08
J5	^{131}I	0.5	62.2	1.52
B1	^{125}I	0.2	18.6	0.22
B1	^{125}I	0.5	73.3	0.76
B1	^{131}I	0.5	37.6	1.0
T1	^{125}I	0.25	19.4	0.39
T1	^{125}I	0.5	57.4	1.36
T1	^{131}I	0.5	62.5	1.68

Table 2 *Mean fluorescence intensity of anti-J5, anti-B1, and anti-T1 monoclonal antibodies with human leukemia cell lines*

Cell type	Background	Antibody type		
		J5	B1	T1
Namalwa	45	140	122	57
Raji	34	110	194	52
MOLT-4	31	32	35	150
CEM	31	29	30	175

labeled with 5 mCi of ^{131}I and 5 equivalents of ICl, the efficiency of incorporation of ^{131}I was 62.2, 37.6, and 62.5%, respectively, as shown in Table 1. The specific activities of the ^{131}I-labeled anti-J5, anti-B1, and anti-T1 antibodies were 1.52, 1.06, and 1.68 mCi/mg, respectively.

Chromatograms obtained by HPLC analysis of unlabeled and ^{125}I-labeled anti-J5, anti-B1, and anti-T1 antibodies and gel electrophoresis results indicated homogeneous preparations of unlabeled and radiolabeled products. The ^{125}I- and ^{131}I-labeled products showed minimal (<5%) free iodine in the preparations.

Flow Cytometric Analysis of Anti-J5, Anti-B1, and Anti-T1 Reactivity with Human Leukemia Cells. Reactivity of anti-J5, anti-B1, and anti-T1 antibodies with Namalwa, Raji, CEM, and MOLT-4 cell lines was determined by indirect immunofluorescence analysis. Anti-J5 and anti-B1 bound to Namalwa and Raji cells but not to CEM and MOLT-4 cells, as shown in Table 2. Anti-T1 antibody bound to CEM and MOLT-4 cells, but not to Namalwa and Raji cells. When the anti-J5 and anti-B1 antibodies were subjected to the iodination conditions (without the actual addition of radioiodine), their reactivities by flow cytometric analysis were similar to those of the unmodified antibodies (data not shown). Cells obtained from Namalwa tumors grown in nude mice were found by indirect immunofluorescence to be reactive with anti-J5 and anti-B1 antibodies but were not reactive with anti-T1 antibody (data not shown).

Immunoreactivity of Radiolabeled Monoclonal Antibodies by Live Cell Radioimmunoassay. The immunoreactivity of the radiolabeled anti-J5, anti-B1, and anti-T1 antibody preparations was measured using an *in vitro* live cell radioimmunoassay. The average percentage binding from duplicate samples of ^{125}I-labeled anti-J5 monoclonal antibody to 6×10^6 Namalwa and Raji cells was 31.1 and 17.0%, respectively, while the corresponding binding to CEM and MOLT-4 cells was 3.4 and 2.7%, as shown in Table 3. Namalwa cells bound approximately 7×10^3 molecules of ^{125}I-labeled anti-J5 antibody to the cell surface. The binding of ^{125}I-labeled anti-B1 monoclonal antibody to Namalwa and Raji cells was 11.9 and 26.4%, while the binding to CEM and MOLT-4 cells was 2.6 and 1.4%, respectively. The binding of control ^{125}I-labeled anti-T1 antibody to Namalwa cells was 1.0%, whereas the binding to MOLT-4 cells was 16.3%. In a sequential binding study with ^{125}I-labeled anti-B1 antibody and Raji cells, the binding in 3 successive assays was 26.6, 11.6, and 6.9%, respectively.

Table 3 *Reactivity of ^{125}I-labeled anti-J5, anti-B1, and anti-T1 monoclonal antibodies with human leukemia cell lines by live cell radioimmunoassay*

Leukemia cells (6×10^6) were incubated with 0.04 μCi of radiolabeled antibody, and the average cpm bound per cpm added for several assays each with duplicate samples was determined. The variation between duplicate samples was less than 10%.

Antibody	Radionuclide	% binding			
		Namalwa	Raji	CEM	MOLT-4
J5	^{125}I	31.1	17.0	3.4	2.7
B1	^{125}I	11.9	26.4	2.6	1.4
T1	^{125}I	1.0	4.7	10.2	16.3

Growth of Tumors. In irradiated nude mice given injections of cultured Namalwa and HT-1080 cells, tumors grew in 82 of 104 (79%) mice compared to 29 of 65 (45%) receiving Raji and HT-1080. MOLT-4 tumors grew in 28 of 48 (58%) irradiated mice receiving cultured MOLT-4 and HT-1080 cells. The tumors appeared within 14 days after injection but were quite variable in size and growth rate. The average size of Namalwa and MOLT-4 tumors obtained at the time of dissection (average 34 days after transplantation) for biodistribution and pharmacokinetic analysis was 1.0 g. Necrosis was not seen in tumors of this size.

Biodistribution and Pharmacokinetics of Radiolabeled Monoclonal Antibodies. The relative tissue biodistribution of ^{125}I-labeled anti-J5 monoclonal antibody in antigen-positive, Namalwa tumor-bearing mice at 2, 4, and 7 days after injection is shown in Fig. 1*a*. At 2, 4, and 7 days after i.p. injection, the highest concentration of anti-J5 was in blood and tumor, with relatively small amounts in various normal tissues. Normal lung and spleen had the next highest concentrations of anti-J5 MoAb with peak tissue/blood ratios of 0.6 and 0.7, respectively (Fig. 1*a*). The tumor/blood ratio ranged from 0.7–1.2 over the 7-day period, with the highest ratio at 4 days after injection. There appeared to be no relationship between the percentage of injected dose per g of ^{125}I-labeled anti-J5 in Namalwa tumors and the size of the tumor, for tumors weighing from 0.06–1.56 g. The tissue biodistribution of ^{125}I-labeled anti-J5 in antigen-negative MOLT-4 tumors is shown in Fig. 1*b*. There was no significant uptake of ^{125}I-labeled anti-J5 in MOLT-4 tumors.

