c-erbB2 Protein Overexpression in Breast Cancer as a Target for PET Using Iodine-124-Labeled Monoclonal Antibodies

M. Adel Bakir*, Suzanne A. Eccles, John W. Babich†, Nighat Aftab, Jennifer M. Styles, Christopher J. Dean and Robert J. Ott

Joint Department of Physics and Section of Immunology, Institute of Cancer Research and The Royal Marsden Hospital, Sutton, Surrey, United Kingdom

ICR 12, one of a panel of rat monoclonal antibodies recognizing the external domain of the human c-erb B2 proto-oncogene product, (Styles, 1990) was chosen as a candidate for radiolabeling with ^{124}I for positron emission tomography of selected patients with breast cancer. By using N-bromosuccinimide (NBS), optimal labeling conditions were established using ^{125}I. The labeling efficiency was determined using instant thin-layer chromatography (ITLC) and gel filtration (HPLC). The antibody was then labeled with the positron emitter ^{124}I, and a labeling efficiency of 96% and immunoreactivity of 80%–90% was obtained. The product was stable, with less than 5% of the radiolabel being eluted after six days storage in plasma at 37°C. Immunolocalization studies were performed in athymic mice bearing human breast carcinoma xenografts overexpressing the c-erb B2 gene product using as controls ^{125}I labeled isotype-matched rat antibody, and antigen-negative tumors. Good uptake of ^{124}I-labeled ICR12 was obtained in c-erb B2 expressing tumors (up to 12% injected dose per gram at intervals up to 120 hr), with localization indices of 3.4–6.2. Tumor xenografts of 6 mm diameter were successfully imaged with high resolution at 24, 48 and 120 hr using the RMH/ICR MUP-PET camera. We suggest that ^{124}I-labeled ICR12 is a suitable agent to image and quantify immunolocalization in patients whose tumors overexpress the c-erb B2 proto-oncogene product.

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The human c-erbB-2 proto-oncogene product is a transmembrane glycoprotein of approximately 185–190 kilodaltons molecular weight with extensive structural homology to the EGF-receptor (*1*). A putative ligand has recently been described, (*2*) and the fact that the ligand has been shown to be produced by certain mammary carcinoma cell lines raises the possibility that the c-erbB-2 protein may function as an autocrine growth factor receptor in breast cancer. This feature may contribute to the poorer prognosis of patients whose tumors over-express the gene (*3–5*). Similar correlations between c-erbB-2 expression and prognosis have been suggested in patients suffering from adenocarcinomas of the lung (*6*), endometrium (*7*), ovary (*8*) and possibly bladder (*9*), rendering this a most significant tumor marker in a variety of carcinomas. It has also been reported that breast tumors overexpressing c-erbB-2 are frequently unresponsive to endocrine treatment and chemotherapy (*10,11*), which has led us to develop a number of monoclonal antibodies with specificity for the external domain of the protein with a view to their eventual exploitation for better detection and treatment of this clinically important sub-group. Such antibodies may provide operationally 'tumor-specific' reagents, since evidence suggests that they do not bind in significant amounts to normal cells expressing only a single gene copy (*12*).

Apart from their prognostic use in immunohistochemical analysis (*4,5,13,14*), these antibodies may be useful for tumor localization by immunoscintigraphy, and possibly for immunotherapy. As a first step in their pre-clinical evaluation, the present study was undertaken to determine their usefulness in PET imaging. PET provides enhanced spatial resolution and sensitivity compared with conventional gamma scintigraphy, as well as the ability to quantify radioisotope distribution in vivo. This may extend to quantitation of c-erbB-2 expression in situ, and the ability to detect small secondary tumor deposits in high risk patients.

Our initial approach to antibody imaging using PET focused on the need to label ICR12 effectively with a radionuclide that possessed suitable chemistry and half-life. As optimal localization occurs 24 hr or more after administration, ^{124}I (positron emitter, $T_{½}$ = 4.2 days) was our first choice radionuclide. In order to provide a labeled antibody of the highest possible purity and immunoreactivity for clinical use, a study was undertaken to optimize the labeling parameters of ICR12 using radioiodines. This

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For reprints contact: Dr. Suzanne A. Eccles, Dept. of Immunology, Haddow Laboratories, Institute of Cancer Research, Downs Rd., Sutton, Surrey SM2 5NG, UK.

* Present address: P.O. Box 11865, Damascus, Syria.

