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Clinical Radioimmunolocalization with a Rat Monoclonal Antibody Directed Against c-*erb*B-2

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ABSTRACT

Lymph node status is still the single most important prognostic factor in breast cancer and surgery remains the only reliable means of providing this information. This study evaluates using a highly specific radiolabeled monoclonal antibody to provide equivalent information.

The optimum labeling conditions for radiolabeling a monoclonal antibody against the gene product of the protooncogene c-*erb*B-2 with Tc99m were established. This immunoconjugate was next evaluated in a mouse model system and averaged 20% localization of the total injected dose per gram of tumor at 24 h.

Ten patients have had this immunoconjugate, with planar and tomographic reconstructed images being obtained at 24 h. The resulting images were compared to histopathological examination of the surgical specimens. Three patients acted as normal controls, two patients were selected on the basis of inappropriate sampling of adjacent ductal carcinoma *in situ*, three patients demonstrated only moderate antigen expression, and two patients demonstrated excellent tumor localization in both breast primary and regional node metastases.

The high specificity of this antibody, ease of labeling, and excellent localization performance with a good antigen target encourage the development of this system as a method of localization and a potential means of antibody-guided therapy.

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Index Entries: Axillary lymph nodes; breast cancer; c-*erb*B-2; disease staging; monoclonal antibody; radioimmunolocalization.

INTRODUCTION AND AIMS

Lymph node status is still the single most important prognostic factor in breast cancer. At present, axillary surgery remains the only reliable means of providing this necessary information for determining the requirements of an individual for adjuvant therapy. This study evaluates the potential of clinical radioimmunolocalization using a highly specific radiolabeled monoclonal antibody against the gene product of the c-*erb*B-2 protooncogene to provide equivalent information by a noninvasive technique.

Successful localization of an antibody to the target antigen relies firstly on there being a high level of antigen expression by the tumor relative to normal tissues (*1,2*), and on the availability of highly specific antibodies that recognize the target antigen. This tumor specificity is itself an essential prerequisite to any future development of immunotherapeutic applications (*3,4*). By selecting the antibody for radioimmunolocalization studies on the basis of prior immunostaining of fine needle aspirates of each tumor, we aimed to identify the best antibody to be used for the investigation of each individual patient. We present the results from the first of our antibodies, ICR12 that has been fully evaluated both in vitro, in vivo, and in clinical cases (*5*). ICR12 is a rat IgG2a monoclonal antibody directed against the extracellular domain of the 185 kDa product of the c-*erb*B-2 protooncogene; a transmembrane tyrosine kinase related to the receptor for epidermal growth factor. Amplification of this gene and overexpression of the product has been found in some 20% of breast cancers and overexpression has been found to correlate with poor prognosis in these patients (*6,7*). The minimal levels of expression of this antigen in most normal tissues make this a highly tumor specific target (*8*). ICR12 was selected from a panel of rat monoclonal antibodies to p185 (*9*) on the basis that it binds both to frozen and formalin fixed paraffin embedded tumor sections that overexpress p185 and it localizes with high efficiency and stability to p185 expressing breast and ovarian cancer xenografts in athymic mice (*10*).

PATIENTS AND METHODS

Patients were recruited for inclusion into this study from staging clinics at The Royal Marsden Hospital, Sutton. All patients had primary breast cancer, were aged between 35–80 yr, and were recruited prior to surgical management of the axilla. Written consent was obtained from each patient following approval of the protocol by The Royal Marsden Hospital Ethical

Committee. The diagnosis of breast cancer relied on the triple approach of clinical findings, mammography, and positive fine needle aspiration cytopathology or core biopsy histopathology. Immunostaining was by the indirect immunoperoxidase technique (*11*) and the results obtained were finally verified by staining of fixed paraffin embedded tissue obtained from the surgically resected specimen (*11*). Strong staining was defined as dense immunoperoxidase brown staining of greater than 50% of malignant cell membranes.

ICR12 was labeled with Tc99m using a modification of the mercaptoethanol reduction method (*12,13*). Briefly, the optimal conditions were achieved by treatment of the antibody by part reduction with 2-mercaptoethanol (2ME) at a molar ratio of 500 parts 2ME:1 part antibody, for 30 min at room temperature. The final stage of labeling involved the further reduction of the partly reduced antibody using a standard bone scanning kit "Osteoscan HDP" (Mallinckrodt Medical, London) at 100 μL from the reconstituted kit (stannous chloride and disodium oxidronate, one vial reconstituted with 5 mL phosphate buffered saline) per mg of the reduced antibody in the presence of 1000MBq of Tc99m. In the preclinical studies, the effects of differing conditions of radiolabeling on the immunoreactivity of the antibody were established using competitive radioimmunoassay techniques (*10*). ICR12 labeled with Tc99m was compared with 125-iodine labeled ICR12 for its capacity to compete with unlabeled ICR12 for binding to the human ovarian carcinoma cell line SKOV3, which overexpresses the target antigen (*10*). Standard competitive radioimmunoassay techniques were employed with doubling dilutions of each test antibody against the native antibody down the cell plate. Further determination of the effect of labeling on immunoreactivity of the radiolabeled antibody was performed by immunoprecipitation studies of Tc99m labeled antibody binding to Sepharose 4B beads coated with an excess of the p185 antigen. The resultant trapping of the active-labeled antibody to the antigen coated beads was measured in an automated gamma counter. This method of testing demonstrates the combined effects of both labeling efficiency and immunoreactivity, rather than testing each function in isolation.

