VIA MEDICA

Nuclear Medicine Review 2003
Vol. 6, No. 2, pp. 99–103

ISSN 1506–9680

Original

Uptake of radiolabelled herceptin by experimental mammary adenocarcinoma

Rafał Wiercioch[1], Ewa Balcerczak[1], Ewa Byszewska[2], Marek Mirowski[1, 2]

[1]Department of Pharmaceutical Biochemistry, Molecular Biology Laboratory, Medical University, Łódź, Poland
[2]Radioisotope Centre POLATOM, Otwock-Świerk, Poland

[Received 24 VI 2003; Accepted 20 X 2003]

Abstract

BACKGROUND: The aim of this study was to examine the biodistribution of ^{131}I-herceptin in C3H/Bi mice with transplantable mammary adenocarcinoma with a high frequency of C-erbB2 receptor expression.

MATERIAL AND METHODS: Mice C3H/Bi with subcutaneously transplanted mammary adenocarcinoma were used as animal model to study the interaction between C-erbB2 receptor and hercepin, a humanized anti-C-erbB2 monoclonal antibody. The expression of the gene encoding C-erbB2 receptor in the tumours was studied by the RT-PCR technique.

RESULTS: Expression of this gene was found in 66% of the studied cases. Similarly, the presence of the C-erbB2 receptor in 77% of the tumours was detected by a Western blot analysis with the use of herceptin. Biodistribution experiments of iodine-labelled herceptin in mice C3H/Bi with adenocarcinoma revealed its maximal accumulation in the tumours at 48 hours since the *i.v.* injection (7% ID/g). The tumour/muscle radioactivity ratio reached its highest value (above 20) also at 48 hours after the injection.

CONCLUSIONS: C3H/Bi mice with this adenocarcinoma may be a good experimental model to study herceptin, or its fragments, labelled with different radionuclids for preliminary evaluation of their usefulness in the therapeutic and diagnostic aspects of breast cancer.

Key words: herceptin, C-erbB2, receptor binding, breast cancer

Correspondence to: Marek Mirowski
Department of Pharmaceutical Biochemistry, Molecular Biology Laboratory, Medical University
ul. Muszyńskiego 1, 90–151 Łódź, Poland
Tel/fax: (+48 42) 677 91 30
e-mail: mmirowski@pharm.am.lodz.pl

Introduction

C-erbB2 (HER2) is a 185 kDa transmembrane glycoprotein with partial homology to epidermal growth factor receptor and with intrinsic tyrosine kinase activity [1–3]. The clinical importance of C-erbB2 became apparent with the recognition of *c-erbB2* gene amplification and the overproduction of the encoding protein in breast cancer patients. The *c-erbB2* amplification occurs in 25–30% of human breast cancers and is connected with the aggressive form of the cancer and with a bad prognosis [4, 5]. High level of C-erbB2 on tumour cells has provided the molecular basis for the use of this receptor as a target for anti-tumour therapy.

A new therapeutic approach to the treatment of C-erbB2 positive breast cancer cases is the use of humanized monoclonal antibody to C-erbB2 marked as herceptin (trastuzumab). This is a murine monoclonal antibody, that was engineered to give a human IgG1 molecule with the murine sequences necessary for binding to the C-erbB2 extracellular ligand-binding domain [6]. The antibody was humanized to minimize the immunogenicity associated with murine protein and to enhance the endogenous immune anti-tumour effects. Preliminary clinical studies provided evidence that herceptin is safe and clinically active in women with C-erbB2-overexpressing metastatic breast cancer [7, 8].

The overexpression of some receptors, including C-erbB2, on different neoplastic cells makes it also possible to use the labelled antibodies raised against them as radiotracers of neoplastic lesions or as carrier systems in targeting anti-cancer drugs. Complexes of radionuclides with such antibodies as herceptin may help to develop new methods of detection, localization and radiotherapy of tumours [9–11].

In the present study, we have investigated the biodistribution of ^{131}I-herceptin in C3H/Bi mice with transplantable mammary adenocarcinoma with a high frequency of C-erbB2 receptor expression.

