The Journal of NUCLEAR MEDICINE

Radioimmunotherapy Targeting of HER2/neu Oncoprotein on Ovarian Tumor Using Lead-212-DOTA-AEI

Eva Horak, Frank Hartmann, Kayhan Garmestani, Chuanchu Wu, Martin Brechbiel, Otto A. Gansow, Nicholas F. Landolfi and Thomas A. Waldmann

J Nucl Med. 1997;38:1944-1950.

The Journal of Nuclear Medicine is published monthly.
SNMMI | Society of Nuclear Medicine and Molecular Imaging
1850 Samuel Morse Drive, Reston, VA 20190.
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)

22. Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. *Science* 1977;197:893–895.
23. Liotta LA, Kleineman J, Saidel GM. Quantitative relationships of intravascular tumor cells, tumor vessels and pulmonary metastases following tumor implantation. *Cancer Res* 1974;34:997–1004.
24. Terranova VP, Hujanen ES, Martin GR. Basement membrane and the invasive activity of metastatic tumor cells. *J Natl Cancer Inst* 1986;77:311–316.
25. Fishman AJ, Khaw BA, Strauss HW. Quo vadis radioimmune imaging. *J Nucl Med* 1989;30:1911–1915.
26. Iwamoto Y, Nomizu M, Yamada-Y, Ito Y, Tanaka K, Sugioka Y. Inhibition of angiogenesis, tumour growth and experimental metastasis of human fibrosarcoma cells HT1080 by a multimeric form of the laminin sequence Tyr-Ile-Gly-Ser-Arg (YIGSR). *Br J Cancer* 1996;73:589–595.
27. Hill RP. Metastasis. In: Tannock IF, Hill RP, eds. *The basic science of oncology*, 2nd ed. New York: McGraw-Hill; 1992:178–195.
28. Hanna N. Role of natural killer cells in host defense against cancer metastasis. In: Nicolson GL, Milas L, eds. *Cancer invasion and metastasis: biologic and therapeutic aspects*. New York: Raven Press; 1984:309–319.
29. Greenberg AH, Egan SE, Jarolin L, et al. Natural killer cell regulation of implantation and early lung growth of H-*ras*-transformed 10T1/2fibroblasts in mice. *Cancer Res* 1987;47:4801–4805.

Radioimmunotherapy Targeting of HER2/neu Oncoprotein on Ovarian Tumor Using Lead-212-DOTA-AE1

Eva Horak, Frank Hartmann, Kayhan Garmestani, Chuanchu Wu, Martin Brechbiel, Otto A. Gansow, Nicholas F. Landolfi and Thomas A. Waldmann

Metabolism Branch and Inorganic and Radioimmune Chemistry Section, Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland; and Protein Design Labs, Inc., Mountain View, California

The specificity, toxicity and efficacy of lead (^{212}Pb) radioimmunotherapy were evaluated in nude mice bearing the SK-OV-3 human ovarian tumor cell line expressing the HER2/*neu* proto-oncogene. **Methods:** The therapeutic agent used was the tumor-specific anti-HER2/neu monoclonal antibody AE1 conjugated to ^{212}Pb, ^{212}Bi being the daughter and thus the source of the alpha-particle and beta emissions. A bifunctional derivative of tetraazacyclododecanetetraacetic acid (p-SCN-Bz-DOTA) was used to couple ^{212}Pb to the anti-HER2/neu monoclonal antibody AE1. The chelating agent did not alter the binding affinity to its antigenic target or the pharmacokinetics and tissue distribution of the AE1 antibody. Toxicity and therapeutic efficacy of ^{212}Pb-AE1 were evaluated in nude mouse ascites or solid tumor models, wherein SK-OV-3 cells were administered i.p. or s.c., respectively. **Results:** The dose-limiting acute toxicity after i.v. administration of ^{212}Pb-AE1 was bone marrow suppression, which was observed at doses above 25 μCi. Therefore, doses of 10 and 20 μCi were used in efficacy trials. The i.p. administration of ^{212}Pb-AE1 3 days after i.p. tumor inoculation led to a significant ($p_2 = 0.015$) prolongation of tumor-free survival. In a second model, i.v. treatment with ^{212}Pb-AE1 3 days after s.c. tumor inoculation prevented subsequent tumor development in all animals treated with 10 or 20 μCi of ^{212}Pb-AE1 ($p_2 = 0.002$ compared to control groups). This efficacy in the adjuvant setting was antibody specific because treatments with equivalently labeled control antibody or unlabeled AE1 antibody or no treatment were less effective. The rate of growth of small (mean tumor volume, 15 mm^3) SK-OV-3 tumors was modestly inhibited. However, tumor growth was not inhibited in mice bearing larger (mean tumor volume, 146 mm^3) SK-OV-3 tumors by the administration of a single dose of 10 or 20 μCi of ^{212}Pb-AE1. **Conclusion:** Lead-212-AE1 as an intact radiolabeled monoclonal antibody may be of only modest value in the therapy of bulky solid tumors due to the short physical half-life of ^{212}Pb and time required to achieve a useful tumor-to-normal tissue ratio of radionuclide after administration. However, the radiolabeled monoclonal antibody may be useful in therapy of tumors in the adjuvant setting. Furthermore, ^{212}Pb may be of value in select situations, including treatment of leukemia, intercavitary therapy or strategies that target vascular endothelial cells of tumors.