† Present address: Dept of Radiology, Massachusetts General Hospital, Boston, MA.

paper describes our findings and the results of localization and PET imaging studies in athymic mice bearing human breast cancer xenografts.

MATERIALS AND METHODS

By using the human breast carcinoma cell line BT474 as an immunogen, rat monoclonal antibodies were raised against the external portion of the c-erbB-2 proto-oncogene product, p185 (*12*). Representative antibodies were shown to specifically immunoprecipitate a 190 kD protein from ^{35}S-methionine-labeled breast carcinoma cells in which the c-erb B2 is amplified (BT474 and MDA MB 361). One of the antibodies (ICR12) was stained with formol-saline fixed sections of breast carcinoma to overexpress the c-erbB-2 gene product and bound to antigen in Western blots. This antibody was selected for further study.

Cell Lines and Xenografts

The human breast carcinoma cell line MDA MB 361 (which overexpresses the c-erbB-2 proto-oncogene), MDA MB 468 and squamous carcinoma cell line LICR-LON-HN5 (which overexpress EGF-R) were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and penicillin, streptomycin and neomycin. Xenograft tumors were established in negative-pressure isolator maintained outbred nu/nu mice by the subcutaneous inoculation of 5.10^6 cells. Subsequent passages of tumors were initiated from trocar fragments for up to 10 generations providing that expression of the relevant antigens remained high (as determined by immunohistochemical examination of randomly selected tumor samples). Each mouse received two trocar fragments, located at midpoint of each flank.

Monoclonal Antibody Preparation and Radiolabeling

ICR12 (*12*) and control antibody ALN/11/53 (*15,16*) are both rat gamma 2a immunoglobulins. They were isolated from hybridoma culture supernatants by precipitation with $(NH4)_2SO_4$ (45% of saturation) and ion-exchange chromatography on Whatman DE-52 cellulose (Whatman Ltd Maidstone, Kent).

Iodine-125 and ^{131}I were obtained from Amersham International at 100 mCi/ml as a reductant-free solution in NaOH pH 7–11. Iodine-124 was produced and supplied by the Radionuclide and Cyclotron Department at King Faisal Specialist Hospital and Research Centre Saudi Arabia (KFSH, SA), using the ^{124}Te(d, 2n)^{124}I nuclear reaction with 15 meV deuterons on 96% enriched ^{124}Te as a target matrix (*17*). The radionuclide and the radiochemical purity were more than 99% and 90% respectively. Iodine-124 (~50 mCi/ml) was supplied in the form of sodium iodide pH 11.

N-bromosuccinimide (NBS), sodium metabisulphite, sodium phosphate, phosphate-buffered saline (PBS) Sephadex G-25 and bovine serum albumin (BSA) were obtained from commercial sources.

Purified IgG2a antibodies were labeled with either ^{125}I or ^{131}I isotopes using the iodogen method (*18*), or with ^{124}I according to the NBS method (*19*) but with varying reaction conditions as follows.

Mass of NBS. Fifty microcuries of ^{125}I in 10 μl PBS pH 7.5 were pipetted into a reaction tube followed by 25 μg of ICR12 in 15 μl PBS. The reaction was started by adding a quantity of NBS (ranging from 0.125 to 10 μg) and terminated 2 min later by the addition of sodium metabisulphite in 0.05 *M* phosphate buffer, pH 7.5, containing the same concentrations of NBS.

Reaction Time. In order to define the optimal reaction time for iodination of ICR12, the procedure was performed with 25 μg of antibody, 50 μCi of ^{125}I and 0.25 μg NBS. The reaction was terminated after intervals of 0.5–20 min and the labeling efficiency estimated.

Quantity of ICR12. Different amounts of ICR12 ranging from 1 to 400 μg were placed in reaction tubes. The radiolabeling was performed using 50 μCi of ^{125}I and 0.25 μg of NBS for 1 min. Two further sets of experiments were carried out using 1 μg and 5 μg of NBS respectively.

pH. The pH profile for radiolabeling 25 μg of ICR12 with 50 μCi of ^{125}I and 0.25 μg of NBS was examined using phosphate buffer in the range of pH 4.4–9.5.

Carrier Iodide. By using 25 μg of ICR12 in PBS and 0.25 μg of NBS, radioiodination was performed with 50 μCi of ^{125}I and increasing amounts of carrier iodine (KI) up to 5 nmol. Results were compared with those obtained in the absence of carrier. Three further sets of experiments were carried out using 1 μg, 5 μg and 10 μg of NBS respectively.