The $C \times t$ profiles of ^{125}I-labeled anti-J5 in blood, Namalwa tumor, and other relevant tissues and whole organs are depicted in a semi-log plot of concentration *versus* time in Fig. 2. At the 48-h time point, the tumor $C \times t$ curve crosses the spleen $C \times t$ curve and the blood was thereafter the only tissue with higher concentrations than the tumor. Pharmacokinetic parameters derived by regression analysis and noncompartmental calculations from the $C \times t$ data are summarized in Table 4. These data best fit a 2-compartment open model after correction for the absorption phase from the peritoneum into the systemic circulation. Peak tissue concentrations of anti-J5 in the normal tissues and organs occurred 12 h after i.p. injection whereas peak levels in the Namalwa tumor occurred at 24 h after injection. Terminal elimination half-lives of anti-J5 from blood, liver, spleen and other tissues ranged from 40–50 h while the elimination $t_{1/2}$ from tumor averaged 65.3 h. Total AUC of anti-J5 in blood was approximately 4–9 times the AUC of the other normal tissues. Spleen was the only normal organ with an AUC close to the AUC of tumor and blood. The AUC of tumor was 2- to 5-fold higher than the AUC of liver, kidney, skin, and muscle. The mean residence time, a noncompartmental calculation analogous to "total body" half-life, was greatest for tumor (100.4 h) compared to blood (63.8 h), liver (64 h), or spleen (56.6 h).

The tissue biodistribution of ^{125}I-labeled anti-B1 in groups of

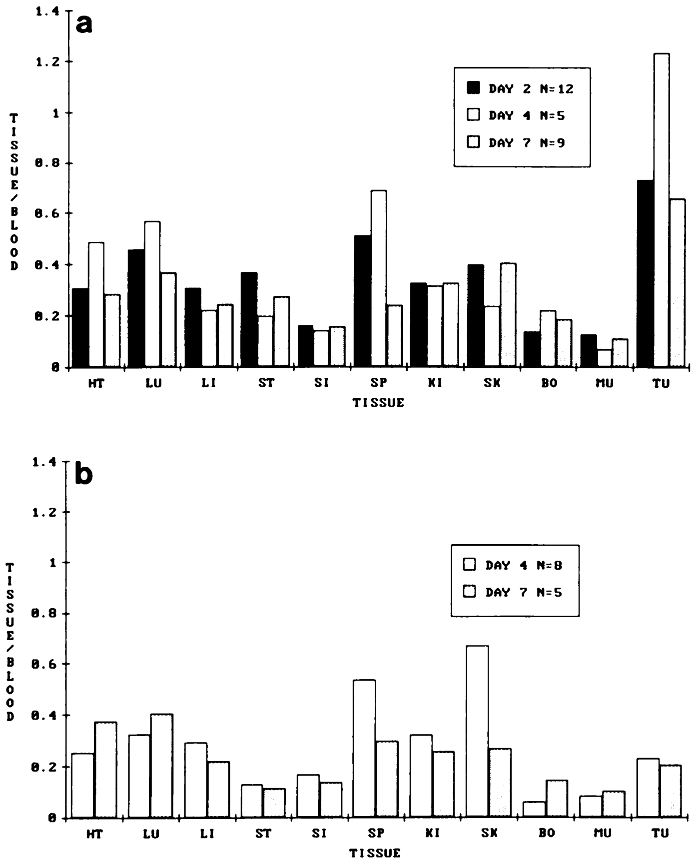

Fig. 1. Biodistribution data. *a*, 0.5 μCi ^{125}I-labeled anti-J5 in athymic nude mice bearing s.c. antigen-positive Namalwa tumors. At 2, 4, and 7 days following i.p. injections tissues were dissected and counted and the ratios of the concentration (cpm per g) in tissues and blood calculated. The SE ranged from 0.02–0.37 at all time points for normal tissues and was 0.12 for tumor uptake at day 2, 0.44 at day 4, and 0.17 at day 7. *b*, 0.5 μCi ^{125}I-labeled anti-J5 in athymic nude mice bearing s.c. antigen-negative MOLT-4 tumors. At 4 and 7 days following i.p. injection the tissues were dissected and counted and the ratios of the concentration (cpm per g) in tissues and blood calculated. The SE for tumor was 0.04. The SE in normal tissues ranged from 0.01–0.40 for both time points. *N*, number of animals per group. *HT*, heart; *LU*, lung; *LI*, liver; *ST*, stomach; *SI*, small intestine; *SP*, spleen; *KI*, kidney; *SK*, skin; *BO*, bone; *MU*, muscle; *TU*, tumor.

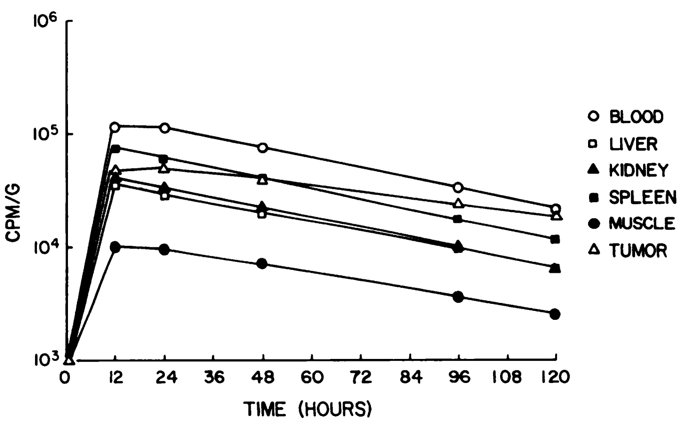

Fig. 2. Pharmacokinetic nonlinear regression analysis of tissue distribution of ^{125}I-labeled anti-J5 in nude mice bearing Namalwa tumors. Five–18 animals/time point were given injections i.p. and the concentrations (*CPM/G*) of radioactivity in tissues and tumor determined.

Namalwa tumor-bearing mice is presented in Fig. 3*a*. The peak concentrations of antibody (at 4 days and later) after administration were in blood and tumor. Six days following i.p. injection more than 5-fold greater activity was found in tumor as compared to normal tissues other than blood. The tumor/blood ratio was 1.2 and 0.7 at 4 and 6 days after injection, respectively.

Table 4 *Pharmacokinetic parameters of tissue concentration (cpm per g) derived by least squares nonlinear regression analysis of ^{125}I-labeled anti-J5 in Namalwa tumor-bearing nude mice*

Mean values of 5–18 animals were determined at 12, 24, 48, 96, and 120 h after injection.

	$t_{1/2}\alpha$ (h)	$t_{1/2}\beta$ (h)	AUC_{0h}^{∞} (cpm/g-h)	Mean residence time (h)	r^a
Blood	3.86	40.4	8.99×10^6	63.8	0.999
Liver	0.81	43.5	2.65×10^6	64.0	0.993
Kidney	0.75	40.8	2.85×10^6	58.8	0.998
Spleen	0.21	39.1	5.23×10^6	56.6	0.992
Muscle	3.52	50.8	0.91×10^6	78.4	0.998
Tumor	5.05	65.3	5.77×10^6	100.4	0.996

[a] Correlation coefficient of regression line.