Received Jul. 23, 1996; revision accepted Feb. 3, 1997.
For correspondence or reprints contact: Thomas A. Waldmann, MD, Metabolism Branch, National Cancer Institute, National Institutes of Health, Building 10, Room 4N115, Bethesda, MD 20892.

Key Words: HER2; *neu* proto-oncogene; alpha-emitting radionuclides; monoclonal antibody therapy; ovarian tumor

J Nucl Med 1997; 38:1944–1950

Monoclonal antibodies targeted to cell surface antigens have been used to treat patients with a variety of cancers. However, unmodified monoclonal antibodies have been relatively ineffective (*1*). One of the factors in this low therapeutic efficacy is that most of the monoclonal antibodies used are not effective cytocidal agents against human neoplastic cells. Furthermore, in most cases, the antibodies were not directed against a vital structure on the surface of malignant cells, such as a growth factor receptor involved in tumor cell proliferation and survival. In an attempt to circumvent these problems, researchers have augmented the cytotoxic action of monoclonal antibodies by arming them with toxins or radionuclides (*2–6*). Furthermore, cell surface antigenic targets, especially receptors for growth factors, have been defined for more effective monoclonal antibody action. In particular, the HER2/neu receptor, the product of the c-*erb*B-2 proto-oncogene, has been the target of monoclonal antibody therapeutic trials (*7–12*). The HER2/*neu* oncogene (*erb*B-2) encodes a M_r 185,000 transmembrane phosphoglycoprotein. The HER2/*neu* gene is overexpressed in 20%–30% of adenocarcinomas of the breast, ovary, lung and stomach and has been linked to poor prognosis (*13–15*). A series of anti-HER2/neu monoclonal antibodies has been generated that includes antibodies without biological activity, as well as those with agonist action in terms of tyrosine phosphorylation of HER2/neu (*7–12*). Preclinical efficacy in mice bearing HER2/neu-expressing human tumor xenografts has been demonstrated with certain antibodies directed toward this receptor.

Although unmodified murine antibodies to the HER2/neu oncoprotein inhibited tumor growth in certain cases, in general it was not sufficient to cure animals of established tumors. This limited efficacy led to alternative approaches that include the humanization of an anti-HER2/neu antibody (*12,16*), the development of a humanized bispecific $F(ab')_2$ fragment for

retargeting cytotoxic T cells (*17,18*), a disulfide stabilized anti-HER2/neu *Pseudomonas* immunotoxin (*11*) and a disulfide-stabilized anti-HER2/neu Fv-beta-lactamase fusion protein for the activation of a cephalosporin doxorubicin prodrug (*19*). In addition, anti-HER2/neu antibodies have been used as carriers of toxic agents, including radionuclides. De Santes et al. (*10*) demonstrated that animals treated with 400–700 μCi of ^{131}I-anti-HER2/neu monoclonal antibodies manifested a marked inhibition of the growth of large tumors. In general, ^{131}I-labeled monoclonal antibodies have been relatively ineffective due to limitations of this radionuclide as a therapeutic agent.

In light of the limitations of radioiodine, metallic radionuclides that can be linked to antibodies may provide a better choice. Antibodies may be armed with beta- or alpha-emitting radionuclides. Future development of radionuclide-conjugated monoclonal antibodies may focus on radionuclides that emit alpha particles, which may be the most effective agents at killing tumor targets without damaging distant normal tissues (*2–6*).