Immunoreactivity of Labeled ICR12. To determine the effect of the conditions of labeling on the immunoreactivity of ICR12, competitive radioimmunoassays were carried out using as a target the antigen present on MDA MB 361 cells. Briefly, ICR12 labeled with ^{127}I was compared with unlabeled ICR12 for its capacity to compete with ^{125}I-labeled ICR12 for binding to the cells. Doubling dilutions of ^{127}I ICR12 or unlabeled antibody were prepared in DMEM-10%FCS and then mixed with an equal volume of ^{125}I-labeled ICR12.

Duplicate aliquots of 50 μl were transferred to MDA MB 361 cells grown to confluence in 96 well plates (Nunc, Gibco Ltd., Scotland). After incubation at 18–20°C for 1 hr, the supernatant was discarded and the plates were washed four times. The cells were lysed by addition of a solution containing 1% sodium dodecylsarcosinate in 0.5 *M* NaOH and the samples counted in a gamma counter. Binding curves were constructed from the data and the amount of active antibody in the experimental sample was determined from the standard curve given by unlabeled ICR12.

Competition assays could not be used with antibody labeled with radioactive iodine. In this case, aliquots of radiolabeled ICR12 (10^4 cpm) were incubated for 1 hr with Sepharose 4B beads coated with an excess of the p185 antigen. After washing four times with PBS containing 0.5% Triton X-100 and 10^{-3}M phenyl methyl sulphonyl chloride, the radioactivity associated with the beads was determined and the percentage of radiolabeled antibody bound was calculated. Antigen-coated beads were prepared as described by Styles et al. (*12*) using ICR13 as the capture antibody.

In Vitro Stability of ICR12 Radiolabeled with ^{124}I. ICR12 was labeled with ^{124}I using NBS as an oxidizing agent at a specific activity of 2 μCi/μg. The stability of the label was determined as a function of time, temperature, and suspension medium (PBS or plasma) for up to six days after preparation. Aliquots of labeled ICR12 were stored at 4°C in PBS or at 37°C in human plasma. At various intervals, radiochemical purity was measured by ITLC and HPLC.

In Vivo Biodistribution Studies

Groups of athymic mice bearing bilateral MDA MB 361 (antigen-positive) tumors or bilateral LICR LON HN5 (antigen-negative) tumors were inoculated intravenously with a mixture of 5 μg each of ^{124}I-labeled ICR12 and ^{125}I-labeled control isotype-

matched ALN/11/53 at a specific activity of 1 μCi per μg. Groups of four to six mice were killed by CO_2 asphyxiation at 24, 48 and 120 hr postinoculation. Radioactivity in weighed samples of blood, lungs, liver, spleen, kidneys and tumors was determined using a Packard autogamma spectrometer. Computerized "spill-over" corrections were applied to determine the relative quantities of the two simultaneously administered isotopes in each sample. The results were expressed as the percentage injected dose per gram of tissue (%ID/g) for both specific and nonspecific antibody; as tumor-to-normal tissue ratios and as a localization index (LI) calculated as follows:

$$LI = \frac{\%ID/g \text{ specific Mab in tumor}/\%ID/g \text{ in blood}}{\%ID/g \text{ nonspecific Mab in tumor}/\%ID/g \text{ in blood}}.$$

In Vivo Imaging Studies

For imaging purposes, xenograft-bearing nu/nu mice received potassium iodide in their drinking water 24 hr prior to injection of radiolabeled antibody to minimize the uptake by the thyroid of any released radioiodine. Mice were injected via the tail vein with 100 μCi ^{124}I-ICR12 (10 μCi/μg). The mice were anesthetized with pentobarbitone and imaged using the RMH/ICR MUP-PET positron camera (*20*) at 24, 48 and 120 hr postinjection. Fifty thousand counts per image were collected.

RESULTS

Optimization of Labeling ICR12 with ^{125}I Using NBS

The results of the various procedures used to determine the optimal conditions for labeling ICR12 were expressed as the percentage labeling efficiency as a function of the different labeling parameters.

The results in Table 1 indicate that the highest radioiodine incorporation into ICR12 (98%) was obtained with 0.125 μg of NBS. This corresponds to an NBS:ICR12 ratio of 4.5:1. The labeling efficiency tended to decrease with increasing amounts of NBS, with a particularly sharp fall between 5 and 10 μg. The peak labeling efficiency (97%) was obtained after a reaction time of 60 sec, although between 2 and 20 min there was no detectable decrease in labeling efficiency indicating that this parameter is not critical above 1 min (data not shown).

