Enhanced Efficacy of Radioimmunotherapy with $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 by Inhibition of Epidermal Growth Factor Receptor (EGFR) Signaling with EGFR Tyrosine Kinase Inhibitor AG1478

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Abstract

**Purpose:** Monoclonal antibodies and tyrosine kinase inhibitors specific for the epidermal growth factor receptor (EGFR) have been shown to enhance the effect of external beam radiation on EGFR-positive tumors. The effect of EGFR signaling abrogation by EGFR tyrosine kinase inhibitor on the efficacy of radioimmunotherapy has not been reported previously. This study investigated the effect of EGFR tyrosine kinase inhibition on the efficacy of radioimmunotherapy in a human cancer xenograft model.

**Experimental Design:** The humanized anti – Lewis Y antibody hu3S193 and the EGFR tyrosine kinase inhibitor AG1478 were studied. BALB/c nude mice were engrafted with A431 squamous carcinoma cells. Initial biodistribution properties of the $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 were evaluated in this model. In therapy experiments, cohorts of four to five xenografted mice were treated with saline as placebo, 0.4 mg AG1478 i.p. (six doses over 2 weeks), single i.v. injections of unlabeled hu3S193, or $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 (12.5, 25, 50, or 100 μCi). The combination of 0.4 mg AG1478 i.p. and 25 μCi $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 i.v. was subsequently evaluated in the A431 model.

**Results:** $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 retained excellent immunoreactivity after radiolabeling. The biodistribution study showed excellent uptake in tumor (90.33 ± 38.84%ID/g) peaking at 24 to 72 hours after injection and with prolonged retention. $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 significantly inhibited A431 xenograft growth at 25, 50, and 100 μCi doses. The combination of 0.4 mg AG1478 with a single dose of 25 μCi $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 resulted in a significant enhancement of efficacy compared with either agent alone ($P = 0.013$).

**Conclusions:** The efficacy of radioimmunotherapy with $^{90}$Y-CHX-A$^\gamma$-DTPA-hu3S193 is significantly enhanced by EGFR tyrosine kinase inhibitor AG1478. Further investigations of dosing regimens using EGFR tyrosine kinase inhibitors and radioimmunotherapy in the treatment of EGFR expressing tumors are warranted.

The Lewis Y (Le$^y$) antigen is a member of a family of blood group–related difucosylated oligosaccharides expressed predominantly during embryogenesis (1). Expression in adult tissues is restricted to granulocytes and epithelial surfaces under physiologic conditions. The overexpression of Le$^y$ by 60% to 90% of human carcinomas of epithelial origin (including those of the breast, colon, non–small cell lung, and prostate) renders it an attractive antigenic target for monoclonal antibody (mAb) immunotherapy (2 – 4). The overexpressed Le$^y$ occurs either as a glycolipid at the cell surface or linked to surface glycoproteins [e.g., members of the epidermal growth factor receptor (EGFR) family (5)]. We are currently investigating the humanized anti-Le$^y$ antibody hu3S193 in phase I/II clinical trials in patients with Le$^y$-positive cancers (6 – 9). The internalization properties of this mAb also make it an attractive candidate for radioimmunotherapy (10). We have shown previously preclinically the generation, biodistribution, and stable antigen-binding properties of hu3S193 radiolabeled with $^{111}$In, with higher tumor uptake observed compared with the $^{131}$I-hu3S193 radioconjugate (10, 11).

The EGFR is overexpressed in many common cancers of epithelial origin function and has been implicated in promoting tumor mitogenesis and metastasis, angiogenesis, inhibition of apoptosis, modification of the cellular response to radiation, and resistance to standard cytotoxic agents (12, 13). Several strategies have been clinically applied to impede the function or expression of the EGFR and affect tumor cell growth, including ligand-blocking anti-EGFR

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antibodies, small molecular weight EGFR-specific tyrosine kinase inhibitors (TKI), and antisense EGFR to inhibit the expression of the receptor (12, 14, 15).

