Therapeutic Efficacy of Anti-LewisY Humanized 3S193 Radioimmunotherapy in a Breast Cancer Model: Enhanced Activity When Combined with Taxol Chemotherapy

Kerrie Clarke, Fook-Thean Lee, Martin W. Brechbiel, Fiona E. Smyth, Lloyd J. Old, and Andrew M. Scott1

Tumour Targeting Program, Ludwig Institute for Cancer Research, Melbourne Branch, Austin and Repatriation Medical Centre, Victoria 3084, Australia [K. C., F-T. L., F. E. S., A. M. S.]; Radioimmune and Inorganic Chemistry Section, Radiation Oncology Branch, NIH, Bethesda, Maryland 20892 [M. W. B.]; and Ludwig Institute for Cancer Research, New York Branch, Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [L. J. O.]

ABSTRACT

Monoclonal antibody therapy may provide new treatment options in the management of metastatic breast cancer by selectively targeting tumors and producing a therapeutic effect, by delivering radiation or other toxins directly to tumor cells, or by producing an intrinsic immune inflammatory response. The effect of 131I-labeled humanized anti-LewisY monoclonal antibody 3S193 (hu3S193) was compared with that of placebo and radiolabeled huA33 control antibody in a series of radioimmunotherapy experiments in a MCF-7 xenografted BALB/c nude mouse breast cancer model. The maximum tolerated dose of 131I-labeled antibody occurred at 200 μCi/mouse, at which dose level three of six mice that received 131I-hu3S193 showed significant tumor growth inhibition in contrast to no responses in the comparable 131I-huA33 control treatment arm. Breast cancer is an ideal model to test the efficacy of combined modalities given its known sensitivity to both radiotherapy and chemotherapy. The synergy between radioimmunotherapy and chemotherapy was therefore also explored using a combination of 131I-labeled hu3S193 antibody and Taxol using subtherapeutic doses of each agent. The combination of Taxol and 100 μCi of 131I-hu3S193 produced significant tumor inhibition in 80% of mice, whereas no responses were seen with either treatment modality alone or the combination of Taxol and 131I-huA33. These results support a potential therapeutic role of radiolabeled hu3S193 in the treatment of breast cancer, including combination therapy with Taxol, and warrants further investigation of this promising new agent.

INTRODUCTION

Breast cancer is a radiation- and chemotherapy-sensitive malignancy, but despite new agents and approaches to treatment, the median survival of patients with metastatic breast cancer has not altered significantly in recent years (1). In metastatic disease, the duration of chemotherapy response may be limited by the emergence of resistant tumor cells. The role of radiotherapy in the management of breast cancer has been largely restricted to palliation of symptomatic sites of metastatic disease, in particular bone deposits, because of concerns regarding toxicity. mAb2 therapy may provide new treatment options in the management of metastatic breast cancer by selectively targeting tumors and producing a therapeutic effect mediated through growth factor receptors, by delivering radiation or other toxins directly to tumor cells, or by producing an intrinsic immune inflammatory response (2, 3).

A number of tumor antigens have been shown to have high expression on malignant breast cancer cells, including Her2/neu, MUC, carcinoembryonic antigen, and LeY (Ref. 2). The LeY antigen is a member of a family of blood group-related difucoylated oligosaccharides (4). The LeY antigen has been shown to be expressed by 60–90% of human carcinomas of epithelial cell origin, including breast, pancreas, ovary, colon, gastric, and lung cancer, but it is present in only a few normal tissues (5–7). The high frequency of LeY-expressing tumors, its high density and altered expression on the surface of tumor cells, and its relatively homogenous expression in primary and metastatic lesions have led to its selection as an antigenic target for a range of epithelial tumors, including breast cancer (8–10).

3S193 is a mAb produced using a standard hybridoma technique after immunization of BALB/c nude mice with LeY-expressing MCF-7 breast cancer cells. The murine antibody has been shown to have high specificity for LeY and reacted strongly in rosetting assays and cytotoxic tests with LeY-expressing cells (11, 12). 3S193 has been humanized (hu3S193) and reactivity for LeY confirmed to be similar to the murine version (11, 12). Biodistribution studies of the 125I-, 111In-, and 90Y-labeled antibody showed that the antibody can be labeled, retains sta-

Received 1/14/00; revised 6/23/00; accepted 6/26/00.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom requests for reprints should be addressed, at Ludwig Institute for Cancer Research, Austin and Repatriation Medical Centre, Heidelberg, Victoria, 3084, Australia. Phone: 613-9496-5876; Fax: 613-9496-5892; E-mail: ams@austin.unimelb.edu.au.