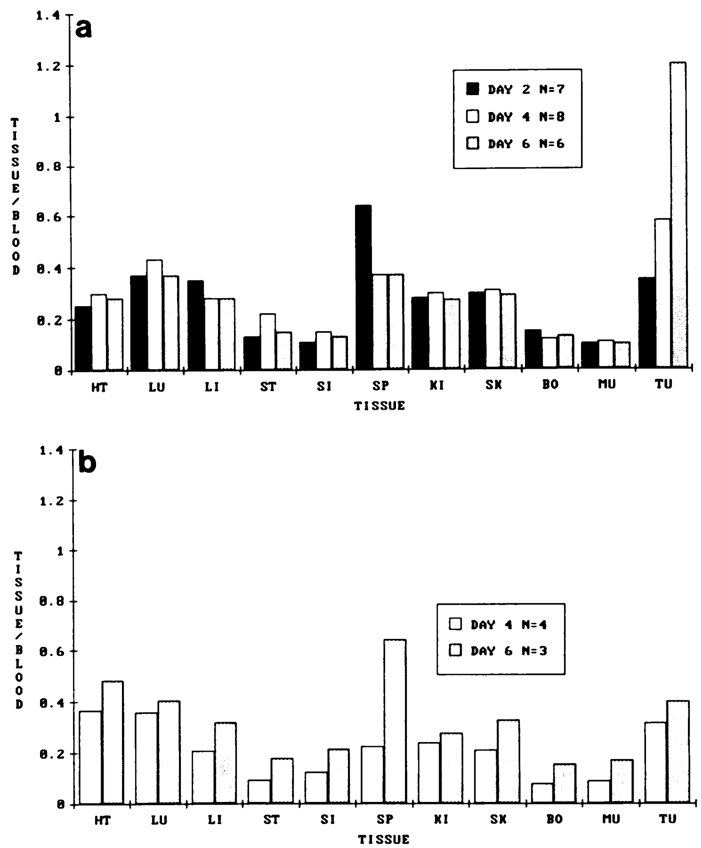

Fig. 3. Biodistribution data. *a*, 0.5 μCi ^{125}I-labeled anti-B1 in athymic nude mice bearing s.c. antigen-positive Namalwa tumors. At 2, 4, and 6 days following i.p. injection tissues were dissected and counted and the ratios of the concentrations (cpm per g) in tissues and blood calculated. The SE for tumor was 0.06, 0.10, and 0.24 at the respective time points and the range for normal tissues at all time points was 0.01–0.07. *b*, 0.5 μCi ^{125}I-labeled anti-B1 in athymic nude mice bearing antigen-negative MOLT-4 tumors. At 4 and 6 days after i.p. injection of labeled antibody, tissues were dissected and counted and the ratios of the concentration (cpm per g) in tissues and blood calculated. The range of SE was 0.01–0.03 for all tissues other than tumor. The SE for tumor uptake on day 4 was 0.04 and on day 6 it was 0.20. *N*, number of animals used per group. *HT*, heart; *LU*, lung; *LI*, liver; *ST*, stomach; *SI*, small intestine; *SP*, spleen; *KI*, kidney; *SK*, skin; *BO*, bone; *MU*, muscle; *TU*, tumor.

The biodistribution of ^{125}I-labeled anti-B1 monoclonal antibody in antigen-negative MOLT-4 tumors is depicted in Fig. 3*b*, showing minimal background uptake in this T-cell tumor.

Biodistribution studies with "control" ^{125}I-labeled anti-T1 showed little uptake in B-cell Namalwa tumors, as shown in Fig. 4. There was 3.5-fold less concentration in Namalwa tumors at 4 days compared to the "targeted" anti-J5 and anti-B1 antibody. The localization of ^{125}I-labeled anti-B1 and ^{131}I-labeled anti-T1 administered as a paired-label mixture in nude mice bearing Namalwa tumors is shown in Fig. 5. The tumor/blood ratio for ^{125}I-labeled anti-B1 was 0.7 and 1.0 at 4 and 6 days after injection, whereas the tumor/blood ratio of ^{131}I-

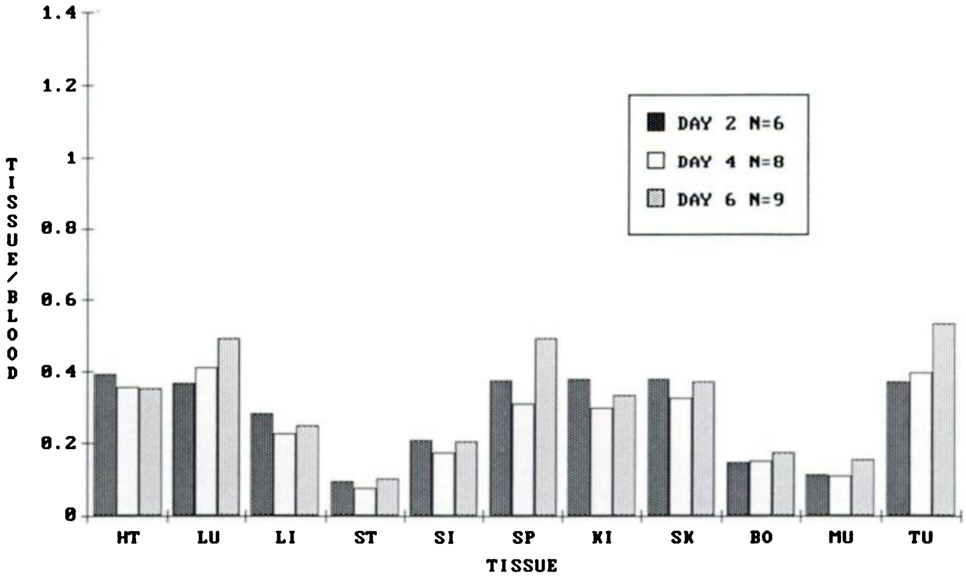

Fig. 4. Biodistribution of 0.5 μCi [125]I-labeled anti-T1 in athymic nude mice bearing s.c. antigen-negative Namalwa tumors. At 2, 4, and 6 days after injection the tissues were dissected and counted and the ratios of the concentration (cpm per g) in tissues and blood calculated. The SE for tumor was 0.05, 0.06, and 0.06, respectively, while the SE for normal tissues ranged from 0.02–0.16. *N*, number of animals used per group. *HT*, heart; *LU*, lung; *LI*, liver; *ST*, stomach; *SI*, small intestine; *SP*, spleen; *KI*, kidney; *SK*, skin; *BO*, bone; *MU*, muscle; *TU*, tumor.