The tyrosinase AG1478 is a small-molecule TKI of the EGFR (14, 16) and has antitumor activity in vitro and in vivo models (16, 17). Although single-agent antitumor activity has been shown with AG1478 against several different xenografts overexpressing the EGFR or the truncated tumor-associated de2-7 EGFR in nude mice, the most promising preclinical data have been generated by combining AG1478 with standard chemotherapy or disparate inhibitors to the EGFR (17). Nontherapeutic doses of AG1478 significantly enhanced the cytotoxicity of both cisplatin and temozolomide in these animal models and effected supra-additive antitumor activities in combination therapy with an antibody that binds to a unique epitope on the EGFR, mAb 806 (17–20). Accordingly, the interaction of EGFR inhibition combined with conventional cytotoxic therapies shows great promise in the treatment of a variety of solid tumors that overexpress this receptor.

The targeted systemic delivery of radiation to a tumor through radiolabeled-mAb (radioimmunotherapy) offers several potential advantages over external beam radiotherapy, including the ability to specifically target multiple sites of disease, avoid or minimize normal tissue toxicity, and cause cell death of adjacent tumor cells that are adjacent antigen negative or antibody inaccessible. As tumors can overexpress both the Lea antigen and EGFR, we explored the possibility of achieving more potent antitumor activity through combination therapy of AG1478 and 90Y-CHX-A-DTPA-hu3S193 in an animal model of epithelial cancer.

**Materials and Methods**

**Cell lines and antibodies.** The human squamous cell carcinoma A431 cells have been described previously (18). The A431 cells express >106 Lea antigen and 104 EGFR molecules on the cell surface. These cells were cultured in DMEM/F-12 with Glutamax (Life Technologies, Melbourne, Victoria, Australia) supplemented with 10% FCS (IRH Biosciences, Melbourne, Victoria, Australia). SW1222, a Lea-negative and EGFR-negative human colon cancer cell line was obtained and cultured as described previously (11). Cell viability in all experiments, as determined by trypan blue exclusion, exceeded 90%.

The humanized anti-Lea antigen mAb, hu3S193 (IgG1), which has been described previously (6), and the isotype control antibody, humanized anti-A33 mAb (huA33), which specifically recognizes the A33 antigen and does not bind A431 cells (28, 29), were produced in the Biological Production Facility (Ludwig Institute for Cancer Research, Melbourne, Victoria, Australia).

**AG1478 preparation.** The synthesis of the EGFR TKI tyrophostin AG1478 [4-(3-chloroanilino)-6,7-dimethoxyquinazoline] has been previously described (30). The mesylate form of AG1478 was manufactured by the Institute of Drug Technology (Boronia, Victoria, Australia). For in vivo studies, the AG1478 was formulated in 100 mmol/L Captisol (sulfolubutyl ether β-cyclodextrin: Cydex, Overland Park, KS) at a concentration of 400 μg/100 μL (9.7 mmol/L).

**Antibody labeling.** Antibody labeling with 99mTc and was achieved via a bifunctional metal ion-chelating agent, C-functionalized trans-1,4-cyclohexyl diethylenetriaminepentaacetic acid (CHX-A-DTPA), as described previously (10). In brief, 99mTcCl (MDS Nordion, Kanata, Ontario, Canada), converted to its acetate form, was bound to CHX-A-DTPA-hu3S193 or CHX-A-DTPA-huA33 antibody conjugate under mildly acidic conditions (pH 5.5) for 30 minutes; then, the pH was raised to 7 by the addition of small aliquots of 2.0 mol/L sodium acetate followed by 10 mmol/L EDTA. The radiolabeled mixture was purified by desalting on a Sephadex G50 column (Pharmacia, Uppsala, Sweden) equilibrated with saline. Radiolabeling was done on the day of injection into mice.

**Quality assessment of radioconjugates.** Before injection, the percentage of unbound radionuclide content was determined by instant TLC (31), and the immunoreactive fraction of the final radiolabeled hu3S193 product was tested by a Lea-positive cell binding assay according to Lindmo et al. (32). For the control radioconjugate huA33, an A33 antigen-positive colon carcinoma cell line, SW1222, was used.

**Serum stability study.** Freshly prepared 99mTc-CHX-A-DTPA-hu3S193 was incubated at 37°C in healthy donor serum for 6 days to evaluate the stability of the radioconjugate. On days 0, 3, and 6, samples were collected and the immunoreactivity and radiochemical purity were determined as described above.