Material and methods

Animals

In the experiments, female C3H/Bi mice with transplantable mammary tumours were used. The animals were obtained from the Institute of Oncology, (Warsaw, Poland) and kept under standard conditions: temperature 22°C, a 12 h light-dark cycle, and allowed tap water containing 0.1% KI (to minimize free radioiodide uptake by the thyroid) and rodent chow *ad libitum*. Mice with tumours over 0.5 cm in diameter were used for biodistribution

studies. All experiments on living animals were carried out according to the protocols approved by the local ethical committee.

Tumour implantation

The mammary tumour bearing C3H/Bi mice were anaesthetized and killed by cervical dislocation. The tumours were removed and, after washing with cold and sterile 0.9% NaCl, sliced into 2–3 mm pieces and homogenized (1:9 w/v). Tumour cell suspension was filtered through aseptic gauze and then injected subcutaneously (150 μl) into three different areas of the dorsal region of healthy mice lightly anaesthetized with vetbuthal (pentobarbital sodium salt, 60 mg/kg body weight, intraperitoneal injection).

Tumour membrane preparation

The homogenate was prepared from tumours after discarding the necrotic fragments. The tissue was homogenized in an ice-cold 0.01 M Tris-HCl buffer, pH 8.0 containing 0.005 M phenylmethylsulfonylfluoride (PMSF) in ratio 1 : 9 and then centrifuged at 2000 rpm for 20 min at 4°C. The supernatant was recentrifuged at 25000 rpm for 30 min at 4°C. The pellet was resuspended in the same buffer and spun again at 25000 rpm. The pellet was then dissolved in 0.01M Tris-HCl buffer, pH 8.0 containing 0.15 mM NaCl, 1 mM EGTA and 0.005 M PMSF and protein concentration was measured by Lowry's method using bovine serum albumin as a standard.

Electrophoresis of membrane proteins

Electrophoresis was performed in 7.5% polycrylamide gel under reduced conditions (SDS-PAGE). Aliquots of membrane proteins (20 μl, about 50 μg of protein) were mixed with the same volume of sample buffer (0.0625 M Tris-HCl, pH 6.8; 4% SDS; 50% glycerol; 20% β-mercaptoethanol and 0.25% bromophenol blue), heated for 1 min at 95°C and run on SDS-PAGE gels. The separated proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane.

Immunoblotting

Proteins transferred to a PVDF membrane [12] were blocked by incubation with a 5% milk solution in TTBS (0.1 M Tris-HCl, pH 7.4 containing 0.9% NaCl and 0.1% Tween 20) for 2 h at room temperature. Then, the herceptin (Hoffmann-La Roche, Germany) at 1:100 dilution in a 5% milk solution in TTBS was added and incubated with PVDF for 1 h at room temperature with constant shaking. After three 15 min washes in TTBS, the PVDF membranes were incubated for 1 h at room temperature with a secondary antibody (goat anti human biotynylated IgG kit, Vector Laboratories, USA) at 1:300 dilution. After being washed three times in TTBS, the PVDF membranes were incubated in ABC reagent (Vector Laboratories) for 30 min in the dark and developed with 0.06% diaminobenzidene and 0.009% H_2O_2 in 0.05 M Tris-HCl buffer, pH 7.5. When the colour had developed, the membranes were washed extensively with distilled water.

RNA extraction

RNA was isolated from mammary adenocarcinomas by Total RNA Prep Plus Minicolumn Kit (A&A Biotechnology, Poland) based on RNA isolation method developed earlier [13]. The isolated RNA had an $A_{260/280}$ ratio of 1.6–1.8.

RT-PCR

cDNA was synthesized by a RevertAid™ cDNA Synthesis Kit (Fermentas, Lithuania). A reaction mixture (total volume 20 μl) containing 1 ml of total RNA (3 μg), 1 ml of oligo(dT) 18 primer (0,5 μg) and 8 ml of deionized nuclease free water was prepared. The mixture was spun down and incubated at 70°C for 5 min, then chilled on ice and the following components were added: 4 μl of 5 $\times$ reaction buffer, 1 μl of ribonuclease inhibitor (20 U/μl) and 4 μl of 10 mM of dNTPs mix. The mixture was incubated at 37°C for 5 min and 1 μl of RevertAid MulV reverse transcriptase (200 U/μl) was added. The mixture was incubated at 42°C for 60 min. The reaction was stopped by heating at 70°C for 10 min. Sets of primers for *c-erbB2* (5'-CCTCTGACGTCCATCATCTC-3', 5'-CG--GATCTCCTGCTGCCGTCGT-3') were planned on the basis of article by Schwartz et al [14]. PCR was carried out as follows: denaturation (94°C, 1 min), annealing (55°C, 1 min) and extension (72°C, 2 min) for 30 cycles. As a reference the primers for *β-actine* gene (5'-TGTATGCCTCTGGTCGTACCAC-3', 5'ACAGAG--TACTTGCGCTCAGGAG-3') were used [15].