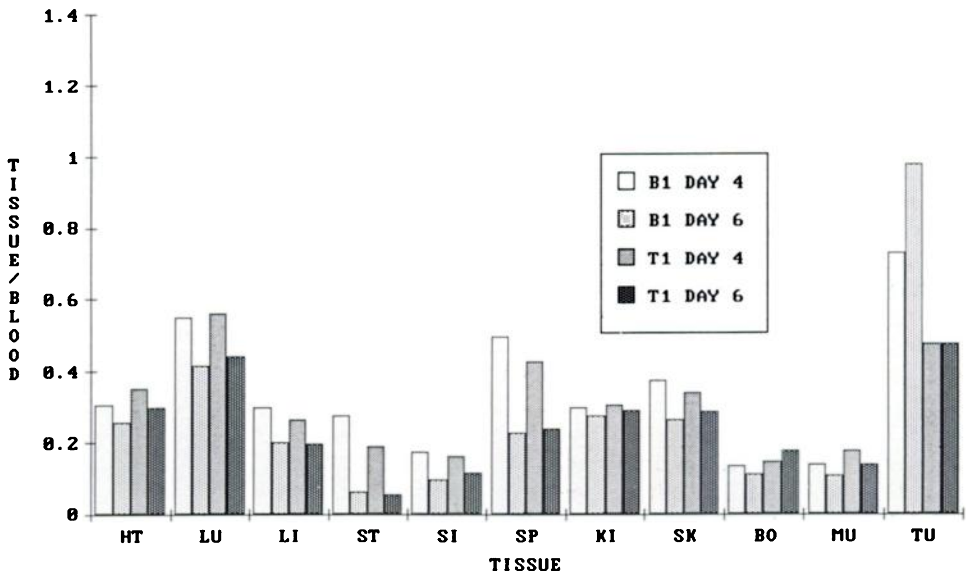

Fig. 5. Biodistribution data for a paired-label experiment in which athymic nude mice bearing Namalwa tumors were given injections i.p. of 0.5 μCi [125]I-labeled anti-B1 and 0.5 μCi of control [131]I-labeled anti-T1. The tissues were dissected and counted and the ratios of the concentration (cpm per g) in tissues and blood calculated. Counts (cpm per g) were corrected for spillover. The SE for tumor uptake by anti-T1 was 0.12 on day 4 and 0.15 on day 6 whereas the SE for anti-B1 was 0.17 on day 4 and 0.26 on day 6. Three animals were used for each time point. *HT*, heart; *LU*, lung; *LI*, liver; *ST*, stomach; *SI*, small intestine; *SP*, spleen; *KI*, kidney; *SK*, skin; *BO*, bone; *MU*, muscle; *TU*, tumor.

labeled anti-T1 was 0.45 at both time points. There was 1.5-fold less concentration of ^{131}I-labeled anti-T1 in Namalwa tumors as compared to ^{125}I-labeled anti-B1 in both the paired-label and unpaired biodistribution studies. The biodistribution of ^{125}I-labeled anti-B1 and ^{131}I-labeled anti-T1 were very similar in normal tissues of Namalwa tumor-bearing nude mice, but the concentrations of anti-B1 in tumor were significantly higher.

Imaging of ^{131}I-labeled Anti-J5, Anti-B1, and Anti-T1 Monoclonal Antibodies in Nude Mice Bearing Namalwa Tumors. The scintigraphic images obtained at 4, 8, and 11 days after injection of ^{131}I-labeled anti-J5 in a nude mouse bearing a Namalwa tumor is shown in Fig. 6, *a–c*. The images show a reduction in the blood pool and normal tissues from 4–11 days after antibody administration. The *T/NT* ratios with anti-J5 were 36, 59, and 74% at 4, 8, and 11 days. Scintigraphic images obtained with ^{131}I-labeled anti-T1 at 4 and 8 days after injection are shown in Fig. 6, *d* and *e*. Fig. 7 shows mouse images obtained after i.p. injection of ^{131}I-labeled anti-B1 (Fig. 7, *a* and *b*) and ^{131}I-labeled anti-T1 (Fig. 7, *c* and *d*). *T/NT* ratios with anti-B1 antibody were 33 and 39% (days 2 and 4). Anti-B1 antibody showed marked preferential tumor uptake while anti-T1 antibody did not. After the imaging, the mouse was dissected and the tissues counted in a well-type gamma counter. The biodistribution of ^{131}I-labeled anti-B1 was similar to that obtained with ^{125}I-labeled anti-B1 which is shown in Fig. 3*a*.

DISCUSSION

The results of the present study indicate that both anti-J5 and anti-B1 radiolabeled MoAbs localized Namalwa tumors growing in athymic nude mice. The tissue distribution of the radiolabeled anti-J5 and anti-B1 to Namalwa tumors was selective in that tumor concentrations far exceeded highly vascularized normal tissue or organ concentrations. The elimination half-lives of the normal tissues and organs were also much shorter than the elimination rate from the antigen-positive s.c. tumor. Tissues such as bone, muscle, and skin showed minimal uptake and more rapid clearance rates compared to the tumor. Peak tumor/blood ratios in the s.c. Namalwa tumors occurred at about 4 days after i.p. injection of anti-J5 and anti-B1 MoAbs. There was no correlation between the uptake of ^{125}I-labeled anti-J5 or anti-B1 in Namalwa tumors and the size of the tumors. Control studies with radiolabeled anti-T1 MoAb in

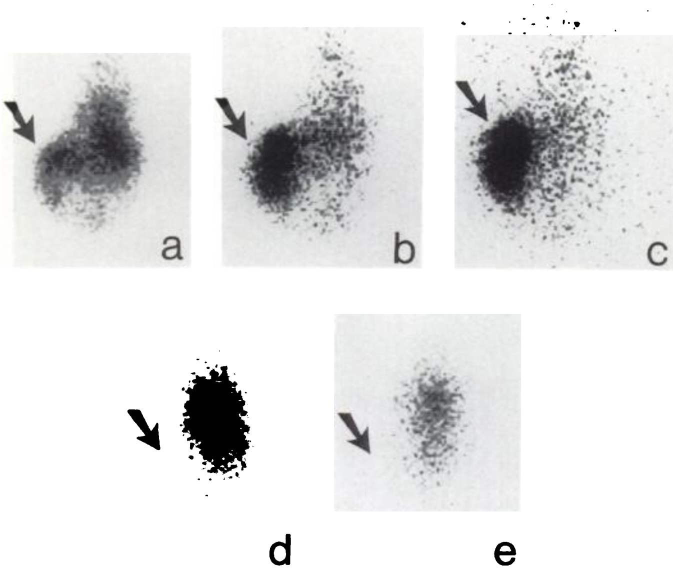

Fig. 6. Whole body scintigraphic images of athymic nude mice bearing s.c. Namalwa tumors (*arrows*) given injections i.p. of 300 μCi of ^{131}I-labeled anti-J5 or ^{131}I-labeled anti-T1 antibody. Dorsal images were taken at 4 (*a*), 8 (*b*), and 11 (*c*) days after administration of labeled anti-J5. Anti-T1 dorsal images were taken at 4 (*d*) and 8 (*e*) days after injection.

