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Radioimmunodetection and Therapy of Breast Cancer

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Breast cancer is the second most-common cause of cancer death in women in the United States. Although more than 60% of patients can now be cured by initial treatment, the rest, although perhaps receiving palliation with currently available therapy, will die of their disease. Early detection of micrometastasis and improved treatment strategies are needed. Monoclonal antibody (mAb)-based imaging and tumor targeted therapy holds the potential to impact these problems. The most significant results of systemically administered antibody-based radiopharmaceuticals for detection and targeted therapy (radioimmunotherapy [RIT]) of breast cancer give strong evidence that this potential can be realized. Interest in immunoimaging recently has focused on small mAb modules used with ^{18}F, ^{64}Cu, or ^{124}I to detect minimal disease in breast cancer by positron emission tomography or single-photon emission computed tomography. Reported therapy trials in advanced breast cancer have yielded objective responses and minimal toxicity. These studies have spanned several radionuclides as well as several mAb, fragments and approaches, including dose intensification with bone marrow support; combined therapy with other modalities (ie, CM-RIT); biodegradable peptide linkers; and pretargeting. RIT evaluated in clinical breast cancer trials has delivered as much as 4000 cGy to metastatic breast cancer per therapy dose with marrow stem cell support. Preclinical studies have demonstrated further promising strategies for breast cancer. RIT studies must address the key issue: enhancing the therapeutic index (tumor effect verses most sensitive normal tissue (bone marrow) effect). Approaches now include newly engineered mAb, scFv modular constructs, blood clearance on demand, enhanced pretargeting, applications of both alpha and beta emitting radionuclides, and combination therapy using molecular triggers for therapeutic synergy. These strategies for detection and treatment of metastatic breast cancer should lead to notable clinical impact on management and cure of breast cancer.
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Single-agent radioimmunotherapy (RIT) is now used to deliver effective systemic tumor targeted radiation therapy for hematologic malignancies, particularly non-Hodgkin's lymphoma. Although promising, RIT has been less effective for solid tumors, in part because they are less radiosensitive. However, early micrometatasis of breast cancer have been demonstrated to be radiosensitive because the initial use of conservative surgery followed by external beam radiation therapy to microscopic residual disease in the breast produces the same 8- to 10-year regional control, disease-free survival, and overall survival rates as modified radical mastectomy.[1-4] The radiosensitivity of normal tissues has prevented the administration of similar doses of external beam radiation therapy to the entire body, either alone or as part of combined modality therapy for metastatic breast cancer.

The detection of micrometestatic disease at the time of initial treatment or as residual disease after therapy should allow selective use of further therapeutic options in this key early interval. However, therapy with a much better therapeutic index (TI) is needed to make further progress in treating this disease. The TI of systemically administered, tumor-targeted RIT has been enhanced during the past decade. It is now possible to deliver to tumor deposits throughout the body between 3 and 30 times more than the highest normal tissue radiation dose.[5] RIT agents in clinical trials for breast cancer can deliver of 2000 to 4000 cGy to metastatic tumors per cycle of therapy when autol-

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ogous peripheral blood marrow stem cell support is used. This makes combined modality therapy with RIT for breast cancer a realistic and compelling goal. The major dose-limiting effect is myelosuppression; other toxicities have been minimal, although renal, lung, and liver toxicities are likely at higher doses. Dose-escalation studies have not reached levels of second-organ toxicities. Total dose to metastatic breast cancer tumor as high as 11,200 cGy has been reported by 3 cycles of ^{131}I-ChL6 multi-cycle therapy with stem cell support (150 mCi/m^2), whereas the highest dose to a normal organ, a total of 3100 cGy to lung, produced no evidence of toxicity.[6] Approaches using pretargeted mAb-based constructs that effectively bind subsequently injected small radioactive modules provide further possibilities for positron emission tomography (PET), single-photon emission computed tomography imaging and substantial enhancement of the TI of radionuclide therapy of breast cancer.[7,8]

Phase I/II trials of RIT specifically for breast cancer have reported clinically relevant (though transient) response rates of 30% to 60% in heavily treated patients with advanced disease. The highest dose to a normal organ is to liver, lung, or kidney depending on the radionuclide used, antigen target, antibody or antibody fragment carrier of the radionuclide and method of linkage. Thus, all characteristics of the radiopharmaceutical play a role in the pharmacokinetics, dosimetry, TI, toxicity and efficacy of this treatment modality. However, the clinical impact of imaging and targeted radionuclide therapy on patient management and ultimate cure of breast cancer, depends on early identification of disease or minimal residual disease, and use of the optimal sequence and timing of RIT in combined synergistic therapies.

Molecular Targets for Imaging and Targeted Therapy of Breast Cancer

Theoretically, an ideal target for radionuclide detection and therapy of metastatic breast cancer would be tumor-specific, generously expressed on all the breast cancer cells breast cancer patients, and not released into the circulation. Further useful characteristics of the antigen target include mAb-target interactions that trigger responses sensitizing tumor cells to radiation. Although, perhaps, the ideal tumor-specific targets for imaging or RIT have yet to be found, excellent and useful cancer cell targets have been identified. Many were studied by immunohistopathogy in metastatic breast cancer patient's biopsies (Fig. 1).