**Xenograft model.** Xenografts were established in 4- to 6-week-old female BALB/c nu/nu mice (Biological Resource Centre, University of New South Wales, Sydney, New South Wales, Australia). Mice were maintained in autoclaved microisolation cages housed in a positive pressure containment rack (Thoren Caging Systems, Inc., Hazelton, PA).

Mice were identified by earmarks. A431 tumor cells (5 × 105) in 100 μL PBS were inoculated s.c. into the flank of 4- to 6-week-old mice. Tumor volumes in mm3 were measured using the formula: \( V = \frac{L \times W^2}{2} \times 0.5 \) (10), where two perpendicular measurements of diameter of palpable tumors were obtained. Therapeutic agents were administered 12 days later when xenograft volumes were 75 to 200 mm3. All animal studies were approved by the Animal Ethics Committee of the Austin Hospital.

**Biodistribution of 99mTc-CHX-A-DTPA-hu3S193.** Female BALB/c nude mice with established A431 xenografts received a single i.v. dose of 99mTc-hu3S193 (1.25 μg 2.8 μg μCi). Groups of mice (n = 3) were sacrificed at 4, 24, 48, 72, 120, 168, and 288 hours following 99mTc-hu3S193 injection. Control groups of mice (n = 3) bearing A431 xenografts received a single injection of 99mTc-huA33 isotype control antibody and were sacrificed at 24 and 72 hours after injection. Blood was collected by cardiac puncture. Tumors and organs [liver, spleen, kidney, muscle, skin, bone (femur), lung, heart, stomach, brain, small bowel, and tail] were excised, blotted, and weighed. All samples were counted as described previously (10). Standards prepared from the injected material were counted each time with tissues and tumors, enabling calculations to be corrected for the isotopic physical decay. Results of radiolabeled antibody distribution over time were expressed as the percentage of injected dose per gram tissue (%ID/g) as well as tumor-to-blood ratios.

**Radioimmunotherapy dose escalation of 99mTc-CHX-A-DTPA-hu3S193.** Groups of four to five mice with established A431 xenografts (mean ± SD = 142.28 ± 59.35 mm3) received an i.v. dose containing a sterile filtered mixture of 49 μg 99mTc-CHX-A-DTPA-hu3S193 (12.5, 25, 50, or 100 μCi) or isotype control 99mTc-CHX-A-DTPA-huA33 (12.5, 25, 50, or 100 μCi) in 0.1 mL saline. This protein concentration was selected based on the specific activity (2.3 mCi/mg) of 99mTc-hu3S193 where 100 μCi equals a protein dose of 49 μg. Accordingly, where appropriate, unleabeled mAbs were added to the radiolabeled conjugates to adjust for the selected protein dose. Control animals received saline or equivalent protein dose of unlabeled antibody. The day of antibody injection was designated day 0 of study. Tumor volumes were determined regularly and mice were euthanized when tumor volumes reached 1,000 mm3. Postmortem examination was done whenever possible if mice died unexpectedly or after mice were culled for reasons of toxicity. Data were expressed as mean ± SE tumor volume for each treatment group.
Differences in tumor volume between therapy and control groups at day 11 were tested for statistical significance using a Student’s t test as this corresponded to the day of termination of vehicle control and unlabeled hu3S193 groups. The optimal subtherapeutic radioimmunotherapy dose was determined and then used for combination therapy studies.

Combination therapy. A dose of 400 μg AG1478 mesylate in Capisol on six occasions over 10 days has been shown previously as subtherapeutic in our laboratory (17). The therapeutic efficacy of AG1478 alone (400 μg on days 0, 2, 4, 6, 8, and 10) or in combination with subtherapeutic 90Y-CHX-A”-DTPA-hu3S193 radioimmunotherapy (10.9 and 25 μCi) was investigated using the established A431 tumor model. A fourth treatment arm examined the therapeutic efficacy of the AG1478 regimen combined with a single dose of unlabeled hu3S193 (10.9 μg). Therapeutic agents were administered by the i.p. route and all control animals received i.p. injections of the saline vehicle. Toxicity was monitored by daily observations of the animal’s activity, appearance, and body weight. Differences in tumor volume between groups at given time points were tested for statistical significance using a Student’s t test.

