Lung Carcinoma Imaging Using a Synthetic Laminin Derivative Radioiodinated Peptide YIGSR

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The synthetic laminin pentapeptide tyrosyl-isoleucyl-glycyl-seryl-arginine (YIGSR) binds to a metastasis associated high-affinity laminin receptor. The aim of this study was to investigate if the radiolabeled peptide can be considered as a basis for a potential tumor-imaging radiopharmaceutical. **Methods:** Iodine-131-labeled YIGSR was injected in mice inoculated with Lewis Lung carcinoma, as well as in normal controls. The experimental animals were imaged on a gamma camera 10 hr after peptide administration. The same peptide was also labeled with ^{125}I and administered to tumor-bearing and normal mice. At various time-points after peptide administration, the experimental animals were killed, and the radioactivity in the tumor, lung, liver and spleen was measured. Microscopic autoradiography was performed in histological sections of the same tissues. **Results:** The tumor and the spleen of tumor-bearing animals were imaged on a gamma camera. No significant blood-pool background was detected. No other organ except urinary bladder and thyroid was imaged in normal animals. The peptide was retained on tumor and spleen of tumor-bearing animals. Twenty-four hours after peptide administration, the tumor, lung, liver and spleen of animals with tumors contained significantly more radioactivity than the same tissues of equally treated normal controls. The radiolabeled peptide YIGSR was detected by microscopic autoradiography on the surface of certain tumor cells, but not on the surface of any normal cell. **Conclusion:** Although extensive research is still required, the peptide YIGSR can be considered as a basis for the development of a receptor specific radiopharmaceutical useful for the in vivo estimation of the metastatic potential of tumors. This radiopharmaceutical may be helpful in staging and prognostic-related decisions on cancer treatment.

Key Words: radiolabeled peptides; tumor imaging; metastasis; extracellular matrix; laminin peptides; receptors

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The detection of metastasis and the assessment of the metastatic potential of tumors is a crucial step for cancer staging. A radiopharmaceutical that accumulates on metastasis-associated receptors and, thus, detects metastatic cancer cell populations might be useful for cancer staging purposes.

Basement membrane binding capacity is strongly related to the metastatic potential of cancer cells (*1,2*). The interactions between the metastatic cell surface and laminin, the major cell binding molecule of basement membranes (*3*), play a critical role during metastasis (*4*). This is attributed to the existence of specific laminin binding sites on the cancer cell surface (*5,6*).

Proteolytic fragments of laminin can inhibit experimental metastasis by covering these laminin binding sites (*7*). Various laminin-derived synthetic peptides have shown a similar antimetastatic activity (*2*). One of these peptides, the nonapeptide glycine-phenylalanine-aspartic acid-glycine-tyrosine-isoleucine-glycine-serine-arginine (GPDGYIGSR), is binding to a high-affinity, laminin receptor that is strongly associated with the metastatic ability of cancer cells (*8–10*). The pentapeptide tyrosine-isoleucine-glycine-serine-arginine (YIGSR), a part of the above mentioned nonapeptide, showed a higher affinity to the metastasis-associated receptors (*11*) and can also inhibit experimental metastasis (*12*). Metastatic cells possess the ability to bind with high-affinity on basement membranes (*13*), having on their surface specific binding molecules (*1*). For example, human MCF-7 breast carcinoma cells possess 10000–50000 high-affinity laminin binding sites per cell with an estimated Kd of 50-2.2 n*M*. (*14*). As shown in human colon carcinoma cell lines, the binding of cancer cells to laminin is enhanced in less differentiated cells with high metastatic ability (*8*). The experimental tumor Lewis Lung carcinoma (3LL) contains about 2000 high-affinity laminin binding sites per cell with an estimated Kd of 0,6 n*M*. (*10*). When 3LL tumor cells were treated with indomethacin, laminin binding sites and metastatic ability were increased. The increased metastatic ability of the indomethacin-treated cells could be reversed if the specific receptors were occupied by the peptide YIGSR (*10*). The same peptide also has been used for the selection of melanoma cell lines in vitro with high metastatic ability (*15*) because of the significant difference on high-affinity laminin receptor number between metastatic and nonmetastatic cells. Thus, metastatic cells contain a large number of high-affinity receptors binding to a small and easily radiolabeled molecule.