HER-2/Neu Antigen Target

Overexpression of the HER-2/neu proto-oncogene has been shown to correlate with poor clinical prognosis in breast cancer. The gene encodes a transmembrane phosphoglycoprotein-bearing extensive structural homology to the epidermal growth factor receptor. A viral oncogene encoding a truncated epidermal growth factor receptor, the human homologue of neu, was identified and designated c-erbB-2 or

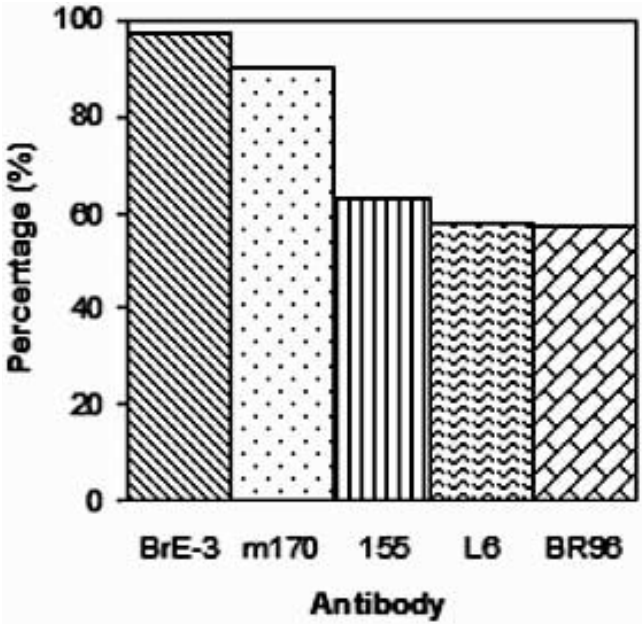

Figure 1 Metastatic breast carcinoma biopsy samples staining with BrE-3, m170, 155, L6, and BR96 antibodies by immunohistopathology and graphed as a percentage of patients' samples positive. Fifty percent of the tumor cells staining in the biopsy specimen was considered positive.[29]

HER-2. Several studies have now documented that amplification of the HER-2/neu gene occurs in approximately 25% to 35% of breast and ovarian adenocarcinomas and is uniformly associated with expression of oncogene protein p185. Multiple mAb to this protein have been developed; a humanized anti-HER-2/neu mAb (Herceptin) has been approved by the Food and Drug Administration and, clinically, is frequently used in "naked antibody" therapy.[9]

Radioactive anti-HER-2/neu rhuMAb are considered attractive agents for radioimmunodiagnosis and radioimmunotherapy of aggressive HER-2/neu-positive breast carcinomas. Several preclinical radioimmunopharmaceuticals targeting HER-2/neu are under development and study.[10-12] mAb-based scFv constructs of anti-HER-2/neu rhuMAb have provided noteworthy preclinical imaging studies, both using a clinical PET system by Robinson and coworkers with ^{124}I conjugated anti-HER2 diabody molecules[12] (Fig. 2A) as well as micro PET imaging with ^{64}Cu-anti-HER2 minibodies (Fig. 2B).[13] The highly specific tumor targeting that can be achieved with engineered antibody-based constructs matches well the needs of PET-based imaging strategies. This is particularly true for the smaller engineered constructs such as noncovalent single-chain Fv (scFv) dimmers or diabodies that are rapidly eliminated through the kidneys.

When properly applied, PET-based molecular characterization methods could provide a powerful tool to both determine the potential utility of a particular therapeutic regimen and to assess the response of breast cancer patients after treatment. For example, the efficacy of treatment with Herceptin depends to a large degree on presence of 2+ to 3+ expression of its target antigen HER2. The decision to treat a