Results

Production and characterization of 90Y-radioconjugates. The 90Y-CHX-A”-DTPA-hu3S193 and -huA33 were both prepared to a specific activity of 2.3 mCi/mg protein. The radiochemical purity of 90Y-labeled antibodies was confirmed to be >99% of 90Y bound to the antibody. The immunoreactivity of 90Y-CHX-A”-DTPA-hu3S193 was 46.1% to 55.2% for Le”-positive A431 cells. The calculated Kd of 90Y-CHX-A”-DTPA-hu3S193 by Scatchard analysis was 7.4 × 10^-6 mol/L. The control 90Y-CHX-A”-DTPA-huA33 was determined to have 60.5% immunoreactivity for A33-positive SW1222 colon carcinoma cells (data not shown).

Serum stability. A 6-day serum stability study observed a 40% drop in 90Y-CHX-A”-DTPA-hu3S193 immunoreactivity over 3 days at 37°C. By day 6, the immunoreactivity of the radioconjugate had decreased to 16.1%. Radiochemical purity remained high (>85%) over the 6-day incubation.

Biodistribution of 90Y-CHX-A”-DTPA-hu3S193. The biodistribution of 28 μCi 90Y-CHX-A”-DTPA-hu3S193 in tumor and normal tissues is presented in Table 1 and Fig. 1A and B. Specific A431 tumor uptake was observed from 4 hours, peaked at 24 hours (mean ± SD; 90.3 ± 38.8%ID/g; Fig. 1A), and was retained for over 7 days (Fig. 1B). This prolonged tumor localization was reflected in the tumor-to-blood ratios of 1:0.5 at 24 hours and a peak of 6.8:1 on day 7. Some uptake of 90Y-CHX-A”-DTPA-hu3S193 was observed in normal organs, which was associated with the blood pool of the radioconjugate, whereas bone accumulation of the radiometal was low. 90Y-CHX-A”-DTPA-hu3S193 uptake in control SW1222 colon tumor was low and nonspecific (data not shown). The biodistribution of isotype control 90Y-CHX-A”-DTPA-huA33 was determined in A431 tumors and normal organs at 24 and 72 hours (Fig. 1A). The biodistribution was associated with blood pool activity only with low nonspecific uptake (8.5 ± 0.5%ID/g at 24 hours) observed in A33 antigen-negative A431 tumors (Fig. 1A).

90Y-CHX-A”-DTPA-hu3S193 dose escalation therapy study. Results for the 90Y-CHX-A”-DTPA-hu3S193 dose escalation therapy study in mice bearing established A431 xenografts are shown in Fig. 2A and B. Animals with established tumors received a single treatment on day 0. Mice that received saline or unlabeled hu3S193 were euthanized at day 11 due to large tumor burden. Doses of 12 and 25 μCi 90Y-CHX-A”-DTPA-hu3S193 delayed tumor progression slightly compared with controls (Fig. 2A), with the 25 μCi dose being significantly different to the unlabeled hu3S193 group at day 11 (P = 0.016); animals in both groups were euthanized at day 18 due to large tumor burden. Mice that received 50 μCi 90Y-CHX-A”-DTPA-hu3S193 exhibited significant tumor growth inhibition until day 18 before exponential growth of the tumor resumed (day 11; P = 0.003), whereas a single dose of 100 μCi 90Y-CHX-A”-DTPA-hu3S193 caused marked delay in tumor growth over the period of study (65 days; day 11; P < 0.001; Fig. 2A). No deaths or significant toxicity were observed in any mice as a result of 90Y-CHX-A”-DTPA-hu3S193 therapy. The growth of A431 xenografts in animals treated with isotype control 90Y-CHX-A”-DTPA-huA33 was not inhibited at 12.5 μCi compared with vehicle control animals at day 11 (P = 0.08; Fig. 2B). At higher doses of radiolabeled control huA33, some retardation of tumor growth was observed compared with nontreated controls. However, the antitumor effects were not as marked as the comparable hu3S193-treated groups, indicating the specificity of the radioimmunotherapy being.