2 The abbreviations used are: mAb, monoclonal antibody; LeY, Lewis Y antigen; TV, tumor volume; CR, clinical response; PR, partial response; MTD, maximum tolerated dose.
bility in vivo, and localizes to MCF-7 xenografts in imaging studies (13).

In this study, a BALB/c nude mouse human breast cancer xenograft model was used to test the therapeutic potential of \(^{131}\text{I}\)-labeled hu3S193 and to explore the effects of combining chemotherapy with radioimmunotherapy. Taxol was selected for the combination therapy based on its relative ease of administration (i.p.) and the well-defined dosing profile in nude mice, which enabled subtherapeutic doses with negligible toxicity to be chosen. The sequence of radioimmunotherapy and chemotherapy administration has been reported to be of importance in achieving optimal tumor response (14, 15). In the current study, Taxol was administered i.p. 24 h after injection of radiolabeled antibody, based on previous studies in a xenografted mouse model demonstrating superior response rates for this regimen compared with Taxol given prior to radioimmunotherapy (14).

MATERIALS AND METHODS

mAbs

The generation of murine 3S193 using standard hybridoma technique after immunization of BALB/c mice with Le\(^{5}\)-expressing MCF-7 breast cancer cells and its subsequent humanization and characterization have been described previously (11, 12). hu3S193, a CDR-grafted IgG1 version of 3S193, was produced by Scotgen (Aberdeen, Scotland) in conjunction with the Ludwig Institute for Cancer Research. hu3S193 used for the experiments outlined was obtained from both the New York Branch and the Biological Production Facility of the Melbourne Branch of the Ludwig Institute for Cancer Research. huA33, an IgG1 humanized antibody (16) directed against a novel antigen found in \(>95\%\) of colorectal cancers, was supplied by the New York Branch of the Ludwig Institute and used as a subclass-specific control.

Cell Lines

MCF-7, a Le\(^{5}\)-expressing human breast adenocarcinoma cell line originally derived from the pleural effusion of a 69-year-old lady with estrogen receptor-positive metastatic breast cancer (17), was obtained from the American Type Culture Collection (Rockville, MD). SW1222, a Le\(^{5}\)-negative human colonic cancer cell line, was a gift from the tumor cell bank of the New York Branch of the Ludwig Institute and was used as a control cell line.

Cells were grown in 175 cm\(^2\) plastic flasks (Nalge NUNC International, Roskilde, Denmark) and maintained in log-phase growth in RPMI 1640 (Trace Chemicals, Sydney, Australia) supplemented with 10% (MCF-7) or 5% (SW1222) FCS (MultiSer; Trace Biosciences, Australia), 100 units/ml penicillin, 100 \(\mu\)g/ml streptomycin, 0.25 ml/l insulin, 2 mM glutamine, and essential amino acids. Cells were cultured at 37°C in a 5% CO\(_2\) incubator (Forma Scientific Inc, Marietta, Ohio) and passaged with 0.05% EDTA-PBS (BDH Chemicals, Sydney, Australia.). Cell viability in all experiments, as determined by trypan blue exclusion, exceeded 90%.

Mouse Model

Xenografts were established in 5- to 6-week-old BALB/c nude mice, homozygous for the nul/nul allele and bred by the SPF Facility, University of South Australia. To establish MCF-7 human breast xenografts, mice were supplemented with exogenous estrogen (18). After light ethrane anesthesia, a 60-day slow release estrogen pellet (0.72 mg of estradiol/pellet; Innovative Research of America, Sarasota, FL) was inserted using aseptic techniques into a small s.c. pocket fashioned between the shoulder blades. The surgical incision was closed with a single stitch (6–0 absorbable dacron or silk). MCF-7 cells (25 \(\times\) 10\(^6\)) in 100–150 \(\mu\)l of medium were subsequently injected s.c. into the left inguinal mammary line. Mice were maintained in autoclaved microisolator cages housed in a positive pressure containment rack (Thoren Caging Systems Inc., Hazelton, PA). Mice were identified by ear marks and were observed daily for tumor growth and monitoring of surgical site for infection. Mice were divided into groups stratified to ensure an even distribution of tumor sizes within each group, thereby compensating for the individual variability of xenograft growth rates.