Our present studies focused on the radionuclide ^{212}Pb, which has a 10.6-hr half-life. Lead-212 is the parent of ^{212}Bi, which is the source of an alpha particle as well as beta and gamma emissions. In this study, we used ^{212}Pb-tetraazacyclododecanetetraacetic acid-AE1 (^{212}Pb-DOTA-AE1) directed toward SK-OV-3 tumors in our therapeutic trials. The goals of this study were: to determine specificity and pharmacokinetics, including biodistribution of the DOTA chelate AE1 anti-HER2/neu monoclonal antibody in our experimental tumor model; to define the toxicity of the ^{212}Pb-DOTA-AE1 monoclonal antibody conjugate and, thereby, establish the maximum tolerated dose; and to define the efficacy, specificity and toxicity of ^{212}Pb radioimmunotherapy of a human HER2/neu-expressing tumor SK-OV-3 in nude mice.

MATERIALS AND METHODS

Cell Lines and Monoclonal Antibodies

The SK-OV-3 cell line (ATCC HTB 77), derived from a human ovarian adenocarcinoma, was used. AE1 is an IgG2a murine monoclonal antibody directed against the extracellular domain of the HER2/neu receptor. The previously described anti-Tac, an IgG2a murine monoclonal antibody that binds to the human IL-2Ralpha subunit, but not to murine SK-OV-3 cells, was used as a negative control antibody (*20*).

Radioiodination of Monoclonal Antibodies

Radioiodinations of AE1 for bindability studies with ^{125}I were performed using a modification of the chloramine-T method; the resultant specific activity was 4.0–6.5 μCi/μg. Ninety-seven percent of the iodinated AE1 was precipitable with 20% trichloroacetic acid.

Conjugation of the Chelate to Monoclonal Antibodies

To permit labeling with ^{212}Pb or ^{88}Y, the monoclonal antibody AE1, as well as anti-Tac, which served as the control antibody for all studies, was first conjugated with 2-(p-SCN-Bz)-DOTA (*21,22*). For convenience, the chelate will be referred to as DOTA. Typically, 8–10 mg of antibody at a concentration of about 5 mg/ml were dialyzed against bicarbonate buffer, pH 8.6, for 6 hr. The antibody preparation was then conjugated to ^{14}C-(p-SCN-Bz)-DOTA as described (*21,22*). The average number of DOTA chelates per molecule of antibody was 1.2, determined as described previously (*22*). Each lot of chelated AE1 antibody was compared with the unmodified antibody and was shown to have an unaltered binding capacity using a competitive binding assay (Fig. 1). Briefly, 5×10^5 SK-OV-3 cells were incubated on ice with

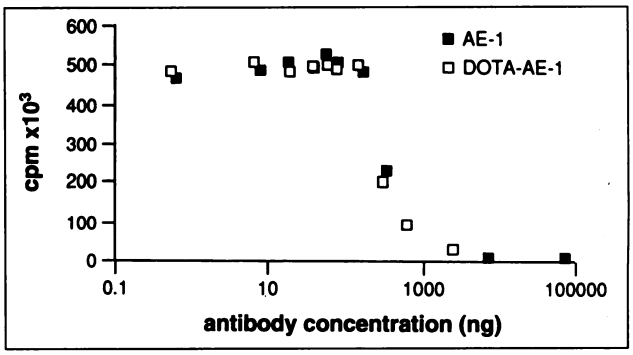

FIGURE 1. Assay of the effect of AE1 and DOTA-AE1 on the binding of ^{125}I-AE1 to SK-OV-3 cells. ■, unmodified competitor; □, DOTA-AE1 competitor.

increasing concentrations of unmodified (AE1) or chelated (DOTA-AE1) antibody (ranging from 0 to 75,000 ng/vial or from 0 to 2,688 ng/vial, respectively) in the presence of a constant nonsaturating amount of ^{125}I-AE1 antibody (6.25 ng/vial). After 3 hr of incubation, cells were centrifuged through an oil cushion, and cell-bound radioactivity was determined.

Procedure for Radiolabeling of Chelated Antibody

Carrier-free ^{88}Y was purified from metal contaminants by column extraction chromatography and incorporated into the chelated antibody as described (*23*).