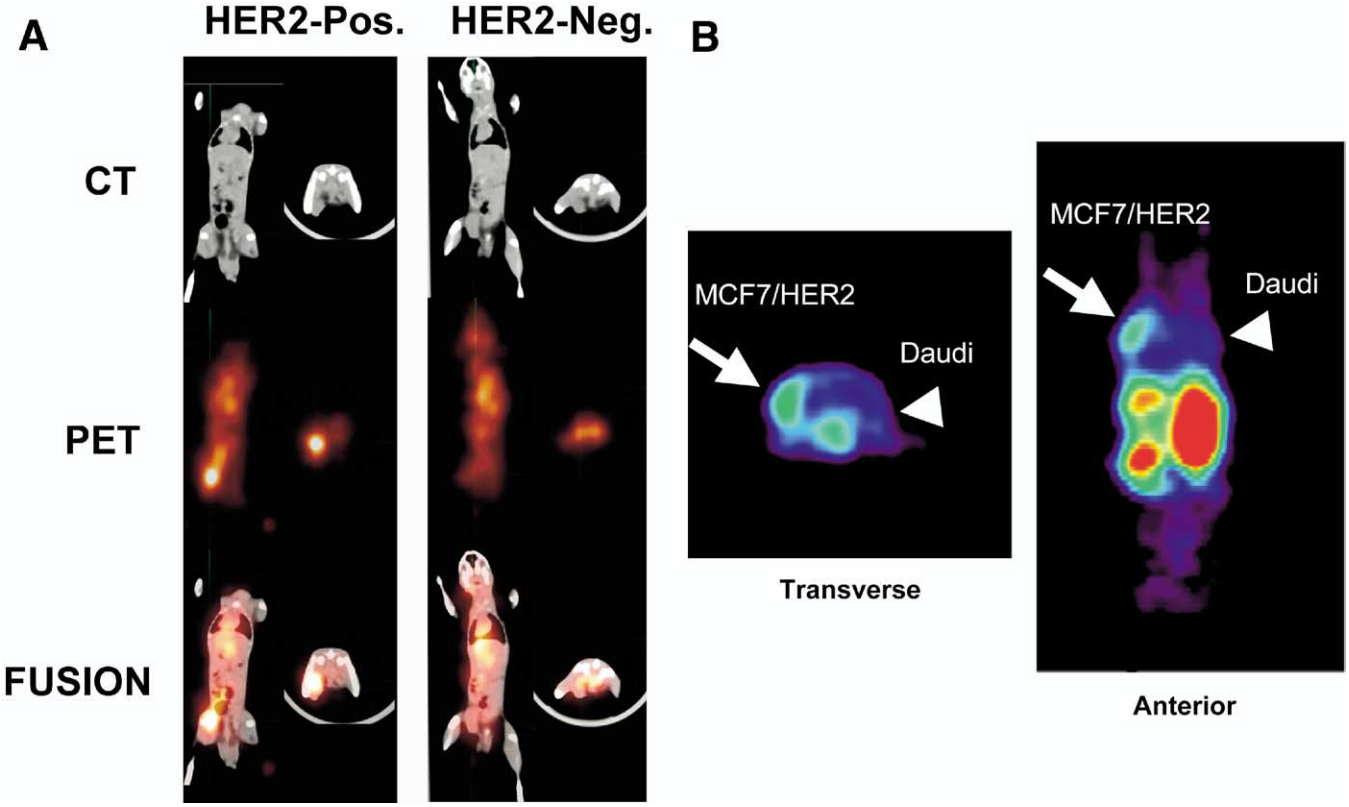

Figure 2 (A) ImmunoPET imaging with ^{124}I conjugated anti-HER2 C6.5 diabody. Immunodeficient mice bearing s.c. HER2-positive human SK-OV-3 tumors (left) or HER2 negative human MDA-MB-468 tumors (right) imaged on a G.E. Discovery LS clinical PET/CT scanner 48 h after the IV administration of I-124 conjugated C6.5 diabody reveals specific tumor localization only in the HER2 positive tumors. For each mouse, a coronal and transaxial image is provided.[12] (B) Intermediate-sized antibody fragments such as minibodies (single-chain Fv-C_h3 fusion proteins, 80 kDa) labeled with the positron-emitting radionuclide, ^{64}Cu ($t_{1/2}$ = 12.7 h) shows high-resolution microPET images of xenografted mice. The trastuzumab (Herceptin) minibody evaluated in MCF-7/HER2 xenografted mice 4 h post injection showed a tumor uptake of 4.6 ± 0.5% ID/g.[13] (Color version of figure is available online.)

patient with Herceptin is made based on the analysis of biopsy material that does not necessarily reflect the HER2 status of other sites of metastasis. ImmunoPET imaging with an anti-HER2 C6.5 diabody could provide the means to noninvasively assess the likelihood that the sites of known disease express sufficient HER2 antigen to respond to Herceptin therapy. Similarly, loss of HER2 expression following therapy, as detected by ImmunoPET imaging, may correlate with response.

Carcinoembryonic Antigen Target (CEA)

Expression of CEA has been reported in 10% to 95% of breast cancer. First described by Gold and Freedman in 1965, CEA was thought to be a specific marker for colon adenocarcinoma. However, subsequent studies demonstrated CEA expression in other human adenocarcinomas including the surface membrane of breast cancer cells. The incidence of CEA expression reported with a well-characterized monoclonal antibody (mAb), T84.66, having high affinity and specificity for a CEA epitope, is 56% of 202 breast cancers with the CEA epitope on more than15% of the tumor cells; 33% of these tumors demonstrated staining of more than 90% of cells (Fig. 1).[14]

Many anti-CEA antibodies have been used for radioimmunodetection, and for phase I/II therapy trials in patients with various cancers.[14-16] NP-4 belongs to the murine IgG_1 subclass and is specific for CEA, reacting with a class III peptide epitope of the CEA molecule. Phase I/II dose escalation studies of tumor targeted ^{131}I-NP-4 therapy have been reported in a mixed adenocarcinoma patient group with some therapeutic responses noted, including responses in breast cancer.

Therapy studies specifically in breast cancer have also been performed with T84.66.[14] T84.66 does not crossreact with any other molecule of the large CEA gene family and has been classified in the Gold I group according to its epitope reactivity.[14,17,18] A chimeric form (mouse-human mAb) has been used in clinical studies for the scintigraphic detection of mammary breast cancer and in phase I/II therapy trials,[18,19] and smaller scFv-based anti-CEA constructs are under study.[20] The maximum tolerated dose for chimeric ^{90}Y-DTPA-cT84.66 without marrow support was 22 mCi/m^2 (grade 3 reversible myelosuppression). Higher activities of ^{90}Y-DTPA-cT84.66 using autologous peripheral blood stem cell support after therapy[17] was given to 6 patients after tumor imaging. A single cycle of ^{90}Y-cT84.66 at 15mCi/m^2 (3 patients) and 22.5 mCi/m^2 (3 patients), with all patients having marrow recovery after stem cell reinfusion. Patients dem-