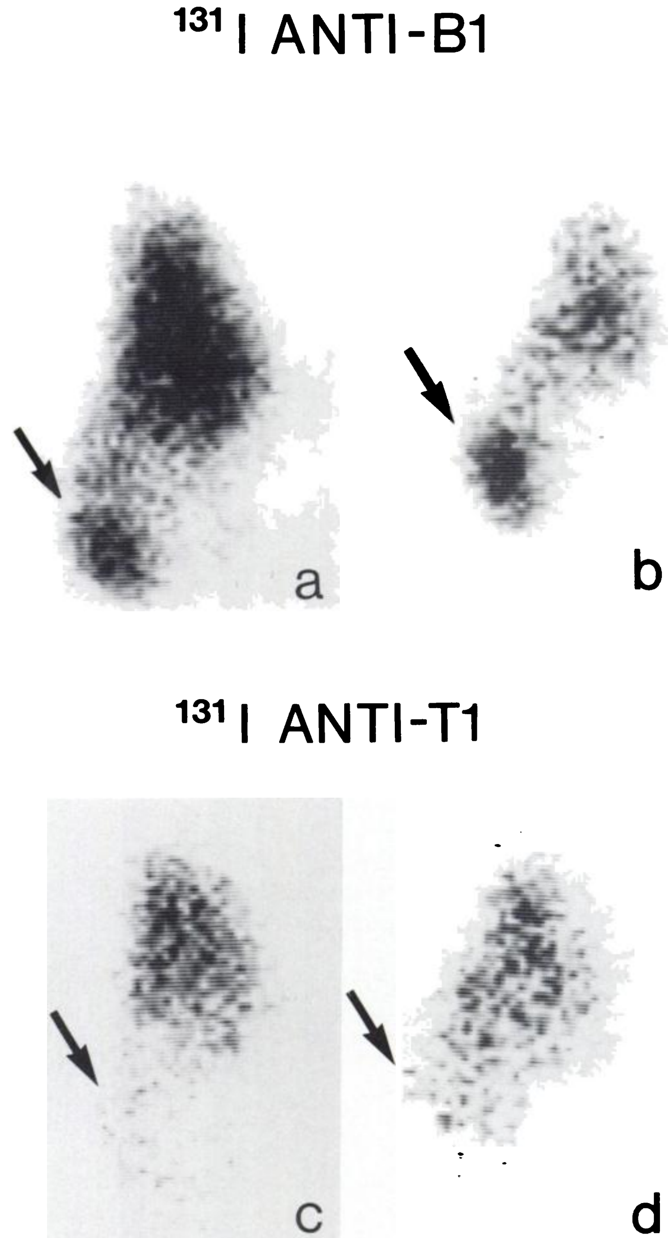

Fig. 7. Whole body scintigraphic images of athymic nude mice bearing s.c. Namalwa tumors (*arrows*) given injections of 30 μCi of ^{131}I-labeled anti-B1 (*a* and *b*) or ^{131}I-labeled anti-T1 (*c* and *d*). Dorsal images were taken at 2 (*a* and *c*) and 4 (*b* and *d*) days after i.p. injection.

athymic mice bearing Namalwa tumors and radiolabeled anti-J5 and anti-B1 MoAbs in mice bearing MOLT-4 tumors indicate that the localization of anti-J5 and anti-B1 in B-cell Namalwa tumors was antibody specific. A similar specificity of binding was obtained against tumor cells *in vitro*.

The immunoreactivity of the various MoAbs after iodination was low, which may have affected the *in vivo* distribution resulting in low concentrations in tumor and increased activity seen in normal tissues such as spleen and blood. However, the *in vitro* binding assays were not done at infinite antigen excess which is a method used to determine immunoreactive fraction. Sequential binding studies indicated that significantly more than 11.9–31.1% of antibody is capable of binding to tumor cells. A correlation between immunoreactive fraction and *in vivo* uptake of radiolabeled MoAb in tumor xenografts has been reported (25). With the exception of a small amount of uptake of antibody in spleen 2 days after i.p. injection, neither anti-B-cell MoAb showed substantial uptake in any normal tissue other than blood. This is again reflected by differences observed in the AUC calculations and mean residence time values. Radiolabeled anti-T1 showed minimal uptake and binding to the Namalwa B-cell tumor line.

A feature of many lymphomas is that they are radiosensitive, chemosensitive, and have a better supply of blood than carcinomas and fibrosarcomas (26, 27). The increased exposure (increased AUC and increased half-lives) of the tumor to therapeutic drugs and radionuclides when attached to antibodies directed at antigens on the tumor cells may provide an enhanced therapeutic effect, especially with cycle or phase specific agents like radiation and many chemotherapeutic drugs. The prolonged distribution of radiolabeled antibody in blood would likely result in a significant radiation dose delivered to all vascular organs if a therapeutic dose of radionuclide was administered. The AUC determination may take this into account but may not reflect cumulative dose if a short half-life radionuclide is used for conjugation. Tumor localization of radiolabeled polyclonal antibodies has been demonstrated in experimental lymphoma model systems (28, 29). Radiolabeled MoAbs have been used to localize experimental murine lymphoma (30) and Burkitt's lymphoma in athymic nude mice (31). MoAbs labeled with ^{131}I have been used for radioimmunotherapy of leukemia/lymphoma in experimental animal models (30, 32–34). Clinical localization and therapy studies have been reported with ^{131}I- and ^{123}I-labeled Lym-1 antibody against human B-cell lymphomas (31, 35–37).