The amplification of the *c-erbB2* receptor and *β-actine* genes was performed in the same tube (multiplex PCR, MPCR) as follows: denaturation (94°C, 30 s); annealing (60°C, 30 s); extension (72°C, 30 s) during 35 cycles. The PCR products were separated by electrophoresis in 2% agarose gel.

Radiolabelling of herceptin

The labelling of herceptin with [^{131}I] was carried out using the chloramine T method [16]. Labelled herceptin was purified from free radioiodine with the use of a molecular-sieving column (PD-10) and filtered through a 0.22 mm filter.

Biodistribution

Biodistribution studies were carried out on C3H/Bi mice weighing about 30 g, which had developed three tumours with a weight from 0.3 to 0.6 g.

Tumour bearing mice were injected in the tail vein (*i.v.*) with approximately 0.3 μCi (11 kBq) of ^{131}I-herceptin (specific activity c.a. 3.0 MBq/μg of herceptin). The radioactivity in various organs of the mice was determined at intervals of 0.5, 2, 6, 24, 48, 72, 96, 120 and 144 h. For each interval four mice were used. After anaesthetizing, the blood was obtained by heart puncture. All tissue samples were removed, washed in cold PBS, weighed and their radioactivity was measured in a gamma counter. The results were calculated and expressed as a percentage of the injected dose per one gram of tissue (%ID/g) or a percentage of the injected dose (%ID) for radioactivity eliminated with urine.

Results

C3H/Bi mice loaded with transplantable mammary tumours, histologically classified as adenomacarcinomas [17], were used in the experiments.

The investigation of *c-erbB2* gene expression by the RT-PCR technique was conducted on 18 randomly selected tumours. Twelve of them (66%) showed the presence of *c-erbB2* gene

expression (98 bp fragment). In the same experiment, reference *β-actine* gene was amplified (368 bp) as a positive control of PCR reaction and its expression was stated in all analysed tumours (Fig. 1).

The presence of the investigated receptor in the adenocarcinoma was also confirmed by a Western blot analysis with the use of herceptin as a monoclonal anti-C-erbB2 antibody. For this purpose, the adenocarcinoma cell membranes were subjected to polyacrylamide gel electrophoresis with sodium dodecyl sulfate. Electrophoretically separated proteins were transferred onto a PVDF membrane and analyzed by the Western blot technique, which revealed the presence of C-erbB2 receptor in 14 (77%) out of 18 analyzed tumours (Fig. 2).

Herceptin in SDS-PAGE with 2-mercaptoethanol migrates as two bands; one typical for heavy chains of IgG (about 50 kDa) and the second for light chains (about 24 kDa). Both heavy and light chains were radioactive after herceptin iodination, as determined by electrophoresis followed by autoradiography (data not shown).

Accumulation of herceptin in mouse organs (%ID/g of tissue), as well as its elimination with urine (%ID), have been summarized in Table 1. The highest herceptin concentration in adenocarcinoma was observed 48 h after administration (about 7% ID/g). After this time, a decreasing tendency was observed. However, a high herceptin concentration was noticed in the blood. The percentage of dose per gram of blood was at its highest between 2 and 48 h after the injection. The ratio of tumour to muscle radioactivity reached the highest value (about 20) 48 h after injection. The tumour to blood ratio was at its highest level (2.5) 96 h after the administration of herceptin (Fig. 3).