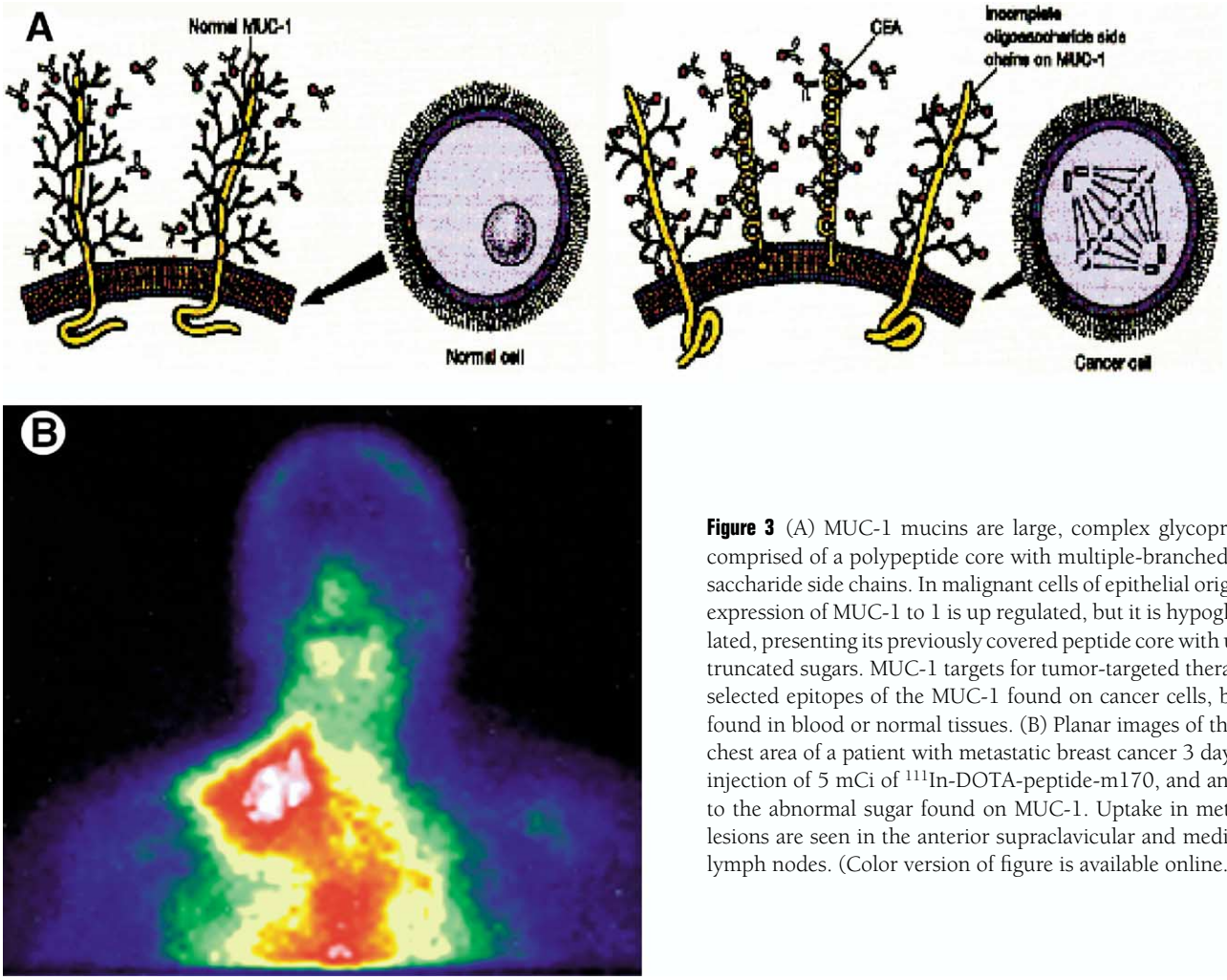

Figure 3 (A) MUC-1 mucins are large, complex glycoproteins, comprised of a polypeptide core with multiple-branched oligosaccharide side chains. In malignant cells of epithelial origin, the expression of MUC-1 to 1 is up regulated, but it is hypoglycosylated, presenting its previously covered peptide core with unique truncated sugars. MUC-1 targets for tumor-targeted therapy are selected epitopes of the MUC-1 found on cancer cells, but not found in blood or normal tissues. (B) Planar images of the mid-chest area of a patient with metastatic breast cancer 3 days after injection of 5 mCi of ^{111}In-DOTA-peptide-m170, and antibody to the abnormal sugar found on MUC-1. Uptake in metastatic lesions are seen in the anterior supraclavicular and mediastinal lymph nodes. (Color version of figure is available online.)

onstrated moderate clinical response: stable disease for 4 months; improvement in bone scans; 50% reduction of metastasis; reduction of malignant pleural effusion for 14 months, bone pain for 1 to 3 months. The results of this trial suggests the potential for antitumor effects of stem cell supported ^{90}Y-cT84.55 therapy in CEA-producing breast cancer.