Imaging with monoclonal antibodies is potentially important for a number of reasons. First of all, improved images could be used for diagnosis, staging, and designing therapies for patients with B-cell lymphomas. Furthermore, they could be used for following patients on therapy. In addition, antibody labeled with radionuclides may be more specific for localizing tumors than current standard techniques, because they may not only discriminate the size and shape of lymph nodes, but also specifically identify tumor cells in lymph nodes (anti-J5). In some cases, lymph nodes are distorted following therapy due to fibrosis yet there are no tumor cells in the node. Current techniques are incapable of discriminating this. Finally, imaging studies with ^{131}I can be extended into therapy trials by increasing the amount of radionuclide conjugated to the antibody and ^{111}In chemistry can be used to model for ^{90}Y which can be used for therapy. Successful preliminary trials utilizing such techniques have been reported (38).

^{131}I-labeled anti-B1 MoAb was used to image B-cells in spleens of rhesus monkeys (39). This antibody does not modulate antigen on the surface of lymphocytes or leukemic cells (40). The anti-J5 MoAb has been used in passive serotherapy studies (14, 15), and it is known to modulate the expression of CALLA on the cell surface of acute lymphoblastic leukemia cells with internalization of antibody and antigen (41, 42). There are reports that the CALLA antigen is shed *in vitro* (43) and that it is present in the circulation of patients (44). The extent to which circulating CALLA antigen may have affected the biodistribution of anti-J5 antibody in the present studies is unknown. The clinical therapeutic trials with anti-J5 and other unlabeled MoAbs against leukemia and lymphoma (13–15, 45–50) have demonstrated that antibody binding to tumor cells and clinical effects can be observed, although usually of short duration. Problems of antigenic modulation, circulating antigen, and host immune response to the mouse MoAb have limited the success of this approach. Nevertheless, the successful treatment with an anti-idiotype MoAb of one patient with recurrent B-cell lymphoma (45) points to the potential promise of this approach.

Diagnostic studies for patients with T-cell non-Hodgkin's lymphoma have been reported utilizing T101 MoAb (anti-CD5). CD5 is expressed on the malignant T-cells associated with CTCL and to a lesser extent on normal T-cells (16), which theoretically should result in more favorable localization of CTCL involved nodes. Diagnostic studies utilizing ^{111}In-labeled T101 antibody clearly demonstrated uptake by CTCL involved nodes with minimal imaging of normal lymph nodes (17).

Therapy trials with radiolabeled MoAbs for patients with B- and T-cell non-Hodgkin's lymphoma have recently begun. In one trial, 5 of 6 cutaneous T-cell lymphoma patients treated with 105–250 mCi ^{131}I-labeled T101 MoAb responded to therapy with 2 partial and 3 minor responses (51). Myelosuppression was the dose limiting toxicity seen in patients receiving the higher therapeutic doses of ^{131}I-labeled T101 antibody (51). Preliminary results with the Lym-1 antibody radiolabeled with ^{131}I also demonstrated responses in 7 of 9 patients with B-cell non-Hodgkin's lymphoma (35–37). While Lym-1 reacts with some normal B-cells, the intensity of reactivity is less than that for malignant B-cells (37). No evidence of radiation toxicity was observed in the marrow, liver, and kidneys of patients receiving ^{131}I-labeled Lym-1 antibody. Similar to the Lym-1 antibody, the anti-B1 antibody also cross-reacts with normal B-cells which could limit its clinical utility. This cross-reactivity is not a problem in the xenograft model because the antibody does not cross-react with normal murine B-cells. Another MoAb recognizing an antigen expressed by mature human normal and malignant B-cells is the MB-1 antibody (52). Despite MB-1 antigen expression on mature B-cells, Press *et al.* (53) reported therapeutic efficacy in 2 patients with B-cell lymphoma administered ^{131}I-labeled MB-1 antibody (250 and 480 mCi). Dose limiting toxicity was neutropenia and thrombocytopenia. One possible limitation in the use of the anti-B1 MoAb for clinical diagnosis and therapy of B-cell lymphomas is its cross-reactivity with normal B-cells. However, these results plus the experience with other T- and B-cell MoAbs which also cross-react with normal lymphocytes suggest that the anti-B1 antibody may still be useful clinically. However, more specific MoAbs would be expected to show improved localization in patients with lymphoma. These encouraging clinical results by other investigators support our ongoing preclinical trials with antibodies directed against B-cell lymphomas and will lead to clinical trials with these antibodies.