In this study, the peptide YIGSR was labeled with radioiodine and administered to Lewis Lung carcinoma, inoculated, experimental animals. Metastatic cell populations were detected by in vivo scintigraphic imaging, as well as by autoradiography in histological sections.

MATERIALS AND METHODS

Balb C57BL mice, 8–10 wk old, were inoculated with experimental tumor (Lewis Lung carcinoma), by intramuscular injection in the forceps muscle of the right lower extremity, of 25-μl cell suspension in normal saline containing about 1 million cells. The experimental animals were killed 24 days after inoculation.

Imaging

Laminin peptide YIGSR was purchased from Sigma (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany). The peptide was radiolabeled with ^{131}I by using the chloramin T method (*16*). After iodination, the radiolabeled peptide was purified from free iodine and chemicals using a SEP-PAK C 18 column (Waters Associates, Milford, MA) as described elsewhere (*6*). The specific activity of the peptide YIGSR used in the experiments was about 555 MBq/mg. The purity of the peptide before and after iodination was verified by HPLC chromatography (Shimadzu) on a C-18 reverse phase column that was eluted by a 0%–100% acetonitrile gradient in 0.1% TFA-water. Iodine labeling did not cause any significant changes to the peptide, and no significant amount of free iodine was found in the preparation before administration.

Two groups of experimental animals were used. The first group included six normal animals. The second group included six Lewis

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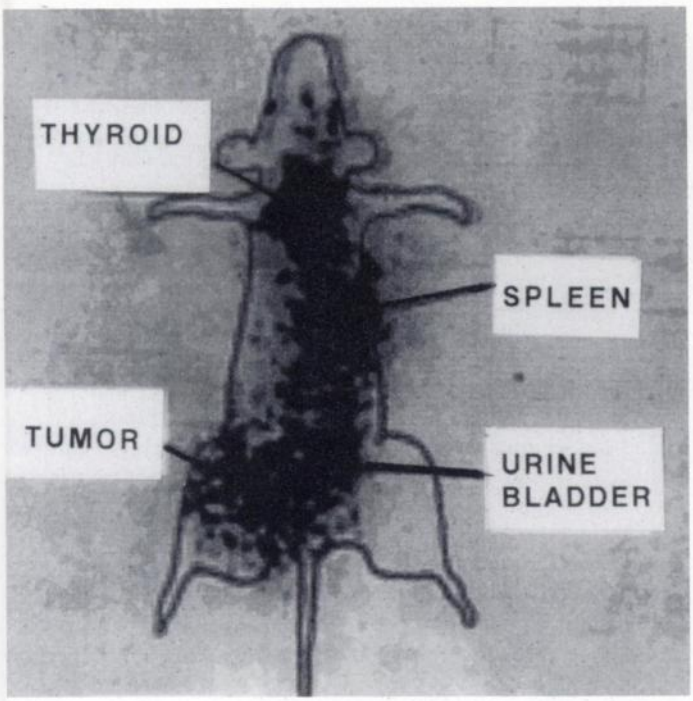

FIGURE 1. Image of a tumor-bearing mouse with Lewis Lung carcinoma 10 hr after intraperitoneal injection of ^{131}I YIGSR. The radioactivity does accumulate on the area of the tumor and on the spleen. The thyroid gland and urine bladder visualization is evidence of in vivo degradation and dehalogenation of the peptide.