MUC-1 Antigen Target

MUC-1 mucins are large, complex glycoproteins that have a polypeptide core with multiple oligosaccharide side chains. The mature molecule is anchored within the cell surface by a characteristic transmembrane domain, but most of the mucin is expressed extracellularly (Fig. 3A).[21] In malignant cells, the expression of MUC-1 is elevated, and its orientation within the tissue is no longer just at apical surfaces. MUC-1 mucins released from their surface location have access to the circulation and some cancer related antigen epitopes are present on these circulating molecules. Serial quantitation of these molecules in blood are used to provide a guide to tumor burden, recurrence and response to therapy (eg, CA-15-3).[22]

Several antibodies have been found to react with MUC-1 epitopes that are not present in blood nor available on normal tissues. Reactivity and specificity of 56 MAbs against the MUC-1 mucin have been investigated with a diverse panel of target antigens and MUC-1 mucin-related synthetic peptides and glycopeptides.[21] Most of the antibodies (34 of 56 studied) defined epitopes located within the 20-amino acid tandem repeat sequence of the MUC-1 mucin protein core. Carbohydrate residues were found in the epitopes for 16 antibodies of the remaining 22. The MUC-1 protein core is known to contain variable numbers of the 20-amino acid tandem repeat sequence PDTRPAPGSTAPPAHGVTSA.[21,23] Many antibodies bind rather simple linear peptide motifs of only a few residues in this MUC-1 protein core. This is particularly important because in the malignant cell, aberrant glycosylation may lead to truncated or incomplete oligosaccharide side chains that may be new epitopes or that may expose de novo cancer related determinants within the MUC-1 core. Antibodies to both the peptide core and the aberrant sugar residues have been studied in clinical trials for imaging and therapy of breast cancer (Fig. 3B).[24-28] Two of these, which demonstrated high levels of staining on most breast cancer biopsy specimens,[29] provided excellent RIT tumor targeting on imaging studies in vivo. Pharmacokinetics, dosimetry, and therapy trials of radioimmunoconjugates to these antigens in patients with metastatic breast cancer have been described (Fig. 4).[30-35]

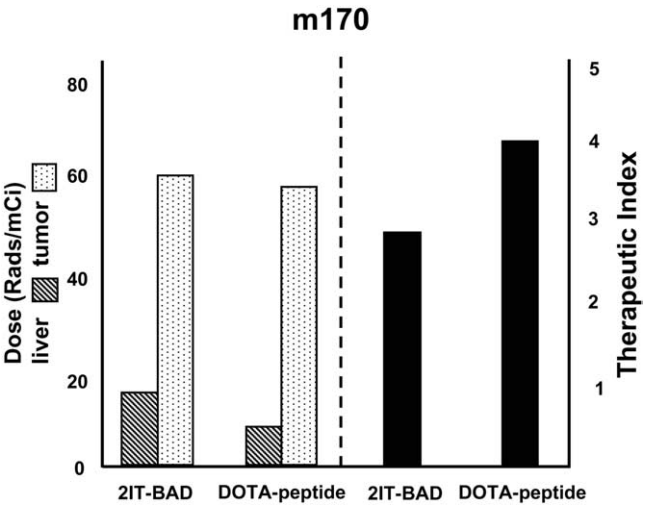

Figure 4 Dosimetry comparison for ^{90}Y-2IT-BAD-m170 to ^{90}Y-DOTA-peptide (p000)-m170 in patients with breast cancer calculated from the ^{111}In imaging. The mean radiation dose to the liver in breast cancer patients was decreased 25% by the use of the p000 linkage for ^{90}Y-DOTA-peptide (p000)-m170. Tumor dose per mCi injected remained unchanged. This increase in the TI would allow approximately 25% more radiation to be delivered to tumors when marrow support is given because the liver is the next-highest normal organ.[37]

BrE-3 antibody and a humanized form (hBvE-3) reacts with an epitope on the tandem repeat of the peptide core of MUC-1. Immunopathology studies of metastatic breast cancer biopsy specimens demonstrated a vigorous reaction of BrE-3 with more than 75% of the cells of more than 95% of the breast cancers.[29] Pharmacokinetics and dose–escalation studies were performed to determine the maximum tolerance dose with ^{90}Y-MX-DTPA BrE-3. In 3 of 6 patients, objective evidence of response to therapy that lasted 3 to 8 weeks.[30] Of 3 patients in the 6.25 mCi/m^2 group, 1 had a partial response (PR) in liver metastasis In a 9.25 mCi/m^2 group, 1 patient had a temporary reduction in skin lesions and arm swelling, and another had a measurable reduction in liver tumor that did not meet the criteria for PR. Although patients in this study received only a single, modest ^{90}Y dose, a decrease in measurable disease was observed in three of six patients, although it lasted only briefly. The therapy was well tolerated. The data suggested that multiple cycles of ^{90}Y-MX-DTPA BrE-3 and/or higher doses could result in more frequent and durable responses.

Because the dose-limiting toxicity was myelosuppression, a phase I trial to explore the use of a single, high-dose of ^{90}Y BrE-3 and autologous peripheral blood stem cell support was initiated. Nine women with heavily pretreated disease were enrolled. All of the patients had tumors positive for BrE-3 by immunostaining and were treated with 1 dose of ^{90}Y (15 mCi/m^2, 3 patients; 20 mCi/m^2, 6 patients). ^{111}In-BrE-3 (5 mCi) was given simultaneously for imaging. The only toxicity noted was hematological. Grade 4 platelet toxicity requiring transfusion support developed in four patients. Grade 4 white blood cell toxicity was seen in 2 patients that resolved in 3 to 9 days. All hematological nadirs occurred approximately 25 days after treatment. Objective PRs were noted in four of eight (50%) evaluable patients with measurable tumors (four of the total nine patients). Because antibodies to the BrE-3 mouse antibody (human antimonoclonal antibodies, or HAMA) developed rapidly in most patients, such that more than one dose of the therapy could not be considered, a humanized BrE-3 was developed.[31]