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REFERENCES

1. Foon, K. A., and Todd, R. F., III. Immunologic classification of leukemia and lymphoma. Blood, *68:* 1–31, 1986.
2. Ziegler, H. W. L., Frizzera, G., and Bach, F. H. Successful transplantation of a human leukemia cell line into nude mice: conditions optimizing graft acceptance. J. Natl. Cancer Inst., *68:* 15–18, 1982.
3. Dillman, R. O., Johnson, D. E., Shawler, D. L., Halpern, S. E., Leonard, J. E., and Hagan, P. L. Athymic mouse model of a human T-cell tumor. Cancer Res., *45:* 5632–5636, 1985.
4. Leonard, J. E., Johnson, D. E., Felsen, R. B., Tanney, L. E., Royston, I., and Dillman, R. O. Establishment of a human B-cell tumor in athymic mice. Cancer Res., *47:* 2899–2902, 1987.
5. Stashenko, P., Nadler, L. M., Hardy, R., and Schlossman, S. F. Characterization of a human B lymphocyte-specific antigen. J. Immunol., *125:* 1678–1685, 1980.
6. Nadler, L. M., Stashenko, P., Hardy, R., van Agthoven, A., Terhorst, C., and Schlossman, S. F. Characterization of a human B cell specific antigen (B2) distinct from B1. J. Immunol., *126:* 1941–1947, 1981.
7. Brooks, D. A., Beckman, I., Bradley, J., McNamara, P. J., Thomas, M. E., and Zola, H. Human lymphocyte markers defined by antibodies derived from somatic cell hybrids. I. A hybridoma secreting antibody against a marker specific for human B lymphocytes. Clin. Exp. Immunol., *39:* 477–485, 1980.
8. Brooks, D. A., Beckman, G. R., Bradley, J., McNamara, P. J., Thomas, M., and Zola, H. Human lymphocyte markers defined by antibody derived from somatic cell hybrids. IV. A monoclonal antibody reacting specifically with a subpopulation of human B lymphocytes. J. Immunol., *126:* 1373–1377, 1981.
9. Nadler, L. M., Anderson, K. C., Marti, G., Bates, M., Park, E., Daley, J. F., and Schlossman, S. F. B4, a human B lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. J. Immunol., *131:* 244–250, 1983.
10. Ritz, J., Pesando, J. M., Notis-McConarty, J., Lazarus, H., and Schlossman, S. F. A monoclonal antibody to human acute lymphoblastic leukemia antigen. Nature (Lond.), *283:* 583–585, 1980.
11. Ritz, J., Sallan, S. E., Bast, Jr., R. C., Lipton, J. M., Clavell, L. A., Feeney, M., Hercend, T., Nathan, D. G., and Schlossman, S. F. Autologous bone-marrow transplantation in CALLA-positive acute lymphoblastic leukaemia after *in vitro* treatment with J5 monoclonal antibody and complement. Lancet, *2:* 60–63, 1982.
12. Nadler, L. M., Takvorian, T., Botnick, L., Bast, R. C., Finberg, R., Hellman, S., Canellos, G. P., and Schlossman, S. F. Anti-B1 monoclonal antibody and complement treatment in autologous bone-marrow transplantation for relapsed B-cell non-Hodgkin's lymphoma. Lancet, *2:* 427–431, 1984.
13. Press, O. W., Appelbaum, F., Ledbetter, J. A., Martin, P. J., Zarling, J., Kidd, P., and Thomas, E. D. Monoclonal antibody 1FS (anti-CD20) serotherapy of human B cell lymphomas. Blood, *69:* 584–591, 1987.
14. Ritz, J., Pesando, J. M., Sallan, S. E., Clavell, L. A., Notis-McConarty, J., Rosenthal, P., and Schlossman, S. F. Serotherapy of acute lymphoblastic leukemia with monoclonal antibody. Blood, *58:* 141–152, 1981.
15. Ritz, J., Pesando, J. M., Notis-McConarty, J., Clavell, L. A., Sallan, S. E., and Schlossman, S. F. Use of monoclonal antibodies as diagnostic and therapeutic reagents in acute lymphoblastic leukemia. Cancer Res., *41:* 4771–4775, 1981.
16. Bunn, Jr., P. A., Carrasquillo, J. A., Keenan, A. M., Schroff, R. W., Foon, K. A., Hsu, S. M., Gazdar, A. F., Reynolds, J. C., Perentesis, P., and Larson, S. M. Imaging of T-cell lymphoma by radiolabelled monoclonal antibody. Lancet, *2:* 1219–1221, 1984.
17. Carrasquillo, J. A., Bunn, P. A., Jr., Keenan, A. M., Reynolds, J. C., Schroff, R. W., Foon, K. A., Ming-Hsu, S., Gazdar, A. F., Mulshine, J. L., Oldham, R. K., Perentesis, P., Horowitz, M., Eddy, J., James, P., and Larson, S. M. Radioimmunodetection of cutaneous T-cell lymphoma with ^{111}In-labeled T101 monoclonal antibody. N. Engl. J. Med., *315:* 673–680, 1986.
18. Reinherz, E. L., Kung, P. C., Goldstein, G., and Schlossman, S. F. A monoclonal antibody with selective reactivity with functionally mature thymocytes and all peripheral human T cells. J. Immunol., *123:* 1312–1317, 1979.
19. Buchsbaum, D., Randall, B., Hanna, D., Chandler, R., Loken, M., and Johnson, E. Comparison of the distribution and binding of monoclonal

antibodies labeled with 131-iodine or 111-indium. Eur. J. Nucl. Med., *10:* 398–402, 1985.