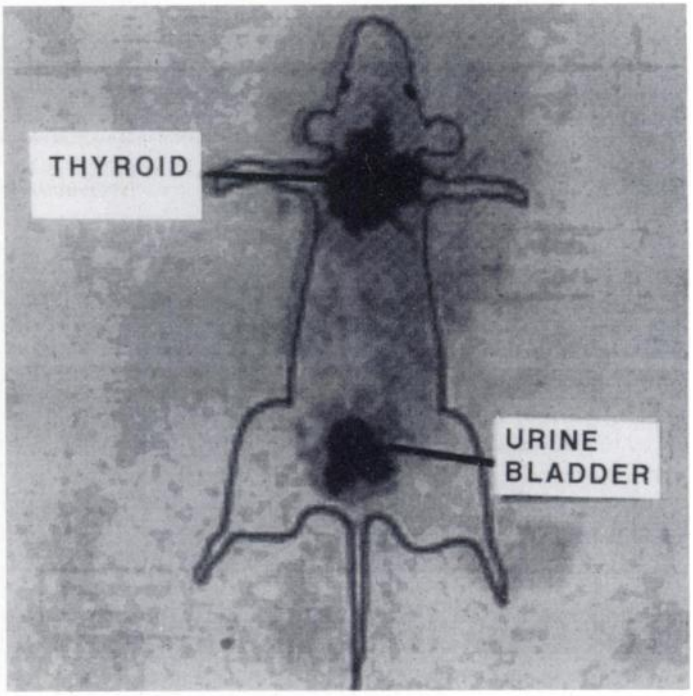

FIGURE 2. Image of a normal mouse 10 hr after intraperitoneal injection of ^{131}I YIGSR. Only the thyroid gland and the urine bladder can be visualized, probably due to degradation and dehalogenation of the peptide. No significant blood-pool background is detected.

Lung carcinoma, inoculated, experimental animals. About 1,8 MBq of the radiolabeled peptide (3,2 μg) was administered intraperitoneusly to the experimental animals. Ten hours after intraperitoneus administration, the animals were anesthetized with ether and imaged on a gamma camera (Sophy Camera) equipped with a parallel-hole, high-energy collimator. The animal was placed on the collimator that was protected by a nylon and absorptive paper diaper; 100000 counts were obtained per image; 4 × magnification was used on a 128 × 128 word image. Imaging time varied from 420–920 sec.

Experiments with Iodine-125-Labeled Peptide

For the ^{125}I-labeled peptide experiments, two groups of experimental animals were used. The first group (Group N) included 20 normal animals. The second group (Group L) included 20 Lewis Lung carcinoma, inoculated, experimental animals.

The peptide was radiolabeled with ^{125}I (ICN) and purified as described above. The specific activity of the peptide YIGSR used in the experiments was about 370 MBq/mg. The purity of the peptide before and after iodination was verified by HPLC chromatography as described above; 0.2 μg of the radiolabeled peptide in a volume of 0,2 ml (about 74 kBq) was administered intraperitoneusly to the experimental animals. At various times after intraperitoneus administration, the animals were killed and blood samples were drawn from the heart and parts of the tumor. The lungs, liver and spleen were removed. The specimens were weighed and the radioactivity was counted on a gamma counter (LKB Wallac). The radioactivity per gram of tissue was then calculated as a percentage of the administered dose.

TABLE 1
Mice Bearing Lewis Lung Carcinoma Tumors*

Time	1 hr (n = 4)	3 hr (n = 4)	24 hr (n = 8)
Blood	8.3 ± 2.34	3.6 ± 2.9	0.014 ± 0.007
Liver	3.98 ± 2.88	2.36 ± 1.18	0.55 ± 0.44
Lung	4.1 ± 0.14	2.1 ± 0.85	1.4 ± 0.44
Spleen	10.28 ± 5.4	8.23 ± 6.1	1.4 ± 1.0
Tumor	1.99 ± 0.06	1.3 ± 0.32	1.15 ± 0.28

*Organ distribution of radiolabeled peptide at various time-points after intraperitoneal injection (percentage of the administered dose per gram of tissue).

Autoradiography

Tumor and other tissue specimens were fixed with 10% formol, dehydrated in ascending alcohol concentrations and included in paraffin; 3–5 μ-sections were cut with a microtom (Jung Multicut 2045). The sections were deparafinized, rehydrated and covered with autoradiography photographic emulsion (Kodak). The sections were kept in a dark and dry place for 20 days and then developed with D19 developer (Kodak), fixed with a fixer fluid (Kodak) washed well with water and covered with Aquamount (Laborimpex). Serial sections of the autoradiographies were stained with eosin-hematoxylin.