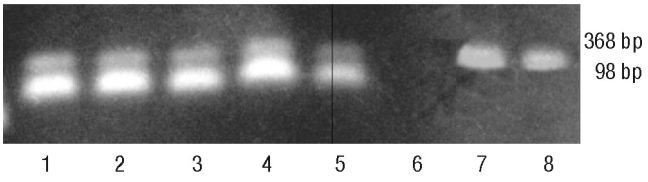

Figure 1. An example of RT-PCR analysis of the *c-erbB2* receptor gene expression (98 bp) in mouse mammary adenocarcinoma. 6 — negative control. 1–5 — *c-erbB2* positive and 7, 8 — *c-erbB2* negative cases. As a PCR control in all analyzed cases *β-actine* gene (368 bp) was amplified.

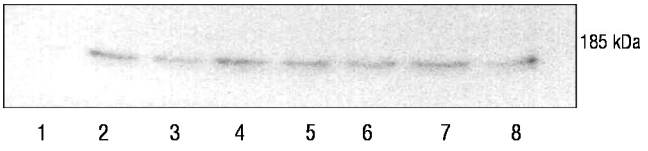

Figure 2. Western blot analysis of C-erbB2 protein in mouse mammary adenocarcinoma. Membrane proteins isolated from tumours were separated by electrophoresis (7.5% SDS-PAGE), transferred onto PVDF and probed with herceptin as a first antibody. 1 — negative control; 2–8 — C-erbB2 positive cases.

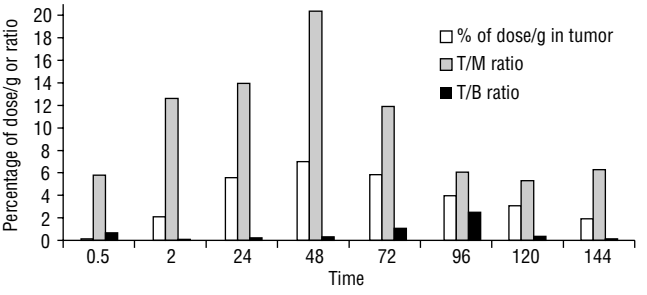

Figure 3. Herceptin accumulation (% ID /g) in mouse mammary adenocarcinoma. The ratio of tumour to muscle (T/M) and tumour to blood (T/B) radioactivity after *i.v.* injection of ^{131}I-herceptin into tail vein.

Discussion

C-*erbB2* gene is over expressed in at least one quarter of human breast cancers [4, 5] and correlates with a poor clinical outcome in women with node-positive and node-negative disease [5, 18]. To target this receptor, monoclonal antibodies directed against C-erbB2 were developed [19]. In preclinical studies these antibodies inhibited the growth of C-erbB2-overexpressing human breast tumour cells and sensitised them to tumour necrosis factor and to cis-diaminedichloroplatinum [20–22].

Taking into consideration the data showing the clinical usefulness of herceptin, we have analysed its biodistribution in mice loaded with transplantable mammary adenocarcinoma with a high frequency of C-erbB2 receptor expression. We first determined, by the RT-PCR technique, the *c-erbB2* gene expression in the tumours. In further studies, herceptin was used as antibody for C-erbB2 receptor detection on the cell membranes prepared from the above-mentioned tumours. The presence of *c-erbB2* amplicon and protein product of this gene in tumour tissue was stated in about 70% of the analyzed cases. However, the frequency of this expression on protein level is higher than at the mRNA level. The specificity of the RT-PCR method is therefore higher than immunoblotting analysis. The recognition of the mouse C-erbB2 receptor by herceptin may suggest that both human and mouse receptors share some antigenic determinants.

In the biodistribution studies, herceprtin was well concentrated in the tumours (Tab. 1). The highest accumulation of this antibody in the tumours was observed 48 hours after its injection. The ratio of radioactivity in tumour and muscle tissues showed a growing tendency with time and its highest value (about 20) was also observed after 48 hours. Taking into account that biodistribution studies were performed on a randomly selected group of tumour bearing mice, and that receptor expression was detected in about 70% of tumours, we might expect the herceptin uptake to be higher by 20–30%, if the experiment was conducted only on mice with *c-erbB2*/C-erbB2 positive tumours.

Unfortunately, also 48 hours after the administration of herceptin, a high concentration of this protein in the blood was noticed. The slow elimination of this antibody from the blood may be partially connected with the probable presence in the sera of the soluble exstracellular domain of the C-erbB2 receptor. Such a domain of this receptor was observed in the sera of breast cancer patients undergoing herceptin treatment [23, 24]. The presence in the blood of extracellular fragments responsible for ligand binding was also documented for other membrane receptors [25, 26].