The humanized V_L and V_H frameworks are 93% and 90% identical to the corresponding human frameworks, respectively. A pharmacokinetic/dosimetry study performed in 7 patients wherein ^{90}Y dosimetry was calculated from ^{111}In MX- DTPA huBrE-3 demonstrated 70 ± 31 cGy/mCi to tumor and 21 ± 12 cGy/ mCi to liver.[32] A phase I study was then of a single dose of ^{90}Y MX-DTPA-BrE-3 followed by granulocyte colony-stimulating factor-mobilized autologous peripheral blood stem cell (PBSC) support in patients with refractory metastatic breast cancer.[32] Patients received 10mCi/m^2 (n = 3), 20mCi/m^2 (n = 3), or 33mCi/m^2 (n = 3) of ^{90}Y-MX-DTPA-hBrE-3 followed 14 days later by PBSC support. No nonhematologic noninfectious toxicities were seen in any of the patients, despite the fact that seven of the nine had failed autologous stem cell transplant. Radiation absorbed dose estimates for ^{90}Y in the first two patients, extrapolated from ^{111}In, were 2.81 and 2.94 rads/mCi for the whole body. Of the nine patients, four had measurable disease. In these patients, one PR (liver lesion), one PR (nodes and chest wall PR with stable liver disease), one mixed response, one stable disease was reported.

Muc-1 MoAb 170H.82 was derived against a synthetic asialo GM1 terminal disaccharide associated with the cell membrane and is related to the Thonsen-Friedereich disaccharide.[36] ^{99}Tc and ^{111}In radioimmunoconjugates of 170H.82 (m170) are effective for imaging primary and metastatic breast cancer and have been shown to detect lesions less than 1 cm in size with an overall clinical accuracy of 92%.[37] Of 99 metastatic breast cancer biopsy specimens, 89 (90%) demonstrated abundant staining with m170 (Fig. 1).[29]

The ^{90}Y-m170 therapy studies to date have been preceded by ^{111}In-m170 pharmacokinetic studies to determine the maximum dose of ^{90}Y-m170 that can be administered without exceeding an 800 cGy non marrow normal organ limit for each of 3 therapies of patients in level 1 and 1000 cGy for any non marrow normal organ for patients at level 2. Sufficient autologous PBSCs are harvested and frozen pretherapy for infusion after each therapy dose. The mean and range of calculated doses (cGy/mCi) for all studies (n = 10) are whole body 2.2 (2.1-2.4), liver 17.4 (12.7-22.2), lung 6.3 (4.8-7.2), kidney 8.1 (6.3-11.5), marrow 3.3 (1.9-4.4), and tumors (n = 33) 81.1 (14.1-141.5). Of the patients treated with sufficient follow-up for analysis, and with doses of 37 to 57 mCi of ^{90}Y (level 1), (20-33mCi/m^2), 4 patients proceeded from the dosimetry study to be treated; 1 patient had a PR, 1 had measurable tumor reduction but less than 70%, and 1 had stable disease for more than 1 month.

The use of PBSCs prevented prolonged myelosuppression. The therapeutic responses, coupled with an absence of significant adverse response, suggest that this dosimetry-bases

approach may lead to meaningful therapy when higher ^{90}Y doses are reached.[33] A new linkage in the pharmaceutical as ^{111}In/ 90Y-DOTA-peptide-m170 was studied to evaluate the effect of a radiochelate linkage that could be catabolized in the liver (Figs. 3B and 4). These studies did show that the tumor to liver radiation dose could be improved by 30%, which at therapy levels supported by PBSC, a higher injected dose would be possible.[37]

Because aberrant MUC-1 has provided effective targets for breast cancer, gene-engineered antibody fragments (scFv) have been developed to MUC-1 antigen by phage display immunoglobulin gene libraries from mice immunized with MUC-1 peptide core and MCF-7 membranes. Multivalency of the tumor targeting molecules has been achieved by expression of scFv-SH or di-scFv–SH, containing an engineered (unpaired) cysteine in one of several selected locations, and linkage of these scFv-SH, di-scFv-SH modules to build tumor targeting and pretargeting molecules. ScFv selection and design as modular di-scFv-SH units, and site specific conjugation into larger configurations have been developed, as a new approach to providing tumor binding pretargeting molecules for breast cancer imaging and therapy.[38]

L6 Antigen Target

The L6 cell surface antigen, which is highly expressed on lung, breast, colon, and ovarian carcinomas, is a 24-kDa surface protein containing 3 hydrophobic transmembrane regions that are followed by a hydrophilic region. The L6 antigen is related to a number of cell surface proteins with similar predicted membrane topology that have been implicated in cell growth. Two other members of this family, CD63 (ME491) and CO-029, also are highly expressed on tumor cells.[39] L6 antigen also was found to be expressed in human vascular endothelium but could be covered by an infusion of nonradioactive L6 mAb, so that subsequent radiolabeled L6 MoAb to reach tumor cells.[40]

The chimeric version (ChL6) labeled with ^{131}I was administered in up to 4 monthly cycles to patients with metastatic breast cancer who had failed standard therapy. Ten patients with metastatic breast cancer reactive with L6 by immunohistopathology, received an imaging dose of ^{131}I-ChL6, which was followed 24 hours later by a therapy dose of ^{131}I-ChL6 (20-70 mCi/m^2). Tumor radiation dose was 120 to 3700 rads per therapy cycle; 5 to 30 times higher than the whole body dose. Therapy resulted in minimal acute or subacute toxicity with dose limiting myelotoxicity. Six of 10 patients had clinically measurable tumor responses; 5 had responses that lasted more than 1 month (1.5-5 months).[41] Three additional patients were treated at 150 mCi/m^2 using autologous peripheral blood stem cell support after each dose.[42] Hematological toxicity was modest with thrombocytopenia (25,000 μL) resolving after a maximum duration of 7 days. No significant nonhematologic toxicity was observed. Two of three patients received only a single cycle of RIT because of HAMA. The third patient, treated with cyclosporin A to prevent HAMA, completed all 3 therapy cycles. She received cumulative radiation doses to the lungs and tumor of 3100 and 11,200 cGy, respectively. For 9 months, she had clinically marked reduction in bone pain, a decline in serum tumor markers, and decreased tumor.