TABLE 1
The Effect of NBS Mass on the Labeling Efficiency of ICR12 with ^{125}I

NBS (μg)	Labeling efficiency (%) s.d.*
0.125	98 (0.5)
0.25	97 (1)
0.5	96 (1)
1.0	96 (1.5)
2.0	87 (1)
3.0	87 (1)
4.0	83 (3)
5.0	81 (4)
10.0	50 (4)

* Standard deviation; mean of three experiments.

TABLE 2
The Effect of ICR12 and NBS Mass on Labeling Efficiency with ^{125}I

NBS (μg)	0.25	1	5
ICR12 (μg)	Labeling efficiency (%)		
1	50	20	18
10	92	72	18
100	93	98	91
400	63	96	98

Experiments to determine the effect of pH on the labeling of the antibody showed that the labeling efficiency was lower at acidic pH below 6.0 but was not affected significantly in the range of pH 6.5–9.5 (data not shown).

The absolute quantities and molar ratios of NBS and ICR12 were examined for their effects on labeling efficiency. Labeling efficiency increased initially with molar ratios between 0.5:1 and 2:1, and then remained at a constant optimal level between 2:1 and 45:1, beyond which the labeling efficiency declined sharply (Tables 2 and 3). The effects of adding nonradioactive cold iodide on the labeling efficiency of ICR12 are shown in Table 4. The results indicated that no significant effects were evident when up to 0.25 nmol of "cold" potassium iodide was added, but a further increase in the carrier concentration resulted in a progressive decrease in labeling efficiency. However, by increasing the amount of NBS used, it was possible to maintain a high labeling efficiency with carrier concentrations up to 2.5 nmol, providing that the molar ratio of NBS:ICR12 did not exceed the optimum.

Optimization of Labeling of ICR12 with ^{124}I

The optimal reaction conditions found for ^{125}I were applied to radiolabeling with ^{124}I. In order to obtain an optimal labeling efficiency of 96%, 4 μg of NBS were required for labeling a patient dose of 1.2 mg of ICR12 with 2 mCi of ^{124}I.

For in vivo experiments, labeling was routinely carried out with 2–10 μCi ^{124}I per μg antibody. Under these

TABLE 3
Labeling Efficiency of ICR12 Versus NBS:ICR12 Molar Ratio (MR)

NBS:ICR12 MR	Labeling efficiency (%)
0.52:1	63
2.1:1	93
8.4:1	98
10:1	98
21:1	92
42:1	91
84:1	72
210:1	50
421:1	18
842:1	20
4215:1	18

TABLE 4
The Effect of Carrier Cold Iodide on the Labeling Efficiency of ICR12 with ^{125}I

NBS (μg)	0.25	1	5	10
Carrier I (nmole)	Labeling efficiency (%)			
(NCA)	98	97	82	54
0.025	96	97	80	60
0.25	95	97	84	63
1.25	60	97	84	62
2.5	44	96	84	61
5.0	36	80	80	54

NCA = no-carrier added.

conditions, the labeling efficiency as determined by ITLC and HPLC was 96%, and the immunoreactivity of all preparations was between 80% and 90% of that of the unlabeled antibody.

Reactivity of Labeled ICR12 In Vitro

The effect of the mass of NBS used on the immunoreactivity of the labeled product was assessed using a competitive cell binding assay. The immunoreactivity declined as increasing masses of NBS were used. With NBS:ICR12 molar ratios of 5:1 (which gives optimal labeling efficiency), about 70% of the immunoreactivity was retained; this decreased to 55% and 35% with molar ratios of 10:1 and 25:1 respectively.

Stability of ^{124}I-Labeled ICR12 In Vitro

The stability of labeled ICR12 stored under two different conditions was assessed using two independent techniques. The data indicated that radioiodinated ICR12 was highly stable in phosphate-buffered saline at 4°C, and also that in plasma maintained at 37°C less than 5% of the radiolabel was eluted after six days of storage.