Lead-212 was eluted from a ^{224}Ra/^{212}Pb generator with 1 ml of 2.0 *M* HCl. The solution was passed through a column (2 × 20 mm) of MP-50 resin, pre-equilibrated with 2.0 *M* HCl to remove any breakthrough of ^{224}Ra. The solution was then evaporated to dryness with the addition of 0.5 ml of concentrated HNO_3. The ^{212}Pb activity was dissolved in 0.1 *M* HNO_3, with the pH adjusted to ~4.0 with 3 *M* NH_4OAc. Chelated antibody was added to the ^{212}Pb solution and allowed to react at 35°C for 45 min. Five microliters of 0.1 m EDTA were added to scavenge any free radionuclide. Radiolabeled antibody was purified by high-performance liquid chromatography using a size-exclusion column and 4-morpholinepropanesulfonic acid/Cl buffer as the mobile phase (1 ml/min flow rate). Radiolabeling yields were 65%–75%, and more than 99% of the ^{212}Pb was protein-bound before administration.

Animals

Female 6–8-wk-old athymic-NCR-nude mice were kept in microisolation units on sterilized water and food.

Pharmacokinetics and Tissue Distribution of Radiolabeled AE1

To define the rate of entry of AE1 antibody into a HER2/neu-expressing tumor, mice bearing established s.c. injected SK-OV-3 tumors on their shoulder were injected i.v. with the 2-μCi dose of ^{88}Y-labeled monoclonal antibody. Seventeen mice were injected with the specific anti-HER2 antibody (^{88}Y-DOTA-AE1), and two mice were injected with an irrelevant (^{88}Y-DOTA-anti-Tac) antibody. Three of the mice injected with specific antibody were killed at 2, 6, 10 and 24 hr, and two mice were killed at 48 hr following infusion; their organs were removed for weighing and radioactivity determination using a gamma ray counter.

Toxicity of Lead-212

Lead-212 was used as the radionuclide in the immunotherapeutic studies. The emissions of ^{212}Pb, the decay product daughters and the details of the decay events as they relate to radioimmunotherapy have been described in the literature and are outlined in Figure 2 (*24*).

To establish the toxicity and maximal tolerated dose of the ^{212}Pb-DOTA-AE1, healthy, tumor-free mice were injected with

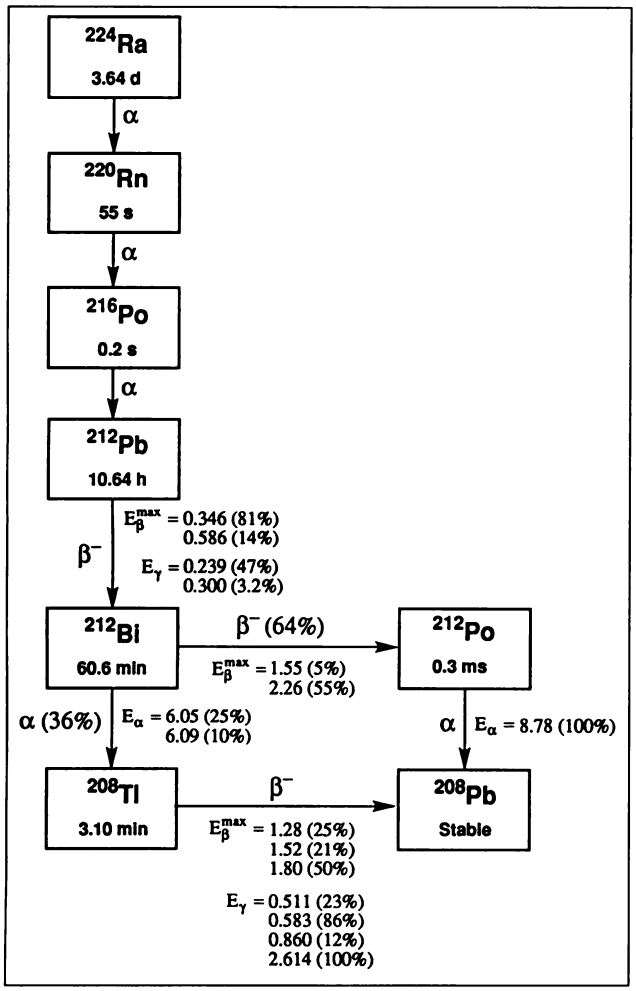

FIGURE 2. Decay series of ^{224}Ra for generating therapeutic doses of ^{212}Pb and ^{212}Bi, with description of the radioactivity emissions of ^{212}Pb and its daughters. The emissions are expressed in MeV.

unmodified AE1 or different doses of ^{212}Pb-DOTA-AE1. Group 1 received only unmodified AE1 antibody, while groups 2, 3 and 4 received 10, 25 and 40 μCi of ^{212}Pb-DOTA-AE1, respectively.