20. Contreras, M. A., Bale, W. F., and Spar, I. L. Iodine monochloride (ICl) iodination techniques. Methods Enzymol., *92:* 277–292, 1983.
21. Minowada, J., Koshiba, H., Sagawa, K., Kubonishi, I., Lok, M. S., Tatsumi, E., Han, T., Srivastava, B. I. S., and Oknuma, T. Marker profiles of human leukemia and lymphoma cell lines. J. Cancer Res. Clin. Oncol., *101:* 91–100, 1981.
22. Pulvertaft, R. V. J. Cytology of Burkitt's tumor (African lymphoma). Lancet, *1:* 238–240, 1964.
23. Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., Boone, B. A., and McCarthy, R. E. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. Cancer (Phila.), *18:* 522–529, 1965.
24. Minowada, J., Oknuma, T., and Moore, G. E. Brief communication: rosette forming human lymphoid cell lines. 1. Establishment and evidence for origin of thymus-derived lymphocytes. J. Natl. Cancer Inst., *49:* 891–895, 1972.
25. Pimm, M. V., and Baldwin, R. W. Comparative tumour localization properties of radiolabelled monoclonal antibody preparations of defined immunoreactivities. Eur. J. Nucl. Med., *13:* 348–352, 1987.
26. Mantyla, M., Kuikka, J., and Rekonen, A. Regional blood flow in human tumours with special reference to the effect of radiotherapy. Br. J. Radiol., *49:* 335–338, 1976.
27. DeVita, Jr., V. T., Hellman, S., and Rosenberg, S. A. (eds.), Cancer, Principles and Practice of Oncology, Ed. 2. Philadelphia: J. B. Lippincott Company, 1985.
28. Korngold, L., and Pressman, D. The localization of antilymphosarcoma antibodies in the Murphy lymphosarcoma of the rat. Cancer Res., *14:* 96–99, 1954.
29. Kitano, M., Mihich, E., and Pressman, D. Antigenic differences between leukemia L1210 and a subline resistant to methylglyoxal-bis-(guanylhydrazone). Cancer Res., *32:* 181–186, 1972.
30. Badger, C. C., Krohn, K. A., Peterson, A. V., Shulman, H., and Bernstein, I. D. Experimental radiotherapy of murine lymphoma with ^{131}I-labeled anti-Thy 1.1 monoclonal antibody. Cancer Res., *45:* 1536–1544, 1985.
31. Epstein, A. L., Zimmer, A. M., Spies, S. M., Mills, S., DeNardo, G., and DeNardo, S. Radioimmunodetection of human B-cell lymphomas with a radiolabeled tumor-specific monoclonal antibody (Lym-1). *In*: F. Cavalli, G. Bonadonna, and M. Rozencweig (eds.), Malignant Lymphomas and Hodgkin's Disease: Experimental and Therapeutic Advances, pp. 569–577. Boston: Martinus Nijoff Publishing Co., 1985.
32. Badger, C. C., Krohn, K. A., Shulman, H., Flournoy, N., and Bernstein, I. D. Experimental radioimmunotherapy of murine lymphoma with ^{131}I-labeled anti-T-cell antibodies. Cancer Res., *46:* 6223–6228, 1986.
33. Adams, D. A., DeNardo, G. L., DeNardo, S. J., Matson, G. B., Epstein, A. L., and Bradbury, E. M. Radioimmunotherapy of human lymphoma in athymic, nude mice as monitored by P-31 nuclear magnetic resonance. Biochem. Biophys. Res. Commun., *131:* 1020–1027, 1985.
34. Redwood, W. R., Tom, T. D., and Strand, M. Specificity, efficacy, and toxicity of radioimmunotherapy in erythroleukemic mice. Cancer Res., *44:* 5681–5687, 1984.
35. DeNardo, S. J., DeNardo, G. L., O'Grady, L. F., Macey, D. J., Mills, S. L., Epstein, A. L., Peng, J.-S., and McGahan, J. P. Treatment of a patient with B cell lymphoma by I-131 LYM-1 monoclonal antibodies. Int. J. Biol. Markers, *1:* 49–53, 1987.
36. DeNardo, S. J., DeNardo, G. L., O'Grady, L. F., Macey, D. J., Mills, S. L., Peng, J.-S., Stewart, S. L., Epstein, A. L., and McGahan, J. P. Radioimmunotherapy of patients with B-cell lymphoma using I-131 LYM-1 MAb. J. Nucl. Med., *27:* 903 Abstr., 1986.
37. DeNardo, S. J., DeNardo, G. L., O'Grady, L. F., Levy, N. B., Mills, S. L., Macey, D. J., McGahan, J. P., Miller, C. H., and Epstein, A. L. Pilot studies of radioimmunotherapy of B cell lymphoma and leukemia using I-131 Lym-1 monoclonal antibody. Antibody Immunoconjugates, and Radiopharm. *1:* 17–33, 1988.
38. Anderson-Berg, W. T., Squire, R. A., and Strand, M. Specific radioimmunotherapy using ^{90}Y-labeled monoclonal antibody in erythroleukemic mice. Cancer Res., *47:* 1905–1912, 1987.
39. Letvin, N. L., Zalutsky, M. R., Lambert, J. M., Chalifoux, L. V., Nadler, L. M., Schlossman, S. F., and Atkins, H. L. Use of radiolabeled monoclonal anti-B1 antibody for B lymphocyte imaging in rhesus monkeys. Nucl. Med. Biol., *14:* 99–105, 1987.
40. Boyd, A. W., Anderson, K. C., Freedman, A. S., Fisher, D. C., Slaugenhaupt, B., Schlossman, S. F., and Nadler, L. M. Studies of *in vitro* activation and differentiation of human B lymphocytes. I. Phenotypic and functional characterization of the B cell population responding to anti-Ig antibody. J. Immunol., *134:* 1516–1523, 1985.
41. Ritz, J., Pesando, J. M., Notis-McConarty, J., and Schlossman, S. F. Modulation of human acute lymphoblastic leukemia antigen induced by monoclonal antibody *in vitro*. J. Immunol., *125:* 1506–1514, 1980.
42. Pesando, J. M., Tomaselli, K. J., Lazarus, H., and Schlossman, S. F. Distribution and modulation of a human leukemia-associated antigen (CALLA). J. Immunol., *131:* 2038–2045, 1983.
43. Komada, Y., Peiper, S., Tarnowski, B., Melvin, S., Kamiya, H., and Sakurai, M. Shedding of the common acute lymphoblastic leukemia antigen (CALLA) by lymphoblastoid cell lines. Leuk. Res., *10:* 665–670, 1986.
44. Losa, G., Heumann, D., Carrel, S., von Fliedner, V., and Mach, J.-P. Characterization of membrane vesicles circulating in the serum of patients with common acute lymphoblastic leukemia. Lab. Invest., *55:* 573–579, 1986.
45. Miller, R. A., Maloney, D. G., Warnke, R., and Levy, R. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. N. Engl. J. Med., *306:* 517–522, 1982.
46. Meeker, T. C., Lowder, J., Maloney, D. G., Miller, R. A., Thielemans, K., Warnke, R., and Levy, R. A clinical trial of anti-idiotype therapy for B cell malignancies. Blood, *65:* 1349–1363, 1985.
47. Nadler, L. M., Stashenko, P., Hardy, R., Kaplan, W. D., Button, L. N., Kufe, D. W., Antman, K. H., and Schlossman, S. F. Serotherapy of a patient with a monoclonal antibody directed against a human lymphoma-associated antigen. Cancer Res., *40:* 3147–3154, 1980.
48. Foon, K. A., Schroff, R. W., Bunn, P. A., Mayer, D., Abrams, P. G., Fer, M., Ocho, J., Bottino, G. C., Sherwin, S. A., Carlo, D. J., Herberman, R. B., and Oldham, R. K. Effects of monoclonal antibody therapy in patients with chronic lymphocytic leukemia. Blood, *64:* 1085–1093, 1984.
49. Dillman, R. O., Shawler, D. L., Dillman, J. B., Royston, R. Therapy of chronic lymphocytic leukemia and cutaneous T-cell lymphoma with T101 monoclonal antibody. J. Clin. Oncol., *2:* 881–891, 1984.
50. Bertram, J. H., Gill, P. S., Levine, A. M., Boquiren, D., Hoffman, F. M., Meyer, P., and Mitchell, M. S. Monoclonal antibody T101 in T cell malignancies: a clinical, pharmacokinetic, and immunologic correlation. Blood, *68:* 752–761, 1986.
51. Rosen, S. T., Zimmer, A. M., Goldman-Leikin, R., Gordon, L. I., Kazikiewicz, J. M., Kaplan, E. H., Variakojis, D., Marder, R. J., Dykewicz, M. S., Piergies, A., Silverstein, E. A., Roenigk, Jr., H. H., and Spies, S. M. Radioimmunodetection and radioimmunotherapy of cutaneous T-cell lymphomas using an ^{131}I-labeled monoclonal antibody: an Illinois Cancer Council study. J. Clin. Oncol., *5:* 562–573, 1987.
52. Link, M. P., Bindl, J., Meeker, T. C., Carswell, C., Doss, C. A., Warnke, R. A., and Levy, R. A unique antigen on mature B cells defined by a monoclonal antibody. J. Immunol., *137:* 3013–3018, 1986.
53. Press, O., Badger, C., Eary, J., Fisher, D. R., Appelbaum, F., Brown, S., Martin, P., Nelp, W., Davis, J., Miller, R., Thomas, E. D., Levy, R., and Bernstein, I. Radioimmunotherapy of refractory human malignant B cell lymphomas (abstract). Blood, *70* (*Suppl.* 1)*:* 248, 1987.