Vascular endothelium was surprisingly found to have a target for L6, which, however, was covered by the initial injection of unlabeled L6 or ChL6, allowing the subsequent radioactive dose to reach tumor tissue. After infusion of L6 or ChL6, patients demonstrated immediate serum complement activation manifested by rapidly decreasing levels of serum complement 3 (C3) and complement 4 (C4). Tumor uptake of a second ^{131}I MoAb dose given after 2 daily injections of 200 mg ChL6 usually was higher than the tumor uptake of the first ^{131}I MoAb given after a single 200-mg infusion of ChL6. Enhanced tumor uptake correlated with greater and more prolonged decrease in serum C3 and C4 and albumin after the second ChL6 infusion. Although serum complement frequently decreased after the first 50 to 100 mg of L6 or ChL6, elevation of soluble interleukin 2 receptor (IL-2R) in serum was only observed in patients receiving 150 mg or more of L6 or ChL6.[40] Patients with therapeutic tumor responses were noted to have had a greater increase in IL-2R levels than patients who did not respond. Transient increase in serum interleukin 2 (IL-2) was only seen in 2 of the 9 treated patients. The absence of pulmonary edema and delayed dose-dependent IL-2R release suggest that targeting of the pulmonary endothelium by L6 or ChL6 is not the major cause of the observed biologic effects. The clinical importance of understanding these mechanisms is emphasized by the occurrence of measurable tumor regressions in 5 of the 9 advanced metastatic breast cancer patients that were treated in this manner. This unique response of a solid tumor to radioimmunoconjugate therapy may be secondary to both the increased delivery of the radioimmunoconjugate to tumor cells caused by enhanced vascular permeability as well as to synergistic effects of radiation and activated effector cell mechanisms.

TAG-72 Antigen Target

The widely described murine monoclonal antibody, B72.3 (satumomab pendetide, OncoScint CR/OV, Cytogen Corporation, Princeton, NJ), was derived from immunization of a nude mouse model with tumor extract obtained of a patient with breast cancer and targets TAG-72. TAG-72, known as tumor-associated glycoprotein, is expressed by most adenocarcinomas.

Antibodies to the TAG-72.3 antigen, particularly B72.3 and chB72.3, were evaluated in patients after careful study in human xenograft mouse models.[43,44] Dosimetry derived from ^{111}In B72.3 pharmacokinetic studies in breast cancer patients suggested that maximum tumor uptake would only be 0.004% ID/g.[43] Therapy studies were conducted in 2 groups of patients with colon cancer with the human IgG4 chimeric version of this MoAb (^{131}I-ch-B72.3). A slight response in 1 of 24 patients was documented but a high incidence of HAMA interrupted therapy.[45]

A newer antibody to a different epitope of TAG-72.3, CC49 radiolabeled with luticium-177 (^{177}Lu), demonstrated

improved tumor uptake in mouse biodistributions.[46] CC49 is a murine IgG_1 monoclonal antibody. Immunohistochemical and immunocytochemical techniques have demonstrated preferential expression of TAG-72 in breast, gastrointestinal and ovarian adenocarcinomas compared with normal tissues, except for the secretary endometrium. At doses less than the LD_{50} of 400 to 500 μCi, a high rate of complete tumor regression was achieved in mouse therapy studies.[46] Imaging studies using ^{131}I-CC49 were then reported showing enhanced targeting following alpha-interferon treatment in women with metastatic breast cancer.[47]

Acute and Subacute Toxicity

Mild-to-moderate clinical toxicity has been anticipated and reported when biologically active monoclonal antibodies or immune targeting molecules combined with or without other biologic response modifiers, (ie, IL-2, IL-6, tumor necrosis factor) are used as part of the radioimmunotherapy.[48,49] It is not surprising that activation of complement, the triggering of normal immune effector cell response, and/or stimulation of other inflammatory mechanisms can cause clinical symptoms. In these instances, fever, chills, urticaria, nausea, headache, hypotension, tachycardia, and muscle aches may frequently be expected as reported with the ChL6 infusions. These are generally mild (grade 1-2), respond to oral antipyretic and antihistamine medications, and are dose rate related. These responses are clearly different from acute hypersensitivity reactions or delayed hypersensitivity reactions, which are almost never seen, but must always be anticipated.

The main toxicities of radioimmunotherapy in patients with metastatic breast cancer are thrombocytopenia and neutropenia. When ^{131}I MoAb is administered intravenously, the majority of the radiation dose that is delivered to the marrow is from radiolabeled antibodies circulating through the marrow unless tumor cells in marrow increase the marrow cell dose by a "bystander" effect. Thus, radiolabeled antibodies with a longer circulation time deliver substantially more radiation to the marrow and produce more myelosuppression per injected dose than radiolabeled antibodies with more rapid blood clearance.