Radioimmunotherapy of HER2/neu-Expressing Tumors with Lead-212 AE1

In all radioimmunotherapy experiments, we used female athymic nude mice (10–12 wk old). In the initial preliminary therapeutic trial, mice were given i.p. injections of 2×10^6 SK-OV-3 cells. Three days later, groups of mice were given i.p. injections of ^{212}Pb-AE1 at dose levels of 10 and 20 μCi (specific activity, 0.6–1.5 μCi/μg), the control radiolabeled monoclonal antibody (^{212}Pb-anti-Tac) at the same dose levels (specific activity, 1.0–2.5 μCi/μg), unlabeled AE1 (10 μg) or no treatment. In the second trial, animals were inoculated with 2×10^6 SK-OV-3 cells s.c. in the back of the neck. Three days later, groups of five mice were treated i.v. with either ^{212}Pb AE1 or ^{212}Pb-anti-Tac at dose levels of 10 and 20 μCi. Control groups again received either i.v. unlabeled anti-Tac or no treatment. In the third trial, groups of five mice with established small-sized s.c. tumors (studied 14 days after s.c. tumor inoculation when mean tumor volume was 15 mm^3) were treated with the same agents under the same i.v. protocol, whereas in the fourth group animals with larger (mean tumor volume, 146 mm^3) tumors were studied with the same agents as used in trials 2 and 3. The primary end point in the initial two trials was tumor-free survival (time from treatment until tumor occurrence). Data were evaluated according to the methods of Kaplan and Meier (*25*) and Mantel and Haenszel (*26*). For the third and fourth studies, the observed parameter was the change in tumor volume calculated as $(L \times W^2)/2$, where L is length and W is width. The tumor volume for each animal at the time indicated was normalized to the tumor volume of the animal that was present on the day of therapy.

RESULTS

SK-OV-3 Murine Model of a HER2/neu-Expressing Human Solid Tumor Model

In our initial studies, we established a murine tumor model for human HER2/neu-directed alpha particle-emitting radioimmunotherapy using the SK-OV-3 human ovarian tumor cell line. The SK-OV-3 cell line expressed approximately 10^6 HER2/neu receptors per cell, as defined by Scatchard analysis using the ^{125}I-anti-HER2/neu antibody AE1. With this cell line, after administration of 2×10^6 cells, tumor occurrence rate was over 95%, with a median time to tumor appearance of 8 days after s.c. injection, and the occurrence rate was 60%, with a median time to appearance of 10–13 days after i.p. inoculation. The s.c. administered tumor remained localized, but grew to a diameter of 1 cm in 15–71 days (mean, 38 days) and to a diameter of 2 cm in slightly over 100 days.

To define the radiosensitivity of SK-OV-3 cells and to provide a baseline for comparison with other tumor models used to evaluate radioimmunotherapeutic agents, we quantitated tritiated thymidine uptake and defined the percentage of remaining cells still capable of colony formation after external irradiation of SK-OV-3 cells at different dose levels (0–10,000 rad). A dose of 520 rad reduced the colony-forming ability of irradiated cells to 2% of that observed with nonirradiated cells.

Biological Activity of DOTA-AE1

To be effective in radioimmunotherapy of HER2/neu-expressing tumors, the chelating agent used to link the radionuclide to the AE1 antibody should not alter the specificity or binding affinity of the monoclonal antibody to its antigenic target. The binding integrity of DOTA-AE1 was assessed by comparing it to unlabeled AE1 in a competitive binding assay (Fig. 1). In this assay, SK-OV-3 cells were incubated with a nonsaturating amount of ^{125}I-AE1, along with increasing quantities of either unmodified or DOTA-AE1. The chelated antibody manifested identical binding activity as unmodified antibody when evaluated in this competition assay.