Table 1. Biodistribution of 90Y-CHX-A”-DTPA hu3S193 in BALB/c nude mice bearing A431 xenografts (mean ± SD %ID/g tissue; n = 3)

<table>
<thead>
<tr>
<th>Organ</th>
<th>4</th>
<th>24</th>
<th>48</th>
<th>72</th>
<th>120</th>
<th>168</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>33.64 ± 2.02</td>
<td>29.15 ± 1.84</td>
<td>15.89 ± 1.30</td>
<td>17.16 ± 1.51</td>
<td>12.32 ± 3.13</td>
<td>5.78 ± 2.11</td>
</tr>
<tr>
<td>A431 tumor</td>
<td>18.62 ± 0.64</td>
<td>90.33 ± 38.84</td>
<td>59.27 ± 10.35</td>
<td>60.57 ± 8.89</td>
<td>52.36 ± 12.73</td>
<td>39.47 ± 11.71</td>
</tr>
<tr>
<td>Liver</td>
<td>7.135 ± 0.03</td>
<td>7.73 ± 1.58</td>
<td>7.55 ± 1.95</td>
<td>5.34 ± 1.28</td>
<td>7.27 ± 1.35</td>
<td>4.48 ± 0.76</td>
</tr>
<tr>
<td>Spleen</td>
<td>6.15 ± 0.30</td>
<td>6.69 ± 2.28</td>
<td>10.53 ± 3.75</td>
<td>4.94 ± 0.65</td>
<td>6.37 ± 1.27</td>
<td>5.39 ± 1.20</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.12 ± 2.13</td>
<td>11.09 ± 0.99</td>
<td>11.37 ± 2.85</td>
<td>8.45 ± 0.53</td>
<td>8.48 ± 0.65</td>
<td>6.71 ± 0.57</td>
</tr>
<tr>
<td>Skin</td>
<td>7.03 ± 1.71</td>
<td>7.21 ± 1.24</td>
<td>6.43 ± 1.80</td>
<td>10.55 ± 6.02</td>
<td>7.74 ± 3.32</td>
<td>4.16 ± 0.70</td>
</tr>
<tr>
<td>Bone</td>
<td>6.87 ± 0.97</td>
<td>7.99 ± 0.89</td>
<td>4.57 ± 2.89</td>
<td>3.53 ± 0.72</td>
<td>6.65 ± 2.58</td>
<td>10.51 ± 1.73</td>
</tr>
<tr>
<td>Lung</td>
<td>16.91 ± 1.29</td>
<td>18.01 ± 2.21</td>
<td>13.97 ± 4.01</td>
<td>9.57 ± 1.68</td>
<td>7.87 ± 2.49</td>
<td>6.97 ± 1.82</td>
</tr>
<tr>
<td>Heart</td>
<td>10.49 ± 0.14</td>
<td>10.32 ± 1.68</td>
<td>5.14 ± 0.81</td>
<td>3.65 ± 0.72</td>
<td>3.81 ± 0.42</td>
<td>2.05 ± 0.71</td>
</tr>
<tr>
<td>Stomach</td>
<td>3.11 ± 1.44</td>
<td>2.63 ± 1.25</td>
<td>3.93 ± 2.41</td>
<td>0.95 ± 0.33</td>
<td>2.49 ± 0.36</td>
<td>0.99 ± 0.16</td>
</tr>
<tr>
<td>Small bowel</td>
<td>4.40 ± 0.64</td>
<td>3.79 ± 0.22</td>
<td>2.95 ± 1.35</td>
<td>1.96 ± 0.24</td>
<td>2.37 ± 0.26</td>
<td>1.16 ± 0.51</td>
</tr>
</tbody>
</table>
delivered by hu3S193 to the Le<sup>+</sup>-positive A431 tumors. Based on these results, and to determine more accurately any additive effect of AG1478 on therapeutic efficacy, a dose of 25 μCi 90Y-CHX-A<sup>00</sup>-DTPA-hu3S193 was selected for the combination studies with AG1478.