RESULTS

Scintigraphic imaging showed that the radiolabeled peptide YIGSR accumulated on tumors. The tumor, spleen, thyroid and urinary bladder of all experimental animals were imaged (Fig. 1). The radioactivity did not accumulate on any organ except the thyroid and urinary bladder of normal animals (Fig. 2). No

TABLE 2
Normal Mice*

Time	1 hr (n = 4)	3 hr (n = 4)	24 hr (n = 8)
Blood	7.5 ± 1.97	3.1 ± 2.1	0.017 ± 0.009
Liver	0.89 ± 0.68	0.95 ± 0.58	0.07 ± 0.02
Lung	1.37 ± 0.94	1.15 ± 0.75	0.14 ± 0.05
Spleen	0.86 ± 0.4	0.57 ± 0.3	0.08 ± 0.04

*Organ distribution of radiolabeled peptide at various time-points after intraperitoneal injection (percentage of the administered dose per gram of tissue).

TABLE 3
Organ Distribution of Radiolabeled Peptide 24 Hr after Intraperitoneal Injection*

Organ	Controls	Lewis Lung	Significance
Blood	0.017 ± 0.009	0.014 ± 0.07	Nonsignificant
Liver	0.07 ± 0.02	0.55 ± 0.44	$p < 0.01$
Lung	0.14 ± 0.05	1.4 ± 0.44	$p < 0.01$
Spleen	0.08 ± 0.04	1.4 ± 1.0	$p < 0.01$

*Comparison between normal and tumor-bearing animals (percentage of the administered dose per gram of tissue). Statistical comparison was performed using the Mann-Whitney U test (n = 8).

significant background activity from the blood pool was observed in any animal.

Thyroid and urinary bladder visualization gave evidence of significant peptide degradation. To estimate the amount of the activity that remained bound on tumor cells, experiments with ^{125}I-labeled peptide were performed.

In pilot experiments, the radioactivity in the liver, spleen and lungs after endoperitoneal injection of the ^{125}I-labeled peptide was measured at 1, 3 and 24 hr (Tables 1 and 2). A high, probably nonspecific, initial binding to the normal organs, as well as in tumors, was observed. At 24 hr, only a minimal fraction of the administered radioactivity was found in the blood and the organs of normal mice. In comparison, the animals with tumor retained significantly higher amounts of radioactivity in the spleen, liver and lungs (Table 3).

Histological sections of the tumor and the organs of the experimental animals were examined by light microscopy and autoradiography. Light microscopy of the tumor, after hematoxylin staining, showed a variety of Lewis Lung carcinoma cell (LLCC) populations in the tumors. The size of these cells ranges from huge to very small. Some cells contain dark-stained nuclei that occupied most of the cell body (Fig. 3A).

Microscopic autoradiography of sections from the primary tumor (Fig. 3B) showed that the peptide did accumulate on the surface of the tumor cells. The peptide accumulation around the cells is very clear and follows the cell surface. The peptide did not bind equally to all cell surfaces at the primary tumor. Some of the tumor cells showed higher activity on autoradiographies than the rest of the tumor and metastases cells. The same pattern of radioactivity distribution follows the LLCC in large metastases (Fig. 4). On the other hand, at small in situ metastatic sites containing a few tumor cells, it seems that most of the metastatic cells accumulate radiolabeled peptide YIGSR (Fig. 5).

DISCUSSION

It has been reported that, after in vivo administration, YIGSR is rapidly eliminated from blood mainly through the urinary system (*17*) and is found almost completely degraded in the urine (*18*). Thus, extensive degradation and dehalogenation of the peptide after in vivo administration was expected. Given the low amount of free iodine in the initial preparation, urine bladder and thyroid scintigraphic visualization can be attributed to in vivo degradation and dehalogenation of the peptide. However, despite the possible degradation, under the experimental conditions described, a sufficient tumor-to-background ratio was achieved so that the primary tumors could be imaged with a gamma camera.