Tumor Measurements

Tumors were measured in the longest axis (L) and the axis at 90 degrees to the longest axis (W) by slide caliper two to three times a week. TV in \(\text{mm}^3\) was calculated by the formula: TV = \((L \times W^2)/2\) (19); the mean TV (\(\pm\) SD) for each treatment group was calculated and graphed. TV was also expressed as percentage of change in TV compared with initial volume on day 0 according to the formula: TV change from time 0 (\(T_0\)), \(\% = \left[\text{TV at time of measurement} - \text{TV at} \ T_0\right] \times 100\), where day 0 is the day of antibody injection (19).

Antibody Labeling

Radioiodination was performed using a modification of a previously published chloramine-T reaction (20), using a 2-fold molar excess of chlorine-T (Merck, Darmstadt, Germany) over antibody dissolved in 0.5 M potassium phosphate buffer (pH 7). After a brief 2-min incubation period, the reaction was stopped by the addition of a 5-fold excess of sodium metabisulfite, again dissolved in a 0.5 M phosphate buffer, and the product was purified through a desalting column (P6DG; Bio-Rad, Sydney, Australia.) equilibrated with PBS. The specific radioactivities of \(^{131}\text{I}\)-hu3S193 and \(^{131}\text{I}\)-huA33 control were 6.8 and 9.8 mCi/mg, respectively.

Radioiodination was performed on the day of injection into mice. Prior to injection, the percentage of unbound radiouclide content was determined by instant TLC (21), and the immunoreactive fraction of the final radiolabeled hu3S193 product was tested by a Le\(^{5}\)-positive cell-binding assay according to Lindmo et al. (22). For the control radioconjugate, an A33 antigen-positive colon carcinoma cell line, SW1222, was used.

Animal Model

\(^{131}\text{I}\) Dose Escalation Study. A single dose of \(^{131}\text{I}\) at 50, 100, 200, or 300 \(\mu\)Ci/mouse, was injected retro-orbitally, bound to either hu3S193 or the subtype-specific control antibody (huA33). Six mice were used for each radiolabeled antibody at each dose level. At all doses, both hu3S193 and huA33 were administered at a protein concentration of 44 \(\mu\)g/mouse. This protein concentration was chosen based on the specific activity of \(^{131}\text{I}\)-hu3S193 (6.8 mCi/mg), where 300 \(\mu\)Ci equals a protein
Table 1  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Mice</th>
<th>TV (mm³) at Day 0 of study (mean ± SD)</th>
<th>Day of study completion</th>
<th>Mean TV ± SD at study completion (mm³)</th>
<th>Mean change in TV at study completion (%)</th>
<th>Response rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>131I-huA33</td>
<td>50 μCi</td>
<td>112.5 ± 33</td>
<td>62</td>
<td>1016 ± 83</td>
<td>973 ± 497</td>
<td>0</td>
</tr>
<tr>
<td>100 μCi</td>
<td>5</td>
<td>127.7 ± 28</td>
<td>85</td>
<td>1254 ± 410</td>
<td>940 ± 252</td>
<td>0</td>
</tr>
<tr>
<td>200 μCi</td>
<td>6</td>
<td>123.0 ± 24</td>
<td>85</td>
<td>516 ± 177</td>
<td>311 ± 98</td>
<td>50% PR</td>
</tr>
<tr>
<td>300 μCi</td>
<td>5</td>
<td>115.2 ± 19</td>
<td>85</td>
<td>53.4 ± 14</td>
<td>−41.2 ± 26 (reduction)</td>
<td>20% CR/80% PR</td>
</tr>
<tr>
<td>131I-hu3S193</td>
<td>50 μCi</td>
<td>115.3 ± 23</td>
<td>42</td>
<td>926 ± 349</td>
<td>616 ± 211</td>
<td>0</td>
</tr>
<tr>
<td>100 μCi</td>
<td>6</td>
<td>125.7 ± 32</td>
<td>62</td>
<td>1115 ± 368 (P &lt; 0.006)</td>
<td>844 ± 346</td>
<td>0</td>
</tr>
<tr>
<td>200 μCi</td>
<td>6</td>
<td>124.4 ± 31</td>
<td>76</td>
<td>1097 ± 367 (P &lt; 0.006)</td>
<td>1097 ± 367</td>
<td>0</td>
</tr>
<tr>
<td>300 μCi</td>
<td>6</td>
<td>122.7 ± 32</td>
<td>85</td>
<td>890 ± 290 (P &lt; 0.001)</td>
<td>800 ± 550</td>
<td>0</td>
</tr>
</tbody>
</table>