**Combination therapy study.** Mice bearing established A431 squamous cell carcinomas (mean ± SD tumor size, 131.74 ± 51.61 mm<sup>3</sup>) received a single treatment of 25 μCi 90Y-CHX-A<sup>00</sup>-DTPA-hu3S193 on day 0 followed by six doses of AG1478 (400 μg) over 10 days as indicated in Figs. 3 and 4. Mice treated with AG1478 alone or AG1478 combined with an equivalent protein dose of unlabeled hu3S193 were culled at day 10 due to tumor burden, and tumor growth was not significantly different to vehicle control mice (Fig. 3). At this time point, a significant difference was observed between the tumor size of the 90Y-CHX-A<sup>00</sup>-DTPA-hu3S193–treated and AG1478 alone–treated mice (P = 0.003), and a greater inhibition of tumor growth was apparent in animals receiving the combination therapy compared with AG1478 alone (P < 0.001; Fig. 3). By day 20, 10 days after the completion of the AG1478 dose regimen, a significant difference in tumor growth was observed between animals treated with a single dose of 90Y-CHX-A<sup>00</sup>-DTPA-hu3S193 and the group receiving the combination of 25 μCi 90Y-CHX-A<sup>00</sup>-DTPA-hu3S193 and 400 μg AG1478 (P = 0.013; Fig. 4). No signs of acute toxicity was evident in the treatment arms compared with vehicle control. Mice were

![Fig. 1. Biodistribution of 90Y-CHX-A<sup>00</sup>-DTPA-hu3S193 and isotype control 90Y-CHX-A<sup>00</sup>-DTPA-huA33 in BALB/c nude mice bearing established Le<sup>+</sup>-positive A431 adenocarcinomas (A) 4 to 72 hours after injection and (B) 120 to 288 hours after injection. Columns, mean %ID/g (n = 3); bars, SD.](image1)

![Fig. 2. Antitumor activity of a single dose of (A) 90Y-CHX-A<sup>00</sup>-DTPA-hu3S193 and (B) isotype control 90Y-CHX-A<sup>00</sup>-DTPA-huA33 administered on day 0 to BALB/c nude mice bearing established Le<sup>+</sup>-positive A431 tumors. Points, mean tumor volume (n = 4-5); bars, SD.](image2)
Euthanized for tumor burden, preventing any long-term toxicity observations. AG1478 did not seem to increase the toxicity of the radioimmunotherapy; the animal behavior was similar between groups and day 20 body weights of the two treatment arms (90Y-hu3S193 ± AG1478) were not significantly different (P = 0.109).

Discussion

In contrast to hematologic malignancies, radioimmunotherapy of solid tumors is often subtherapeutic (33). This is due in part to the relative lack of sensitivity of solid tumors to radiation compared with lymphoma and the inability to deliver sufficient radiation dose to tumor cells without the development of myelotoxicity, which is invariably dose limiting (34). Strategies to enhance the efficacy of radioimmunotherapy are therefore required to achieve therapeutic outcomes. The use of EGFR inhibitors to enhance the efficacy of external beam radiotherapy have been reported both in laboratory and in clinical studies (23, 26, 35, 36); however, there has been no prior study of radioimmunotherapy and the addition of an EGFR TKI to enhance therapeutic efficacy. We have explored previously the concept of combined antitumor therapy using the EGFR-specific TKI AG1478 and showed additive effects with chemotherapeutic agents and synergistic effects with antibodies to the EGFR (17). The study described herein explored the additive effects of targeting two different tumor-associated molecules: the EGFR through the TKI AG1478 and the LeT antigen via radioimmunotherapy. Our study has clearly shown that radioimmunotherapy with 90Y-CHX-A d-DTPA-hu3S193 is significantly enhanced by the EGFR TKI AG1478 in an A431 xenograft animal model, indicating that this strategy may have promise in the treatment of cancer patients. The combination therapy affected marked additive antitumor activity particularly when compared with the control arm of either treatment modality alone or AG1478 combined with unlabeled hu3S193.

The anti-LeT mAb hu3S193 (6) was successfully radiolabeled with 90Y, and the biodistribution and therapeutic efficacy of the radioconjugate was determined in an A431 xenograft animal model. 90Y-CHX-A d-DTPA-hu3S193 was stable with retention of 90Y-hu3S193 ± AG1478 at 400 µCi with no evidence of significant toxicity. In vitro phosphorylation and on downstream signaling targets (e.g., Akt) similar to other EGFR TKIs administered p.o. (17). Treatment of tumor cells with EGFR mAbs (e.g., C225) and TKIs (e.g., Iressa and AG1478) has been shown to induce cell cycle arrest at G1, which combined with radiation effects (primarily arresting cells at G2-M) induces profound cell kill. Inhibition of EGFR by C225 has also been reported to affect DNA-protein kinase, which is a serine/threonine kinase with homology to the catalytic domain of the phosphatidylinositol 3-kinase family and is involved in DNA double-strand break repair (22). The clinical efficacy of combining EGFR inhibitors with external beam radiotherapy has more recently been shown in phase III trials in head and neck cancer and in refractory lung cancer (35, 38, 39).