Biodistribution In Vivo

The percentage of the injected dose per gram of tissues for ^{124}I-labeled ICR12 and control isotype-matched ^{125}I-labeled ALN/11/53 antibodies are shown in Figure 1. Normal tissue levels for both antibodies were comparable at all time points, but ICR12 showed a significant and sustained localization in the antigen-positive MDA MB 361 tumors (12.6% ID/g at 24 hr and 9.0% ID/g at 120 hr). The uptake of control antibody was less than 5% ID/g, falling to 2.4% at 120 hr. The ratios of specific-to-

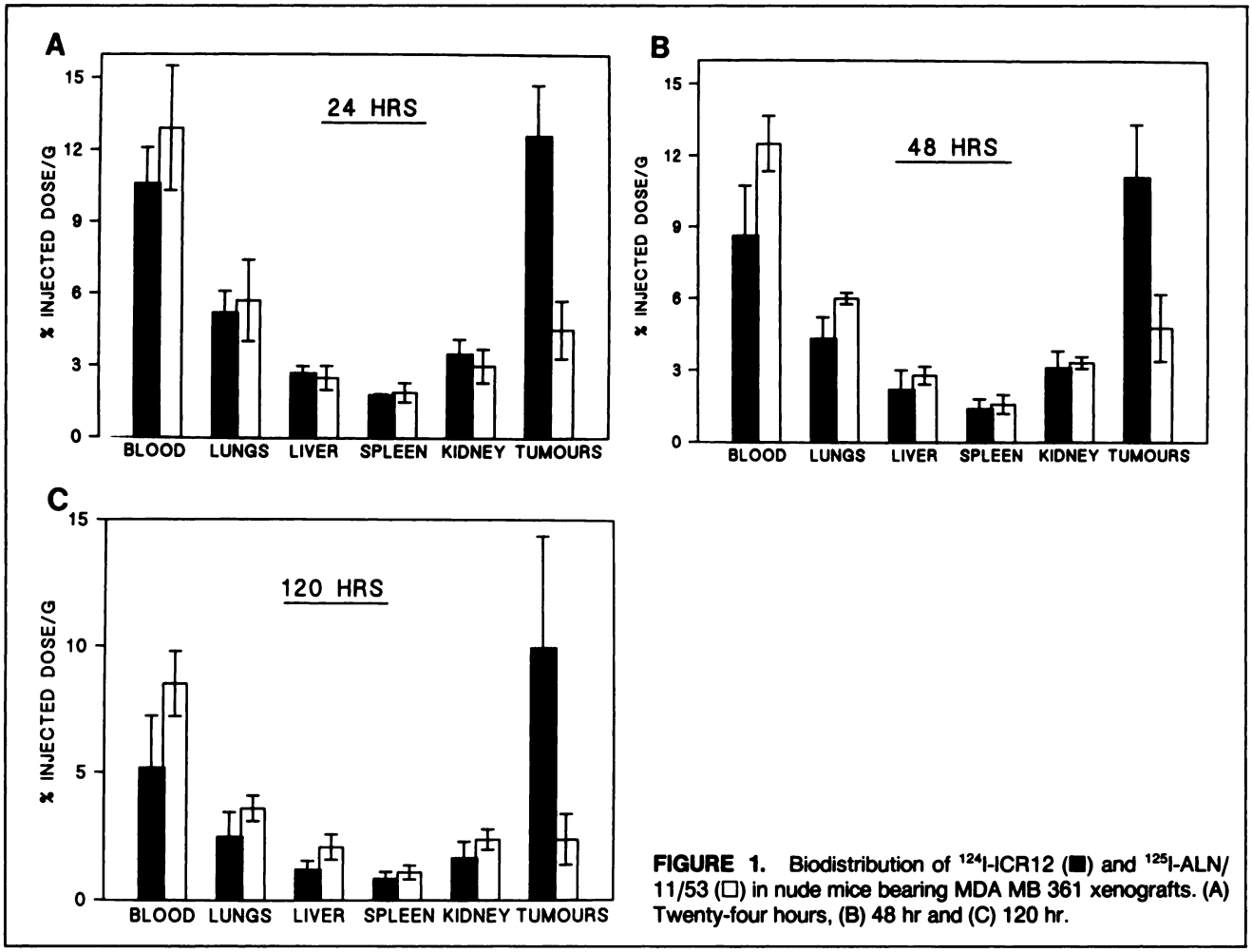

FIGURE 1. Biodistribution of ^{124}I-ICR12 (■) and ^{125}I-ALN/11/53 (□) in nude mice bearing MDA MB 361 xenografts. (A) Twenty-four hours, (B) 48 hr and (C) 120 hr.