Table 1. Biodistribution of ^{131}I-herceptin in mammary adenocarcinoma bearing mice (%ID/g of tissue or blood and %ID eliminated with urine, mean ± SD, n = 4, *i.v.*)

Time	30 min	2 h	24 h	48 h	72 h	96 h	120 h	144 h
Blood	0.2 ± 0.1	25.1 ± 1.4	24.0 ± 2.5	24.7 ± 1.4	6.1 ± 0.3	1.6 ± 0.12	9.7 ± 0.5	7.4 ± 0.5
Lung	0.01 ± 0.01	2.2 ± 0.4	2.5 ± 0.1	2.5 ± 0.15	1.3 ± 0.15	0.5 ± 0.1	1.1 ± 0.1	1.4 ± 0.16
Liver	0.07 ± 0.01	3.7 ± 0.35	1.1 ± 0.05	2.4 ± 0.18	1.2 ± 0.1	1.3 ± 0.14	2.4 ± 0.25	5.3 ± 0.5
Spleen	0.06 ± 0.02	1.6 ± 0.1	0.35 ± 0.1	1.1 ± 0.1	0.8 ± 0.2	0.7 ± 0.08	0.7 ± 0.05	0.1 ± 0.06
Kidney	0.11 ± 0.01	2,2 ± 0.1	0,8 ± 0.1	2.1 ± 0.1	0.8 ± 0.2	0.2 ± 0.02	0.4 ± 0.14	1.3 ± 0.16
Stomach	0.34 ± 0.02	3.4 ± 0.4	0.6 ± 0.1	2.7 ± 0.2	1.4 ± 0.1	0.5 ± 0.1	3.0 ± 0.3	3.8 ± 0.2
Intestine	0.09 ± 0.06	4.9 ± 0.4	1.8 ± 0.1	2.0 ± 0.05	0.75 ± 0.2	0.6 ± 0.2	1.5 ± 0.13	1.1 ± 0.3
Tumour	**0.15 ± 0.01**	**2.1 ± 0.15**	**5.6 ± 0.4**	**7.0 ± 0.16**	**5.8 ± 0.35**	**4.0 ± 0.1**	**3.1 ± 0.2**	**1.9 ± 0.1**
Muscle	0.025 ± 0.04	0.17 ± 0.02	0.4 ± 0.1	0.35 ± 0.1	0.5 ± 0.1	0.65 ± 0.1	0.6 ± 0.08	0.3 ± 0.08
Brain	0.13 ± 0.01	0.35 ± 0.02	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.05	0.15 ± 0.06	0.42 ± 0.1	0.15 ± 0.06
Urine (total)	1.2 ± 0.1	0.5 ± 0.3	4.1 ± 0.2	10.1 ± 1.3	15.0 ± 1.35	25.1 ± 0.18	34.3 ± 2.8	13.4 ± 1.6

One of the main problems for antibody-mediated cancer therapy with radiolabeled antibodies is their slow delivery from the circulation to the target cancer cells [27–29]. Slow tumour-delivery of labelled herceptin was also observed in our study. This may be a source of toxic effects on bone marrow as well as on some organs. In the case of herceptin, it was suggested that radiometal labels are preferable over radioiodine. A scintigraphic image could be obtained as early as 3 h after injection of ^{111}In-labeled herceptin. The accumulation of ^{111}In-herceptin in the WIBC-9 tumors was significantly higher than that of the ^{125}I-labeled herceptin [30, 31].

To enhance perfusion into cancerous tissue, small variants of antibodies were chemically or genetically engineered. A study conducted on Rhesus monkeys showed that full-length herceptin did not penetrate the inner limiting membrane of the retina. In contrast, the Fab antibody fragment diffused through the neutral retina to the retinal pigment epithelial layer [32]. It is also possible that the labelled Fab herceptin fragment will penetrate the C-erbB2 over expressing tumours much more easily than the full-length antibody.