* Day 0, day of radiolabeled antibody injection.

* Percentage of change = (TV at completion of study − TV at day 0)/TV at day 0 × 100.

* Significant t test results for comparisons between the mean TV at study completion of 100–200 and 200–300 μCi dose levels of radiolabeled hu3S193 are shown in parentheses.

* The significant t test results from the comparison between mean TVs at study completion of each 131I-dose level of huA33 control and the comparable 131I-hu3S193 arm from the same day are shown.

mm³. Radiolabeled antibody was injected retro-orbitally to relevant treatment groups on day 0, and Taxol was injected on day 1 of the study, 24 h after injection of radiolabeled antibody. The study was terminated at day 78. As for the other therapeutic studies described, study groups were terminated at earlier times in the event of toxicity or tumor size >1 g. TVs were monitored daily, and the mean percentage of change in TV calculated at study completion as described.

Response and Toxicity Definitions

CR was defined as complete resolution of tumor and PR as a 50% reduction in TV compared with baseline. The MTD was defined as that dose given without causing >15% loss of body weight or >10% mortality in a treatment group (23).

Statistical Analysis

Statistical analysis was carried out with Sigmastat for Windows (Jandel Scientific, San Rafael, CA). t Tests were performed on TVs at day 42 in each study because this corresponded to the day of termination of control group 131I-huA33 at the lowest dose level (50 μCi). For results from the 131I-dose escalation study, a comparison was made among the 131I-dose-escalating groups for each mAb and then a comparison was performed between 131I-radiolabeled hu3S193 and the corresponding dose of control huA33. For results from the combination Taxol and 131I-hu3S193 study, t tests were performed between the day 42 mean TVs for each of the radiolabeled hu3S193 treatment groups and the appropriate controls of Taxol alone and radiolabeled huA33 mAb.

RESULTS

Antibody Labeling

Instant TLC of radiolabeled antibodies prior to injection confirmed that >95% of 131I bound to antibody. The immunoreactivity of 131I-hu3S193 was 60% for Le⁺-positive MCF-7 cells. Control 131I-huA33 was determined to have 73% immu-
3624 131I-hu 3S193 and Taxol Therapy of Breast Cancer Xenografts

Results of the 131I-hu3S193 dose escalation study in mice bearing MCF-7 human breast carcinoma xenografts: day 42 comparison

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TV (mm³) at day 42a</th>
<th>Day 42 t test results within mAb groupb</th>
<th>Day 42 t test results between mAb groupsb</th>
</tr>
</thead>
<tbody>
<tr>
<td>131I-hu3S193</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 μCi</td>
<td>514.9 ± 335.8</td>
<td>vs. 50 μCi of huA33, NS</td>
<td>vs. 50 μCi of huA33, NS</td>
</tr>
<tr>
<td>100 μCi</td>
<td>281.0 ± 132.0</td>
<td>vs. 100 μCi of huA33, NS</td>
<td>vs. 100 μCi of huA33, P = 0.03</td>
</tr>
<tr>
<td>200 μCi</td>
<td>101.8 ± 51.0</td>
<td>vs. 200 μCi of hu3S193, P = 0.03</td>
<td>vs. 200 μCi of huA33, P = 0.001</td>
</tr>
<tr>
<td>300 μCi</td>
<td>7.5 ± 2.1</td>
<td></td>
<td>vs. 300 μCi of huA33, P &lt; 0.001</td>
</tr>
<tr>
<td>131I-huA33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 μCi</td>
<td>926.3 ± 348.8</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>100 μCi</td>
<td>587.9 ± 232.0</td>
<td>vs. 50 μCi of huA33, NS</td>
<td>NA</td>
</tr>
<tr>
<td>200 μCi</td>
<td>362.3 ± 103.9</td>
<td>vs. 100 μCi of huA33, NS</td>
<td>NA</td>
</tr>
<tr>
<td>300 μCi</td>
<td>252.5 ± 85.6</td>
<td>vs. 200 μCi of huA33, NS</td>
<td>NA</td>
</tr>
</tbody>
</table>

a Day 42, 42 days post radiolabeled antibody injection and day of termination for 131I-huA33 50-μCi control group.
b Results of t test are reported as NA, not applicable; NS, not significant; or significant P values for designated comparisons.