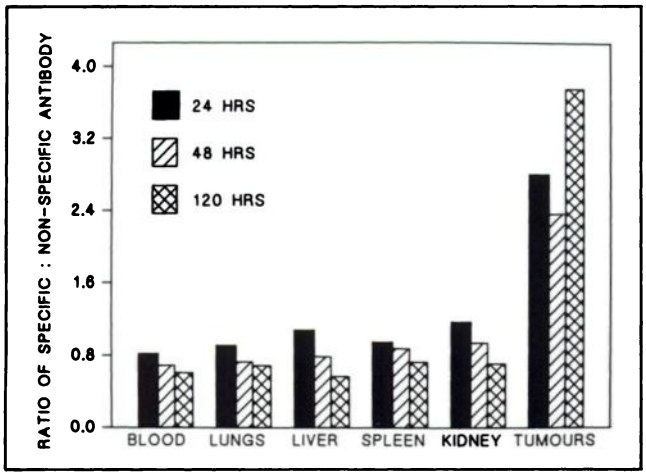

FIGURE 2. Ratios of ICR12:ALN/11/53 monoclonal antibodies in tissues of mice bearing MDA MB 361 breast carcinoma xenografts.

nonspecific antibody localization in all tissues are shown in Figure 2. These values were less than or close to unity for all normal tissues, but between 2.4 and 3.8 for the tumors. The localization indices (LI) are illustrated in Figure 3. Values of 3.4 at 24 and 48 hr rose to 6.2 at 120 hr. No significant localization of radiolabeled ICR12 was obtained in two control tumors which do not overexpress the c-erbB2 proto-oncogene, but which overexpress EGF-R (HN5 and MDA MB 468). Less than 4% ID/g was obtained at any time point, which was not significantly different from that obtained with the control antibody (data not shown).

Imaging Studies In vivo

The MUP-PET scanning of mice inoculated with 10 μg of ^{124}I successfully imaged tumors of 5 mm–7 mm diameter located s.c. in the flanks. Figure 4 shows representative 3 mm thick coronal sections through the mouse at 24 hr

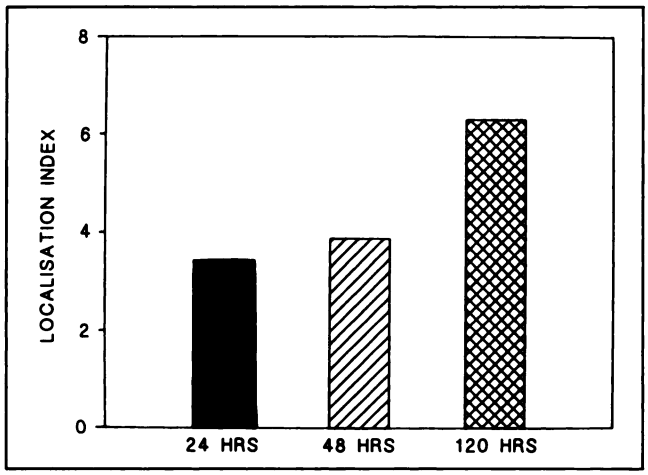

FIGURE 3. Localization indices (LI) as a function of time for ^{124}I-ICR12:^{125}I-ALN/11/53 in nude mice bearing MDA MB 361 breast carcinoma xenografts. Key as in Figure 2.