Herceptin, in the case of C-erbB2 over expressing metastatic breast cancer patients, may serve as a vector for radioimmunodiagnostics or radioimmunotherapy [7, 33]. In the present study it was shown that the mammary adenocarcinoma is characterized by relatively high C-erbB2 receptor expression on protein, as well as mRNA levels. C3H/Bi mice with this adenocarcinoma may be a good experimental model to study herceptin, or its fragments, labelled with different radionuclids for preliminary evaluation of their usefulness in the therapeutic and diagnostic aspects of breast cancer.

Acknowledgements

This work was supported by grants: 503–301–2, 502–13–844 from the Medical University of Lodz, Poland and by the Radioisotope Centre POLATOM (BB–3–11/2001). We wish to thank Prof. Ryszard Wierzbicki for his constructively critical comments and Foreign Language Teaching Center, Medical University of Lodz for help in the correction of the English version of this text.

References

1. Akiyma T, Sudo C, Ogawara H, Toyoshima K, Yarnamoto T. The product of the human c-erbB2 gene: A 185-kilodalton glycoprotein with tyrosine kinase activity. Science 1986; 232: 1644–1646.
2. Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. Science 1985; 230: 1132–1139.
3. Stern DF, Heffernan PA, Weinberg RA. p185, a product of the neu proto-oncogene, is a receptorlike protein associated with tyrosine kinase activity. Mol Cell Biol 1986; 6: 1729–1740.
4. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ulrich A, McGuire WL. Human breast cancer: Correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. Science 1987; 235: 177–182.
5. Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Levin WJ, Stuart SG, Udove J, Ulrich A. Studies of HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science 1989; 244: 707–712.
6. Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME, Shepard HM. Humanization of an anti-p185^{HER2} antibody for human cancer theraphy. Proc Natl Acad Sci USA 1992; 89: 4285–4289.
7. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/*neu* over expressing metastatic breast cancer. J Clin Oncol 1996; 14: 737–744.
8. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA, Slamon DJ. Phase II study of receptor — enhanced chemosensitivity using recombinant humanized anti-p185HER/*neu* monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 1998; 16: 2659–2671.
9. Hopkins K, Chandler C, Bullimore J, Sandeman D, Coakham H, Kemshead JT. A pilot study of the treatment of patients with recurrent malignant gliomas with intratumoral yttrium-90 radioimmunoconjugates. Radiotherapy and Oncol 1995; 43: 121–131.
10. Kalafonos HP, Pawlikowska TR, Hemingway A, Courtenay-Luck N, Dhokia B, Snook D, Sivolapenko GB, Hooker GR, McKenzie CG, Lavender PJ. Antibody guided diagnosis and therapy of brain gliomas using radiolabeled monoclonal antibodies against epidermal growth factor receptor and placental alkaline phosphatase. J Nucl Med 1989; 30: 1636–1645.
11. Papanastassiou V, Pizer BL, Coakham HB, Bullimore J, Zananiri T, Kemshead JT. Treatment of recurrent and cystic malignant gliomas by a single intraactivity injection of ^{131}I monoclonal antibody: feasibility, pharmacokinetics and dosimetry. Br J Cancer 1993; 67: 144–151.
12. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc Natl Acad Sci USA 1979; 76: 4350–4354.