Animal Model

131I-hu3S193 Dose Escalation Study. Results for the 131I-hu3S193 dose escalation study at study completion are summarized in Table 1. The dose-limiting toxicity of 131I occurred at 300 μCi. All mice treated at this dose level had some degree of toxicity manifested by petechiae and bruising of varying degrees of severity, and weight loss. In total, three of five mice that received 300 μCi of 131I-hu3S193 and four of six in the comparable 131I-huA33 group were culled between days 18 and 42 because of such toxicity, which was presumed to be secondary to the radiation dose received.

In the remaining treatment groups, there was only 1 death out of a total of 12 mice treated at 200 μCi of 131I, which occurred in the huA33 group, and 1 death at 50 and 100 μCi of 131I-huA33, respectively, which at postmortem appeared attributable to the presence of metastatic disease. Three mice (one each at 50 and 100 μCi of 131I-hu3S193 and 50 μCi of 131I-huA33) were culled because of weight loss for which no cause was found, although the possibility of micrometastatic disease was not excluded.

Comparative t tests were performed between and within the mAb treatment groups at day 42, the day of termination of the 50-μCi control 131I-huA33 treatment arm (Table 2). A significant dose-response relationship was present for 131I-labeled hu3S193, with higher doses producing marked tumor suppression compared with control 131I-huA33 (Fig. 1 and Table 2). 131I-hu3S193-treated mice at each dose level had slower tumor growth than mice receiving the corresponding dose of 131I-huA33. A significant difference was observed between the day 42 mean TVs of the treatment groups receiving 131I-labeled hu3S193 compared with the group receiving control mAb at 131I-dose levels >50 μCi (Table 2). Four of five mice in the 300-μCi 131I-hu3S193 treatment arm achieved at least a 50% reduction in TV (PR), and one achieved CR (Table 1). No reduction in TV was seen in the comparable huA33 group (Fig. 1D).

At the 200-μCi 131I-dose level, three of six mice in the hu3S193 treatment arm achieved a PR (of 2–22 days duration), and an additional two mice had a maximal reduction in TV of 43 and 46%, respectively (Fig. 1C). No responses or reduction in TV below baseline measurements were observed in the comparable huA33 group, which was terminated early (day 76) because of a mean TV >1 g. The differences between the two treatment arms, 131I-hu3S193 and 131I-huA33, was statistically significant from day 13 until termination (P = 0.006). A dose of 200 μCi of 131I-hu3S193 was established as the MTD.

There was a statistically significant difference in the growth curves at the 100-μCi dose level (P = 0.03 at day 42), with tumor growth being slower in the 131I-hu3S193 arm (Fig. 1B). The huA33 arm was terminated early, on day 62, because of TV >1 g. At the 50-μCi dose level, a marked delay in tumor growth after a single 50-μCi dose of 131I-hu3S193 was observed; however, this difference between xenograft growth curves was not statistical significant at day 42 when the control arm was terminated because of tumor burden, and no responses were observed (Tables 1 and 2). Both unlabeled huS3193 and huA33 treatment arms were terminated early on days 66 and 42, respectively, because of TV >1 g (Fig. 1A).