No significant background uptake or nonspecific uptake in normal animals was observed in experiments with ^{125}I-labeled peptide. However, in pilot experiments using ^{125}I-labeled peptide, a high, probably nonspecific initial binding to the normal organs was obvious. This nonspecific uptake washed out more rapidly from the tissues of normal animals than from the organs of tumor-bearing animals and from tumors. It is suggested that tumor and metastatic sites may retain the specifically bound peptide. This high-affinity binding may protect the peptide from washout and degradation.

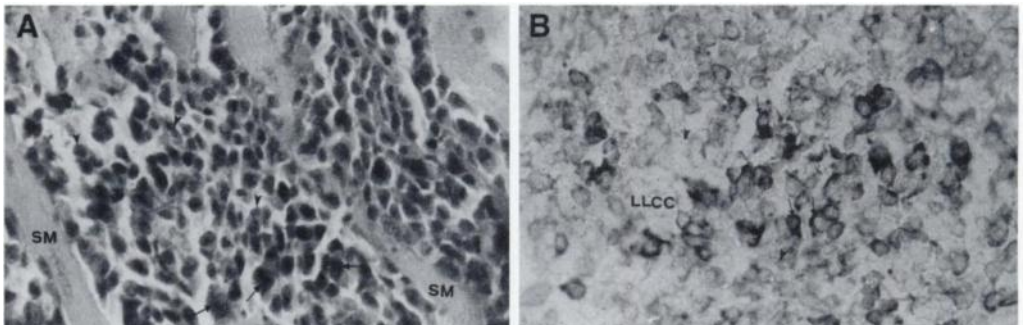

FIGURE 3. (A) Primary tumor section stained with eosin-hematoxylin. Cells varying in size from huge (arrows) to small (arrowheads) are apparent. SM = skeletal muscles of the lower limp (magnification ×100). (B) Microscopic autoradiography of a section from a primary tumor. Some LLCC show intense accumulation of the radioactivity around the cell surface (arrows) and some show very slight or no (arrowheads) LCCC = Lewis Lung carcinoma cells (magnification ×100).

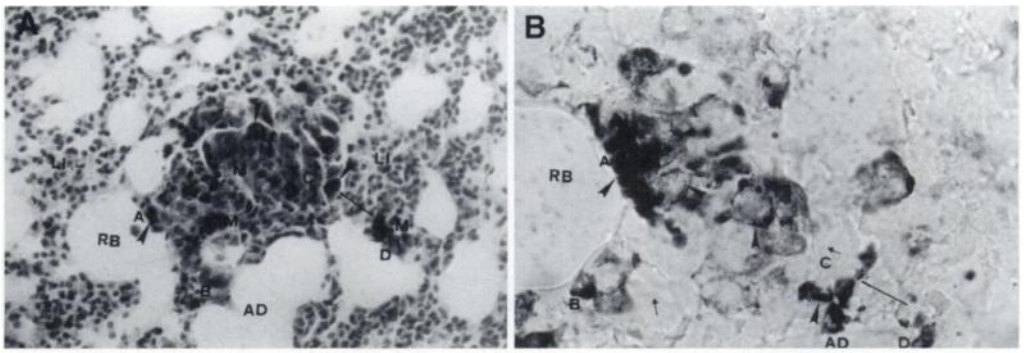

FIGURE 4. (A) Section of a large metastasis in the lung, stained with eosin-hematoxylin. Arrowheads show some metastatic cells with characteristic morphology. (Magnification ×100); M = metastasis; RB = respiratory bronchiole; AD = alveolar duct; LI = leukocyte infiltration. A, B, C, D letters show the corresponding areas between Figure 4A and 4B. (B) Autoradiography of a section serial to the one presented on Figure 4A. Some metastatic cells accumulate the radioactivity very strongly (arrowheads) and some others very slightly or not at all (arrows) (magnification ×400). A, B, C, D letters show the corresponding areas between Figure 4A and 4B. Because of the thickness of the sections (4 μ), the exact shape of the corresponding areas is not identical.