Eight athymic mice bearing established bilateral xenographs of the human breast carcinoma MDA MB-361 were injected intrevenously with 100 μg of Tc99m-ICR12 in order to investigate the in vivo biodistribution of this immunoconjugate. The mice were then killed at 24 h by CO_2 asphyxiation. Radioactivity in weighed samples of blood, lungs, liver, spleen, kidneys, muscle, skin, and tumors was determined in a gamma well spectrometer and the results expressed in terms of percent injected dose per gram of tissue (%ID/g).

Patients were given 2 mg of ICR12 labeled with 700MBq of Tc99m by slow iv injection. Axillary and thoracic regions were imaged at 24 h after injection using a General Electric Maxi gamma camera with a standard collimator. Planar acquisition of approx 200,000 counts was performed

initially, followed by 360° tomography acquiring in 64 projections for 30 s at each angle. Computerized reconstruction was then undertaken to provide tomographic representations in the transaxial, sagittal, and coronal planes. Reconstruction of images was performed using a ramp filter, and these transaxial reconstructions were analyzed further by computer to show levels of activity in regions of interest thus quantifying the imaged results.

RESULTS

The labeled antibody was evaluated in *nu/nu* mice bearing subcutaneous human xenograft tumors that overexpress the gene product. Localization values of the antibody to tumor were four times greater than those of normal tissues and averaged 20.4% of the total injected dose being in a gram of tumor at 24 h. A localization index of greater than 4:1 was found between tumor and any other normal tissue or organ at 24 h with this immunoconjugate.

Following the preclinical investigations, 10 patients received this Tc99m labeled antibody. These patients were selected for suitability by immunocytochemical staining of fine needle aspirate samples taken from the patient's primary breast cancer. After iv administration of the labeled antibody, both planar and tomographic reconstructed images were obtained at 24 h. The results of these images were compared to the histopathological examination of the subsequent surgical resections. Three patients acted as normal controls, 2 patients were selected on the basis of inappropriate sampling of adjacent ductal carcinoma *in situ*, and 2 patients demonstrated excellent tumor localization in both breast primary and regional node metastases. It was possible to demonstrate even solitary nodal metastases of a threshold size of 3 mm transverse diameter (measured from mounted, fixed histopathological specimens) in these strongly c-*erb*B-2 expressing patients. Estimation of tumor localization of the immunoconjugate, calculated from phantom reconstructions of the tumor and measurements of activity per pixel of the obtained images gave values (uncorrected for volume) of 0.005% injected dose per gram of tumor. A further 3 patients demonstrated only moderate antigen expression on pretesting and their images were correspondingly less clear.

DISCUSSION

Our results from the preclinical evaluation experiments and the subsequent clinical evaluation provide support for the hypothesis that the use of a highly specific antibody against a preselected target will improve the prospects for accurate localization of tumor deposits by radioimmuno-

localization. The excellent localizing properties of ICR12 into tumors that strongly overexpress c-*erb*B-2 p185 and the absence of uptake of this antibody in antigen negative tumors and normal breast tissue suggest that it may have a useful clinical role in staging patients and should be included as a constituent member of our eventual completed panel of antibodies for staging breast cancer. Other constituent antibodies might include such as those directed against the epidermal growth factor receptor or surface mucins.

Recent reports (*14,15*) suggest that breast cancer patients with tumors that overexpress the product of the c-*erb*B-2 protooncogene have a poor prognosis because the tumors are more resistant to chemotherapy or hormonal manipulation. The results of our initial study with ICR12 reported in this paper suggest that the good tumor localizing properties of this antibody in those patients that strongly overexpress the target antigen may form a basis for its use in therapeutic applications in these patients, for example, in antibody-directed enzyme prodrug therapy or targeted immunotherapy with either toxins or appropriate radionuclides. In conclusion, not only could ICR12 form part of a panel of antibodies to stage breast cancer and thereby replace axillary surgery as a diagnostic procedure in this condition, but also the antibody may be of use for delivering and directing subsequent therapeutic management in those patients that strongly overexpress the c-*erb*B-2 gene product.

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