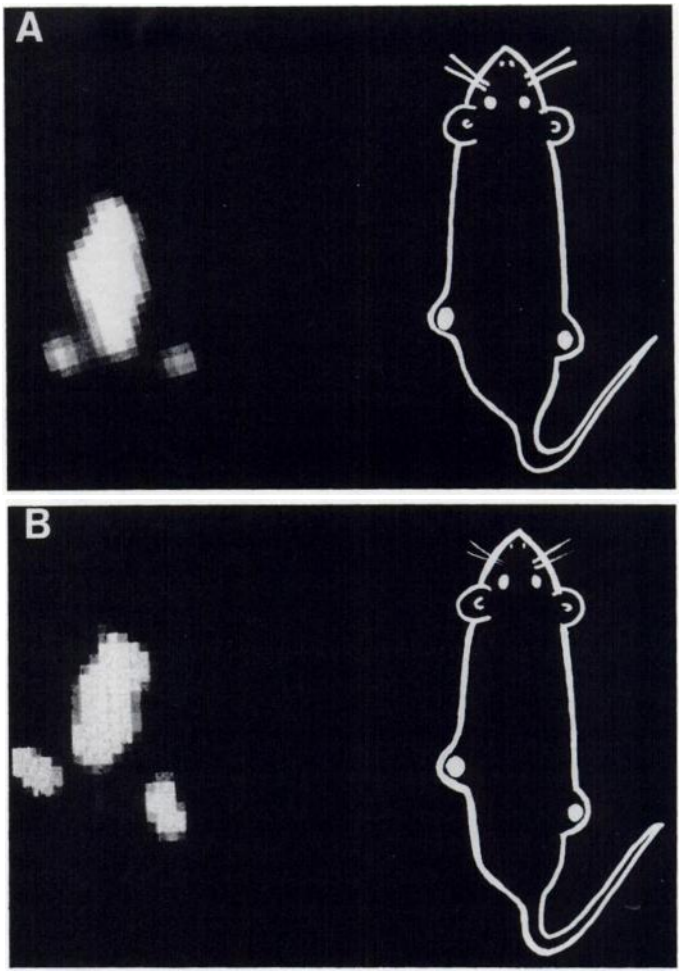

FIGURE 4. PET images of ^{124}I-ICR12 in a nude mouse bearing bilateral flank MDA MB 361 breast carcinoma xenografts of 5–7 mm diameter. Three millimeter coronal sections are shown at (A) 24 hr and (B) 120 hr postinjection.

(A), and 120 hr (B). At both time points, and at 48 hr (not shown), the tumors were easily discernible, and significant blood-pool activity was also present.

DISCUSSION

Amplification of the c-erbB-2 gene (and its associated overexpression at the cell surface) has been correlated with poor prognosis in patients with a variety of adenocarcinomas, most notably of breast and ovary (*3,8*). These patients are therefore at a higher risk of harboring micrometastases which, if detected and treated, may significantly improve their prospects of survival. The fact that the c-erbB-2 gene product is scarcely detectable on normal adult tissues (*21*)—unlike the related EGF-R—makes antibody-guided localization and therapy of such cancers an attractive proposition. The use of monoclonal antibodies labeled with gamma-emitting radionuclides (e.g., ^{131}I or ^{111}In) as diagnostic imaging agents is well established. However, this technique still suffers from some disadvantages which cannot be overcome completely by using single-photon emission tomography (SPECT). The main problems are

difficulty of image attenuation correction and low intrinsic sensitivity and spatial resolution. Positron emission tomography (PET) provides enhanced spatial resolution and sensitivity compared with conventional gammascintigraphy as well as the ability to quantify radioisotope distributions in vivo. The application of PET to monoclonal antibody imaging would therefore be helpful in determining quantitative tumor uptake and may aid in the localization of small tumor deposits.

Our initial approach to monoclonal antibody imaging using PET focused on the need for labeling ICR12 effectively with a radionuclide that possessed suitable chemistry and half-life. As optimal localization of radio-iodinated monoclonal antibodies usually occurs within 24 hr of administration (*22*), we chose ^{124}I ($T_{1/2}$ 4.2 days). In order to provide a labeled product of the highest possible purity, a study was undertaken to optimize the parameters for labeling ICR12. Pilot experiments comparing iodogen and NBS had shown that for iodine isotopes with no-carrier added (NCA) iodide, NBS yielded more consistent results, hence this was selected for future work. Optimal reaction conditions were defined (see Results section) with which a labeling efficiency of 96% was obtained routinely. The radioiodinated product was found to express immunoreactivity in vitro of between 80% and 90% and to remain stably labeled for 6 days in plasma at 37°C. It was considered that this reagent would be ideal for localization/imaging studies in nude mice.

Biodistribution studies demonstrated specific and sustained localization of ICR12 in a human breast carcinoma which constitutively over-expresses the c-erbB-2 gene product (p185) at the cell surface, and high resolution PET scans were obtained at all time points tested. Although several groups have described the development and use of monoclonal antibodies reactive with p185, (*23–26*), we believe that this is the first published demonstration of a successful pre-clinical localization and imaging trial using a human tumor. Saga et al. (*22*) recently described the biodistribution and scintigraphic detection of c-erbB-2 transfected mouse 3T3 cells using a murine antibody designated SV2-61r, but it is not certain that the expression and behavior of human genes will be identical in the two species. By using a radioiodinated anti-p185 antibody, these authors found a maximum uptake of 10.96% ID/g at 12 hr, falling rapidly to levels of less than 4% at 96 hr. This contrasts with our data where levels of at least 9% ID/g were maintained for 120 hr. Saga interprets these results as being due to the internalization of p185 subsequent to monoclonal antibody binding described by Drebin et al., who also used 3T3-transfected cells (*27*), since ^{111}In-labeled monoclonal antibody gave more sustained localization. We would suggest that the down-modulation and internalization of c-erbB-2 p185 is not an inevitable consequence of monoclonal antibody binding since in vitro ICR12 binding to tumor cell monolayers was relatively stable (unpublished observations) and the data for localization in vivo are also consistent with a long half-life at the cell surface. It is therefore possible that the behavior of p185 in 3T3 cells is different from that expressed constitutively in human breast carcinoma cells, or more probably that monoclonal antibodies recognizing different epitopes of the same moiety will engender different biological responses in the target cell. One such example concerns the related EGF-R, in which different monoclonal antibodies have been shown to potentiate or inhibit the effects of ligand binding (Modjtahedi H, *personal communication*). Our data indicate that it is possible to raise antibodies that can be used effectively to target iodine isotopes for scintigraphic or PET imaging and that hold promise for the development of targeted immunotherapy where sustained localization of the monoclonal antibody guided agent will be an advantage.