Combination Taxol and 131I-hu3S193 Study. The results are summarized in Table 3. Overall there was no difference in the mean TV for 100 μCi of 131I-hu3S193 alone or when combined with 300 μg of Taxol, although tumor growth rates of both were slower than matched 131I-huA33 controls (Fig. 2). Increasing the dose of Taxol from 300 to 600 μg resulted in a slower growth curve but no responses when given alone. However, 600 μg of Taxol combined with 131I-hu3S193 led to a marked reduction in tumor growth (P < 0.001 compared with 600 μg of Taxol alone, from day 42 to study completion), with four of five mice in the treatment arm achieving PR and with the response in one sustained to the end of the study (day 78). No responses were observed in the comparable huA33 control group (Fig. 3). These differences in tumor growth curves between the hu3S193 plus 600 μg of Taxol and the comparable huA33 plus Taxol control treatment arm were significant from day 13 until study termination (P = 0.004 at day 42). The mean TV of the treatment group receiving 100 μCi of hu3S193 plus 600 μg of Taxol was significantly less than the corresponding tumors of the treatment group receiving 100 μCi of hu3S193 plus 300 μg of Taxol from day 7 until study completion (P = 0.012 at day 42).
Four mice were culled during the study for reasons other than tumor size. Metastatic disease was identified in one mouse in the 100-μCi 131I-hu3S193 arm sacrificed at day 56, and a second mouse in the 100-μCi huA33 group was culled at day 48 because of failure to thrive with no identifiable cause at post-mortem. Two mice in the 100-μCi 131I-hu3S193 plus 600-μg Taxol arm were culled, one on day 56 and the other on day 71 because of an infected tail and an infected front leg, respectively. Infection occurred at sites of bites inflicted by other mice in the treatment group, highlighting the problem of increased aggression in estrogen-supplemented mice.

**DISCUSSION**

The effectiveness of radioimmunotherapy is dependent on several parameters including the vascularity of a tumor, antigen
expression and accessibility, radiolabeled antibody uptake and retention, and sensitivity of the tumor to radiation (2, 24). As anticipated with a radiation-sensitive malignancy such as breast cancer, we observed a significant dose-response relationship in the 131I-labeled anti-Lea study. The single 50-μCi 131I-hu3S193 dose delayed tumor growth for 35 days compared with the control treatment arm. Higher doses of 131I-hu3S193 (100, 200, and 300 μCi) produced significant reduction in tumor growth compared with 131I-labeled isotype-matched control humanized antibody huA33 over the entire period of study, with the 300-μCi dose achieving PR in four of five mice and CR in one of five mice. The MTD of 131I-hu3S193 occurred at the 200-μCi dose level, similar to other murine model studies with intact radiolabeled antibodies (25). Other groups have been able to deliver higher doses of 131I-labeled antibody when administered as a single dose in a murine xenograft model (26), as in this study. However, the major contributor to toxicity of 131I-labeled antibody is red marrow toxicity, which in turn is dependent on the circulating half-life of the radiolabeled antibody; the differences in blood clearance of radiolabeled antibody would explain the differences in toxicity observed (27, 28). Many parameters affect antibody half-life, including radiolabeling techniques, antibody-antigen binding, and antibody physical properties, and these could also contribute to the discrepancies between studies.

To assess the specificity or tumor-targeting ability of radiolabeled hu3S193 compared with a nonspecific radiation effect, an isotype-matched humanized control antibody (huA33), which does not bind Lea antigen, was included in all studies. In the 131I-labeled antibody study, a significant reduction in TV was seen in mice that received a single dose of radiolabeled hu3S193 compared with 131I-labeled control huA33, thus indicating specific targeting of radiolabel to MCF-7 tumor cells by hu3S193. Using this established MCF-7 tumor model, we previously have shown no effect for hu3S193 alone (five doses of up to 1 mg each) on MCF-7 tumor growth, indicating that the responses seen with radiolabeled hu3S193 are not attributable to Fc-mediated effects of the humanized antibody. hu3S193 was, however, shown to be effective in a prevention MCF-7 tumor model (injection at time of cell inoculation), indicating that in this murine model, antibody alone is effective in a minimal disease setting (11).

The antigenic heterogeneity of solid tumor masses has been well documented. In addition, it has been proposed that loss of target tumor cell antigen expression after unconjugated antibody therapy may lead to selection of resistant tumor cell populations in a manner analogous to hormone- or chemotherapy-induced resistance. The application of radiolabeled mAbs as therapeutic agents may circumvent these problems of tumor escape. Radiolabeled antibodies, in contrast to naked antibodies, can effect cell death without binding to all cells in the tumor mass. With an appropriate radionuclide, binding to one antigen-positive cell may provide a lethal dose of radiotherapy to adjacent cells, whether antigen negative or positive, over a distance specified by the physical properties of the radionuclide chosen. The maximum range of the nonpenetrating β-emissions of 131I is 2.4 mm (28), and survival and repopulation of antigen-negative cells is therefore less likely with radiolabeled mAb therapy approaches.