In Vivo Pharmacokinetics and Tissue Distribution of Radiolabeled DOTA-AE1

The stability of a ^{203}Pb-labeled monoclonal antibody using DOTA as a chelating agent has been examined previously and shown to be stable under in vivo conditions (*24*). To test the in vivo stability of the particular DOTA-AE1 antibody used in this study, we used ^{88}Y rather than ^{212}Pb, in light of its longer physical half-life and gamma emission. The yttrium–DOTA complex has been shown to be and is also generally accepted to be inert and stable in vitro and in vivo, thus providing an unequivocal biodistribution standard for a radiolabeled antibody using a chelated metal. We and others have noted that the indium complex formed with DOTA was not identical with the yttrium complex and, therefore, ^{88}Y was used as an accurate tracer isotope. In these pharmacokinetic studies, animals with established s.c. SK-OV-3 tumors (~1 g) were given i.v. injections of ^{88}Y-DOTA-AE1 (2 μCi per mouse), serial blood samples were collected after injection and the fraction of the dose remaining in the plasma was determined. The terminal

TABLE 1
Tissue Distribution of Yttrium-88-AE1 and Yttrium-88-Anti-Tac in Percentage of Injected Dose per Gram of Tissue

	Yttrium-88-AE1 specific antibody					Yttrium-88-anti-Tac control antibody	Specific-to-control ratio of incorporation
	2 hr	6 hr	10 hr	24 hr	48 hr	24 hr	24 hr
SK-OV-3 tumor	3.70 ± 0.88	7.70 ± 0.28	10.35 ± 0.57	26.05 ± 6.17	24.67 ± 3.88	6.40 ± 2.35	4.07
Blood	35.57 ± 5.05	30.18 ± 4.13	26.65 ± 4.34	23.81 ± 6.01	17.38 ± 3.19	21.50 ± 0.84	1.11
Liver	11.57 ± 0.34	9.90 ± 1.10	8.42 ± 1.30	9.15 ± 1.33	6.42 ± 0.73	6.56 ± 0.00	1.39
Spleen	6.45 ± 0.63	6.02 ± 0.53	5.70 ± 1.25	6.13 ± 1.46	5.45 ± 0.42	6.07 ± 0.20	1.01
Kidney	9.57 ± 0.35	8.44 ± 1.02	7.25 ± 0.53	8.13 ± 1.00	5.79 ± 0.35	6.08 ± 0.17	1.34
Intestine	2.88 ± 0.10	2.58 ± 0.14	2.26 ± 0.00	2.42 ± 0.36	1.83 ± 0.26	2.24 ± 0.19	1.08
Heart	13.82 ± 0.56	10.54 ± 0.48	9.17 ± 1.72	10.80 ± 3.05	8.80 ± 0.72	8.50 ± 1.01	1.27
Lung	11.48 ± 0.70	10.38 ± 0.69	8.50 ± 0.81	10.53 ± 3.06	6.98 ± 0.62	9.14 ± 0.37	1.15
Femur	2.31 ± 0.20	2.05 ± 0.24	1.90 ± 0.20	2.37 ± 0.44	1.94 ± 0.10	2.25 ± 0.00	1.05

half-life (beta) of these studies was 86 hr; the fraction intravascular was 56% with 33% of the i.v. pool catabolized per day as assessed by the method of Matthews (*27*).

The effective treatment of large established tumors with ^{212}Pb-AE1 is dependent in part on the physical half-life of ^{212}Pb (10.6 hr) and the time required to achieve an optimum tumor-to-normal tissue ratio of radioactivity after i.v. administration. To address this issue, animals with established s.c. SK-OV-3 tumors were given i.v. injections of either tumor-specific antibody (^{88}Y-AE1 or of an irrelevant antibody ^{88}Y-anti-Tac). In Table 1, we show the tissue distribution of ^{88}Y-AE1 and the control antibody ^{88}Y-anti-Tac expressed as a percentage of the injected dose per gram of tissue in these tumor-bearing mice, as well as the ratio of specific-to-control antibody incorporation into different organs 24 hr after the i.v. infusion. The values are expressed as the mean ± s.d. of three mice killed at each time point. Within the first 6 hr, there was essentially no enrichment of AE1 in the SK-OV-3 tumors compared with normal tissues. However, by 24 hr, SK-OV-3 tumors manifested an enrichment of ^{88}Y-DOTA-AE1, yielding tumor-to-normal solid tissue ratios of 2.85–11 to 1. Such enrichment was not observed with the control monoclonal antibody anti-Tac. Furthermore, the ratio of incorporated specific antibody (AE-I) to control antibody (anti-Tac) at 24 hr was 4.07 for the tumor, whereas there was no enrichment (ratios of 1.01–1.39) with the normal organs.