13. Chomczyński P, Sacchi N. Single-step method of RNA isolation by acid guanidinum thiocynate-phenol-chloroform extraction. Anal Biochem 1987; 162: 156–159.
14. Schwartz S, Caceres C, Morote J, De Tores I, Rodriguez-Vallejo J, Gonzalez J. Over-expression of epidermal growth factor receptor and c-erbB2/neu but not of int-2 genes in benign prostatic hyperplasia by means of semi-quantitative PCR. Int. J. Cancer 1998; 76: 464–467.
15. Tokunaga K, Taniguchi H, Yoda K, Shimizu M, Sakiyama S. Nucleotide sequence of a full-length cDNA for mouse cytoskeletal β-actine mRNA. Nucleic Acid Res 1986; 14: 2829–2836.
16. Greenwood FC, Hunter WM, Glower JS. The preparation of ^{131}I-labeled human growth hormon of high specific radioactivity. Biochem J 1963; 89: 114–123.
17. Mirowski M, Świtalska J, Wiercioch R, Byszewska E, Niewiadomska H, Michalska M. Uptake of radiolabeled alpha-fetoprotein (AFP) by experimental mammary adenocarcinoma and adenoma: *in vivo* and *in vitro* studies. Nucl Med Commun 2003; 24: 297–303.
18. Ravdin PM, Chamness GC. The c-erbB2 proto-oncogene as a prognostic and predictive marker in breast cancer: A paradigm for the development of other macromoleculear markers — a review. Gene 1995; 159: 19–27.
19. McKenzie SJ, Marks PJ, Lam T, Morgan J, Panicali DL, Trimpe KL, Carney WP. Generation and characterization of monoclonal antibodies specific for the human neu oncogene product p185. Oncogene 1989; 4: 543–548.
20. Hancock MC, Langton BC, Chan T, Toy P, Monahan JJ, Mischak RP, Shawver LK. A monoclonal antibody against the c-erbB2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. Cancer Res 1991; 51: 4575–4580.
21. Harwerth IM, Wels W, Schlegel J, Muller M, Hynes NE. Monoclonal antibodies directed to the erbB2 receptor inhibit *in vivo* tumour cell growth. Br J Cancer 1993; 68: 1140–1145.
22. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ulrich A. p185HER2 monoclonal antibody has antiproliferative effects *in vitro* and sensitizes human breast tumor cells to tumor necrosis factor. Mol Cell Biol 1989; 9: 1165–1172.
23. Payne RC, Allard JW, Anderson-Mauser L, Humphreys JD, Tenney DY, Moerris DL. Automated assay for HER-2/*neu* in serum. Clin Chem 2000; 46: 175–182.
24. Wu JT. C-erbB2 oncoprotein and its soluble ectodomain: a new potential tumor marker for prognosis early detection and monitoring patients undergoing Herceptin treatment. Clin Chim Acta 2002; 322: 11–19.
25. Lee EJ, Oh EJ, Park YJ, Lee HK, Kim BK. Soluble transferring receptor (sTfR), ferritin, and sTfR/log ferritin index in anemic patients with non-hematologic malignancy and chronic inflammation. Clin Chem 2002; 48: 1118–1121.
26. Tesarova P, Kvasnicka J, Umlaufova A, Homolkova H, Jirsa M, Tesar V. Soluble TNF and IL-2 receptors in patients with breast cancer. Med Sci Monit 2000; 6: 661–667.
27. Kobayashi H, Sakahara H, Hosono M, Yao ZS, Toyama S, Endo K, Konishi J. Improved clearence of radiolabeled biotinylated monoclonal antibody following the infusion of avidin as a chase without decreased accumulation in the target tumor. J Nucl Med 1994; 35: 1677–1684.
28. Saga T, Neuman RD, Heya T, Sato J, Kinuya S, Le N., Paik CH, Weinstein JN. Targeting cancer micrometastases with monoclonal antibodies: a binding-site barrier. Proc Natl Acad Sci USA 1995; 92: 8999–9003.
29. Yokota T, Milenic DE, Whitlow M, Schlom J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. Cancer Res 1992; 52: 3402–3408.
30. Behr TM, Behe M, Angerstein C, Griesinger F, Kaufmann CC, Wormann B, Emos G, Becker W. Does immunoscintigraphy allow for prognostic predictions with respect to the toxicity and therapeutic efficacy of herceptin immunotherapy of metastatic breast cancer. Eur J Nucl Med 2001: 976.
31. Kobayashi H, Shirakawa K, Kawamoto S, Saga T, Sato N, Hiraga A, Watanabe I, Heike Y, Togashi K, Konishi J, Brechbiel MW, Wakasugi H. Rapid accumulation and internalization of radiolabeled herceptin in an inflammatory breast cancer xenograft with vasculogenic mimicry predicted by the contrast agent G6-(1B4M-Gd)256. Cancer Res 2002; 62: 860–866.
32. Mordenti J, Cuthbertson RA, Ferrara N, Thomsen K, Berleau L, Licko V, Allen PC, Valverde CR, Meng YG, Fei DT, Fourre KM, Ryan AM. Comparisons of the intraocular tissue distribution, pharmacokinetics, and safety of 125I-labeled full-length and Fab antibodies in rhesus maonkeys following intravitreal administration. Toxicol Pathol 1999; 27: 536–544.
33. Slamon DJ, Leyland-Jones B, Shak S, Fuschs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L. Use of chemotherapy plus monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001: 344: 783–792.