Human Anti-Monoclonal Antibodies (HAMAs)

After exposure to antibodies containing murine proteins, patients may develop HAMAs. A HAMA response usually results in rapid clearance of the therapeutic antibodies for the circulation, thereby reducing tumor uptake. Considerable variability exists in the development of a HAMA response among patients.[49] Imaging with very small amounts of antibody (1-2 mg), or smaller constructs, seldom elicits HAMA. Chimeric and humanized MoAb in moderate doses also have less HAMA response. HAMA develops in approximately half of immunocompetent patients after a single dose of intact murine antibodies; this increases to approximately 90% in patients receiving 3 doses of antibody fragments.[50-53] HAMA can be detected in some patients as soon as 1 week after the administration of murine antibodies and may persist for months or years, precluding tumor targeting with subsequent antibody infusions.

In the presence of HAMA, worldwide experience has generally been that multiple infusions seldom cause clinical problems, if the infusion is given slowly. However, HAMA frequently causes serial therapy to be terminated because the therapeutic agent is no longer able to reach its target. On the other side of the coin, multiple investigators have suggested that with the more common HAMA response, antibodies are sometimes elicited to the immune reactive region of the initial MoAb and then to those antiidiotype antibodies in a cascade capable of creating effective antitumor antibody titers. This suspicion has led individual investigators to postulate that some delayed tumor responses were responses to this "vaccine" like effect of the initial antibody injection.

Cyclosporin A (CSA) in modest doses administered for several weeks after antibody has successfully been used to prevent HAMA by several investigators with minimal or no toxicity.[50-53] In clinical trials in breast cancer patients receiving ^{131}I- or ^{90}Y-DOTA-peptide-(Ch)L6 and CSA "prophylaxis," 4 of f our patients remained HAMA negative after up to 6 antibody exposures.[43,53] This result contrasts with the 100% HAMA observed without CSA in patients receiving intensive dose (Ch)L6 RIT and supports the ability of CSA to facilitate fractionated RIT. The rate of clearance of the antibody may dictate the duration of CSA prophylaxis required to prevent HAMA.[53]

Synergy Studies

Novel, synergistic, multimodality therapy is needed for breast cancer to combat the molecular mechanisms, genetic mutations and epigenetic abnormalities that protect the cancer from therapeutic interventions. Studies combining chemotherapy and RIT are in progress with various agents. Work with the aggressive human breast cancer model HBT3477 and paclitaxel exemplifies the need of combined breast cancer RIT to overcome such cancer cell mechanisms, ie, mutant nonfunctional p53 and high BCL-2 expression.[54,55] Paclitaxel (Taxol) has been shown to have efficacy in ovarian and breast cancers because it stabilizes microtubule formation resulting in mitotic block, bcl-2 dysfunction and activation of apoptosis.[56] Paclitaxel may be even more effective in the presence of mutant p53. Because breast cancer frequently has p53 mutations, the potential synergism between paclitaxel and ^{90}Y-ChL6 was assessed in the HBT3477 breast cancer model. Statistically, there was no tumor response in mice receiving ChL6 or paclitaxel alone. In mice receiving ^{90}Y-ChL6 alone, 79% (15 of 19) tumors responded although none were cured. If paclitaxel was administered 24 to 72 hours before ^{90}Y-ChL6, again, 79% (23 of 29) of tumors responded but 21% were cured. Paclitaxel given with ^{90}Y-ChL6 did not substantially increase toxicity Fifty and 88% of these breast cancer xenografts were cured by this CMRIT when paclitaxel was given 24 and 48 hours respectively, after ^{90}Y-ChL6. In conclusion, paclitaxel seemed to be synergistic with RIT in this human breast cancer model in a sequence dependent manner.

Summary

Immunoimaging and tumor immunotargeted radionuclide therapy are promising approaches for early detection and CM-RIT treatment of metastatic breast cancer. Because of selective biologic concentration of the antibody and thus the isotope in tumor tissue, this modality can deliver substantial doses of radiation to the tumors while minimizing concomitant exposure of normal tissue and target metastases throughout the body in a single treatment. Although excellent results have been reported using RIT in advanced hematologic malignancies,[57-62] successful results in solid tumors, including breast cancer, have been limited.[63-66] However, as noted in this review, antitumor responses have been reported by multiple investigators using a single cycle of moderate dose ^{90}Y linked to BrE3 and ChL6 and higher doses as ^{90}Y BrE3, ^{90}Y hBrE3,^{90}Y cT84.66, and ^{90}Y-m170 with stem cell support. Strategies for RIT as CM-RIT build on current knowledge and promise further enhancement in detection and therapy. It is apparent that the application of enhanced therapeutic index of delivered radiation in the setting of combination therapy is required. Biodegradable peptide linkers between the chelated metal and the antibody have been shown to improve the therapeutic index.

However, clinical impact on the management and cure of metastatic breast cancer will ultimately depend on identification of synergistic therapies. Radionuclide therapy is a continuous, low-dose irradiation and acts mainly through apoptosis; apoptosis often is blocked because most breast cancer metastasis have ineffective p53 and increased BCL. Agents such as paclitaxel (ie, Taxol) are particularly attractive as synergistic agents for RIT because of cell cycle arrest in the radiosensitive G2 mol/L phase and p53 independent apoptosis. Optimal sequence and timing for combined modality treatment with radioimmunotherapy will be critical to achieve maximum synergy and minimize toxicity.

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