Radioactivity–Toxicity Relationship and Maximum Tolerated Dose of Lead-212-AE1

To define an activity-toxicity relationship for ^{212}Pb-AE1, increasing doses (10, 25 and 40 μCi) of ^{212}Pb-AE1 were administered i.v. into tumor-free athymic nude mice, and the effects on hematological and serum chemistry parameters were defined over a period of 180 days. The activity-limiting toxicity was bone marrow suppression in animals receiving a more than 25-μCi dose of lead-radiolabeled AE1 (Fig. 3). Specifically, all animals receiving the 40-μCi dose of ^{212}Pb-labeled monoclonal antibody died within 6–10 days of therapy, manifesting a mean weight loss of 25.8%. Pathological examination of the tissues revealed significant lymphoid depletion and a marked reduction in the size of the spleen (which, on histological examination, was associated with decreased extramedullary hematopoiesis), an acellular bone marrow and pulmonary congestion. Four of the five animals in this group developed septicemia, with bacterial infections affecting the various tissues. All 16 animals receiving 25- and 10-μCi doses of ^{212}Pb monoclonal antibody i.v. survived, but manifested transient granulocytopenia, lymphocytopenia and modest thrombocytopenia (Fig. 3). The animals receiving the 25-μCi dose developed a nadir in their hematopoietic elements in the period spanning days 4–11, with a leukocyte nadir of 400 cells/μl (normal leukocyte range in mice, 2,600–10,700 μl), lymphocyte levels of approximately 250 (normal lymphocyte range in mice, 1,430–9,940 μl) and thrombocytopenia with a nadir of approximately 170,000 μl (normal platelet count range, 592,000–2.97 × 10^6/μl). The platelet and total white blood cell levels returned to the normal range by day 20, whereas the lymphocyte levels were normal

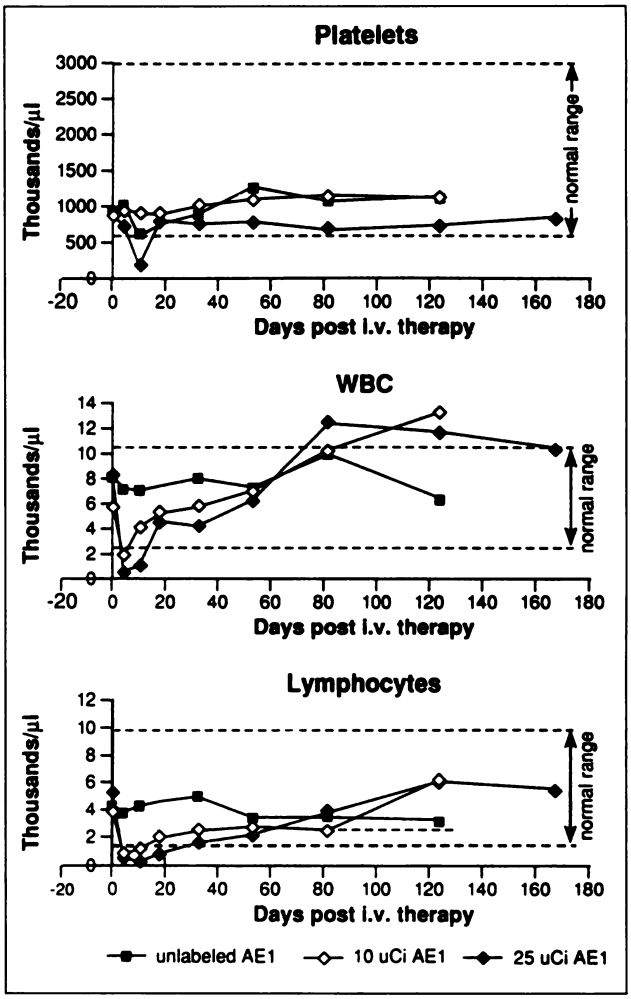

FIGURE 3. The effect of i.v. administered unlabeled AE1 (■), 10 μCi of ^{212}Pb-AE1 (◇) and 25 μCi of ^{212}Pb-AE1 (◆) on platelet, white blood cell (WBC) and lymphocyte levels.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.