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EDITORIAL

Radiolabeled Antibodies to Oncogene-Encoded Molecules for Tumor Imaging and Therapy

The article by Bakir et al. in this issue of *The Journal of Nuclear Medicine* (*1*) demonstrates that a monoclonal antibody (Mab), ICR12, to a protein encoded by the c-erb B2 proto-oncogene, after radiolabeling with ^{124}I localized selectively to c-erb B2 expressing human tumors grows in nude mice. The localization could be detected by positron emission tomography (PET). The specific Mab localized 3-4 times better than a control Mab to tumor and there was up to 12% of the injected dose of ICR12 per gram of tumor tissue. The degree of tumor uptake, as well as the selectivity of specific, versus control antibody was comparable with data reported from similar studies with other antibodies using radioisotopes such as ^{125}I, ^{131}I or ^{111}In (*2*). As emphasized by the authors, the data suggest that ^{124}I-labeled ICR12 will be clinically useful for the diagnostic imaging of some breast carcinomas as well as other neoplasms which overexpress the c-erb B2 proto-oncogene product, and it is likely that the PET scanning technology will have advantages over conventional scintigraphy. A study by De Santes et al. (*3*) describes somewhat similar findings, but using different Mabs and ^{131}I instead of ^{124}I (and PET scanning).

An attractive feature of the work performed by Bakir et al. (and by De Santes et al.) is that the target molecule, p185, is encoded by a proto-oncogene. This has an advantage over tumor-associated differentiation antigens, which are the tumor markers most commonly used so far since the expression of p185 is not only higher in neoplasms than in normal tissues (which is true also for tumor-associated differentiation antigens), but is more intimately associated with the neoplastic state (*4*). Interestingly, overexpression of c-erb B2 correlates with a decreased sensitivity to treatment with hormones or chemotherapeutic drugs (*5,6*). This suggests that diagnostic imaging of tumors using Mabs such as ICR12 may be informative with respect to how aggressive the therapy should be and hence offers an advantage over imaging with Mabs to antigens whose expression does not correlate with prognosis. Furthermore, it may be possible to treat those tumors which can bind anti-p185 Mab by using it as a vehicle to deliver a large dose of radioisotope, and there are reasons to hope that p185 would be less likely to be lost from the cancer cells than antigens which are not encoded by an oncogene. The present study also suggests that certain antigens encoded by other oncogenes, for example, by mutated ras p21, or by mutated suppressor genes, could also prove useful as markers for in vivo diagnostics and perhaps even as therapeutic targets.

While the idea of using antibodies as "magic bullets" for tumor targeting was postulated a century ago by Paul Ehrlich, it has not been until fairly recently that by using Mab technology, antigens have been identified which are both expressed more in tumors than in most normal tissues and are lacking from critical cells such as hematopoetic stem cells. Some encouraging therapeutic findings have been already obtained with lymphomas where complete tumor regressions have been seen in patients given radiolabeled antibodies to tumor-associated differentiation antigens (*7,8*). While similar success has not been achieved when using Mabs to deliver radioisotopes to carcinomas, some provocative data have been obtained quite recently using an anti-carcinoma Mab, L6, to deliver ^{131}I to a small group of patients with breast carcinoma (*9*). Therapeutic studies in man in which anti-cancer drugs or toxins have been delivered via Mabs

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For reprints contact: Ingegerd Hellström and Karl Erik Hellström, Bristol-Myers Squibb Pharmaceutical Research Institute, 3005 First Ave., Seattle, WA 98121.