Previous studies have examined the ability of EGFR inhibitors to enhance the efficacy of radioisotope therapy. In one study of C225 with 90Y-DOTA-ChL6 in a breast cancer xenograft model, similar tumor response rates were observed prior studies of 111In-CHX-A d-DTPA-hu3S193 and higher than 131I-hu3S193, most likely due to the internalization of hu3S193 in LeT-expressing tumor cells and resulting catabolism of radiohalide (11). The therapeutic efficacy of 90Y-CHX-A d-DTPA-hu3S193 was clearly shown, with marked tumor growth inhibition at doses of ≥25 µCi with no evidence of significant toxicity. A subsequent combination therapy study showed that a dose regimen of AG1478 significantly enhanced the antitumor efficacy of a single 25 µCi dose of 90Y-CHX-A d-DTPA-hu3S193 in this animal xenograft model. The use of subtherapeutic doses of AG1478 and 90Y-CHX-A d-DTPA-hu3S193 enabled the additive effect of AG1478 to be more accurately defined. Importantly, no evidence of enhanced toxicity related to either marrow or epithelial tissues from combining AG1478 with radioimmunotherapy was observed.
with $^{90}$Y-DOTA-CHL6 alone versus $^{90}$Y-DOTA-ChL6 with C225 given 24 hours before radioimmunotherapy (34). Interestingly, C225 given 24 hours after $^{90}$Y-DOTA-ChL6 had a lower response rate and higher mortality rate, suggesting that EGFR signaling blockade may have had an effect on radiation sensitivity of normal tissues. In contrast, the addition of AG1478 and $^{90}$Y-CHX-A$^5$DTPA-hu3S193 in our study did not result in any toxicity in treated mice. In a study examining the in vitro effects of gefitinib (Iressa) with $^{211}$At-EGF (ligand) on two EGFR-expressing cell lines, the addition of gefitinib inhibited $^{211}$At-EGF effects on a gefitinib-sensitive cell line and increased efficacy in a gefitinib-resistant cell line (40). These results are discrepant compared with that expected from gefitinib and may be due to the effects of gefitinib on EGFR expression and internalization of the $^{211}$At-EGF molecule, with resultant cell nucleus damage from $^{211}$At. To our knowledge, there has been no study that has examined radioimmunotherapy with an EGFR TKI; certainly, the enhanced antitumor efficacy observed in our study has not been described previously. The Le$^y$ antigen is an attractive target for mAb-based therapy, and we have shown previously that the humanized antibody hu3S193 has efficacy alone and when radiolabeled with $^{131}$I and $^{90}$Y in animal xenograft models (6, 10). The data from this study clearly show that $^{90}$Y labeled to hu3S193 may be used for highly effective tumor cell kill. In phase I trials, hu3S193 has been shown to have excellent targeting properties to epithelial cancers, shows minimal binding to any normal tissue, is nonimmunogenic after multiple infusions, and has a terminal half-life greater than 7 days (7, 41). The optimization of serum half-life for radioimmunotherapy with hu3S193 may be required to achieve improved tumor to red marrow dose for i.v. infusion, although i.p. approaches are being currently explored, and scFv/diabody constructs based on hu3S193 with faster blood clearance have been produced (42, 43). It has also been reported that antibodies to Le$^y$ may inhibit signaling of Le$^y$ modified erbB receptors (EGFR and erbB2; ref. 44), providing a further potential mechanism of efficacy of Le$^y$ targeting antibodies.

Other approaches to increase the efficacy of radioimmunotherapy include the use of fractionated radioimmunotherapy (45, 46), pretargeting (47), and combining radioimmunotherapy with chemotherapy (48). All these strategies have shown promising preclinical data and are currently being evaluated in early-phase clinical trials. The results of our study indicate that combining radioimmunotherapy with EGFR inhibitors may also improve therapeutic efficacy in solid tumors, and these results provide impetus for exploring this combination in future clinical trials.

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