Combined chemotherapy and radiotherapy has proven advantageous in other antibody systems (24). Responses observed with subtherapeutic (100 μCi) doses of 131I-huA33 in mice bearing human colon cancer xenografts were significantly greater after the addition of 5-fluorouracil (with or without leucovorin; Ref. 25). Breast cancer is an ideal model to test the
efficacy of combined modalities given its known sensitivity to both radiotherapy and chemotherapy. Clinically, breast cancer is responsive to a wide range of single chemotherapeutic agents, including the microtubule-stabilizing agent Taxol (1). Taxol was selected for the xenograft studies presented based on its relative ease of administration (i.p.) and the well-defined dosing profile in nude mice, which enabled subtherapeutic doses with negligible toxicity to be chosen. Taxol also arrests tumor cells at the G2-M phase of the cell cycle, which is the most radiosensitive phase and therefore ideally suited to combination therapy with radioimmunotherapy (14). The sequence of radioimmunotherapy and chemotherapy administration has been reported to be of importance in achieving optimal tumor response (14). In our study, Taxol was administered i.p. 24 h after injection of radioimmunolabeled antibody. This schedule was determined from a published study where Taxol administered after radioimmunotherapy with 90Y-ChL6 produced superior response rates compared with Taxol given prior to radioimmunotherapy (14). A dose of 100 μCi of 131I-hu3S193 was chosen for combined therapy studies because of the minimal toxicity and lack of definite responses observed at this dose level in the initial radioimmunotherapy experiments, which would allow additive or synergistic effects of combining this therapy with Taxol (also at subtherapeutic doses) to be evaluated.

The results obtained imply an advantage (additive or synergistic) for combined therapy over radioimmunotherapy alone, with four of five mice in the 600-μg Taxol plus 100-μCi 131I-hu3S193 group achieving a PR compared with no responses with either treatment modality when administered alone. As with the other studies performed, nonspecific, Fc-mediated antibody effect was excluded by the inclusion of an isotype-matched control antibody group. Whereas the group receiving 100 μCi of 131I-huA33 plus 600 μg of Taxol displayed slower tumor growth than other control groups, it was significantly inferior to the group receiving 100 μCi of 131I-hu3S193 plus 600 μg of Taxol, implying that nonspecific (nontargeted) radiation and chemotherapy sensitivity alone were not solely responsible for the responses observed. Although other chemotherapy agents have efficacy in breast cancer and antibody-drug conjugates have also been studied in clinical trials (10), the efficacy of Taxol in breast cancer together with our results suggest that a combined approach could be a logical therapeutic option for Le2-positive malignancies.

Numerous criticisms have been made concerning the validity of animal models. In general, the therapeutic efficacy of antitumor agents tends to be overestimated in the nude mouse model (29). The pharmacokinetic profile in nude mice differs from humans, and the MTD of most antitumor agents for nude mice is greater than the corresponding clinical dose, which is only partly compensated for by higher doses used in human studies (23). In addition, with respect to antibody studies, the lack of expression of antigen in normal tissues in a manner comparable to humans and the consequent reduced nonspecific binding in murine models is known. However, human cancer xenografts in nude mice have been valuable in screening the therapeutic potential of new reagents, with good correlation having been demonstrated between the sensitivity of tumors to a drug in the human body and in BALB/c nude mice (19). The significant responses observed in the therapeutic studies described indicate that hu3S193 is a promising new agent for the treatment of breast cancer, and further exploration of its therapeutic potential is warranted.

REFERENCES


Therapeutic Efficacy of Anti-Lewis\textsuperscript{y} Humanized 3S193 Radioimmunotherapy in a Breast Cancer Model: Enhanced Activity When Combined with Taxol Chemotherapy

Kerrie Clarke, Fook-Thean Lee, Martin W. Brechbiel, et al.


**Updated version**
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/6/9/3621

**Cited articles**
This article cites 28 articles, 15 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/6/9/3621.full#ref-list-1

**Citing articles**
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/6/9/3621.full#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.