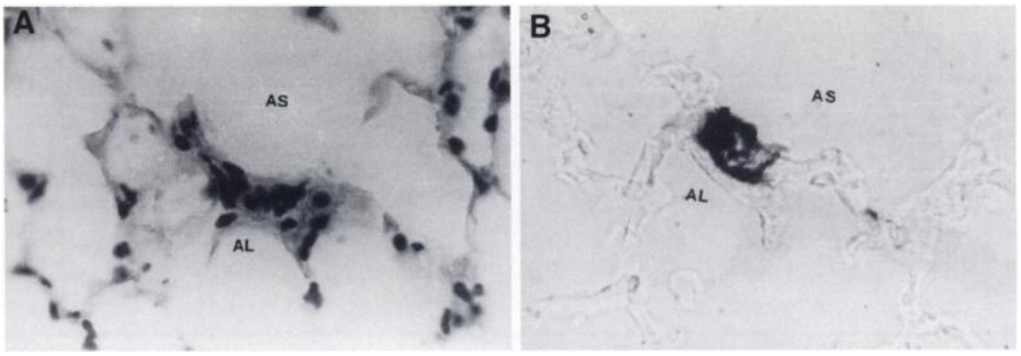

FIGURE 5. (A) Section of an in situ metastasis in the lung, stained with eosin-hematoxylin. (Magnification ×400); AS = alveolar sac; AL = alveolus. (B) Autoradiography of a section serial to the one presented on Figure 5A (magnification ×400). Most of the metastatic cells accumulate the radioactivity very strongly. Because of the thickness of the sections (4 μ), the exact shape of the corresponding areas is not identical.

As shown by autoradiography, the peptide did accumulate on the surface of certain tumor cells, but not on the surface of any normal cell. This fact can be considered as evidence for specific binding of the peptide on cells that are rich on high-affinity metastasis associated laminin receptors. It was obvious that the peptide did not bind equally well on the surface of all tumor cells. Tumors, although originating in one cell (*19*), contain several subpopulations of cells with different properties (*20,21*). Among the various tumor cell subpopulations, there are certain that have the ability to metastasize (*22*). At advanced stages of the disease, these cell populations abandon the primary tumor, circulate in the blood stream and bind to the basement membranes of vascular endothelium (*13,23,24*). To metastasize, cancer cells must possess the ability to bind with high-affinity on vascular endothelium basement membranes (*13*). Cell populations with high metastatic potential have on their surface increased the number of high-affinity basement membrane receptors that bind to the peptide YIGSR (*2,24*). Thus, it is possible that the tumor cell populations detected on autoradiographies of histological sections represent the populations with high metastatic potential. The possibility of assessing the extent of cells with high metastatic potential in the tumors either in vivo using the gamma camera or ex vivo using pathology sections may be clinically useful for the staging and estimation of the metastatic potential of tumors.

The data presented here indicate that, after in vivo administration, the radiolabeled peptide YIGSR can be detected on tumor and metastatic cells. Although imaging could be achieved in small experimental animals bearing relatively large tumors, rapid in vivo degradation may be detrimental to imaging relatively small tumors and metastatic sites in humans (*25*).

Recently, it has been reported that the peptide YIGSR, if coupled with polyethylenglycole, shows better antimetastatic ability than the uncoupled YIGSR peptide (*18*). This effect is due to the higher in vivo stability of the polyethylenglycole-coupled peptide (*18*). Polymeric forms of the peptide provided also increased in vivo stability (*26*). It is possible that these or other forms of peptide YIGSR give superior results in scintigraphy.

The possibility of ^{99m}Tc-labeling should also be taken into consideration. As reported, peptide YIGSR can be efficiently labeled with ^{99m}Tc (*17*). On the other hand, in contrast to the present and previously reported observations, the ^{99m}Tc-labeled peptide does not bind in vivo with B16 melanoma grown in nude mice (*17*). However, it should be considered that tumors implanted in T-cell deficient animals, such as nude mice, often fail to metastasize because the increased numbers of natural killer cells that are present in nude mice act against the growth of metastatic cell lines (*27–29*).

CONCLUSION

Although extensive research is still required, the data presented here permit the suggestion that peptide YIGSR may provide the basis for the development of a receptor-specific radiopharmaceutical useful for in vivo estimation of the metastatic potential of tumors. This radiopharmaceutical may be helpful in staging and prognostic-related decisions on cancer treatment.

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

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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.

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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