Effect of Recombinant α-Interferon on Pharmacokinetics, Biodistribution, Toxicity, and Efficacy of 131I-Labeled Monoclonal Antibody CC49 in Breast Cancer: A Phase II Trial

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ABSTRACT

Preclinical studies have demonstrated that recombinant IFN-α (rIFN-α) can enhance the tumor associated glycoprotein 72 (TAG-72) on tumors. To determine whether rIFN-α could enhance TAG-72 expression in vivo in patients, 15 women with breast cancer were randomized to receive daily injections of rIFN-α (3 × 10^6 units/m^2 for 14 days) beginning on day 1 (group 1 = 7 patients) or on day 6 (group 2 = 8 patients). On day 3, all patients received a 10–20-mCi tracer dose of 131I-CC49, a high-affinity murine monoclonal antibody reactive against TAG-72, followed by a therapy dose of 60–75 mCi/m^2 of 131I-CC49 on day 6. Whole body and single-photon emission computed tomography scans along with whole blood pharmacokinetics were performed following tracer and treatment phases. Hematological toxicity was considerable; reversible grade 3–4 neutropenia and thrombocytopenia was observed in 12 of 15 patients. Twelve of 14 patients tested developed human antimouse antibodies 3–6 weeks after treatment. For group 1 patients, whole blood residence time increased significantly between that predicted from the tracer doses and therapy doses (42.6 ± 4.7 versus 51.5 ± 4.8 h, respectively; P < 0.01). The calculated radiation absorbed dose to red marrow from therapy compared to tracer activity was also significantly higher for this group (1.25 ± 0.35 versus 1.07 ± 0.26 cGy/mCi; P < 0.05). Treatment with rIFN-α was found to enhance TAG-72 expression in tumors from patients receiving rIFN-α (group 1) by 46 ± 19% (P < 0.05) compared to only 1.3 ± 0.95% in patients not initially receiving IFN (group 2). The uptake of CC49 in tumors was also significantly increased in rIFN-α-treated patients. One partial and two minor tumor responses were seen. In summary, rIFN-α treatment altered the pharmacokinetics and tumor uptake of 131I-CC49 in patients at the expense of increased toxicity.

INTRODUCTION

Radioimmunotherapy of solid tumors with MoAbs has met with limited success due to poor tumor targeting of antibody (tumor uptakes of 0.001–0.01 %ID/gram; Ref. 1). Hence, methods to improve tumor uptake of MoAbs and diminish normal organ toxicity are of paramount importance.

Most solid tumors are heterogeneous with respect to surface antigen expression; in fact, some tumors may express little or no antigen, thereby preventing MoAbs or immunoconjugates from binding to a sufficient number of cells to elicit a therapeutic response. Putative methods to overcome antigenic heterogeneity might be the use of antibody “cocktails” (2, 3) or methods to enhance surface antigen expression (4). IFN-α and IFN-γ have been shown to enhance expression of several tumor-specific antigens both in vitro (5–8) and when administered to mice bearing human tumors xenografts (9–11). Among several breast cancer-associated antigens, TAG-72, a high molecular weight glycoprotein, has been shown to be significantly increased on tumor cells in vitro following exposure to rIFN (12–14). Recent studies have shown improved tumor targeting, as well as efficacy, of radiolabeled CC49 (a second-generation, high-affinity murine MoAb against TAG-72) when given in combination with IFN-γ in mice bearing TAG-72-positive tumors (15).

On the basis of the above, the main objectives of this study were to determine the following: (a) whether rIFN-α could enhance TAG-72 expression and uptake of 131I-labeled CC49 (131I-CC49) in breast cancer patients; (b) the toxicity of 131I-CC49 plus rIFN-α; (c) whether rIFN-α could modify the biodistribution and pharmacokinetics of CC49; and (d) the response rate to the combination.

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2 To whom requests for reprints should be addressed, at the University of Texas M. D. Anderson Cancer Center, Box 002, 1515 Holcombe Boulevard, Houston, Texas 77030.
PATIENTS AND METHODS

Patient Eligibility. To be eligible for the study, all patients had to have a Karnofsky performance status of greater than 70% and life expectancy of ≥3 months. Normal hepatic (bilirubin ≤2 mg/dl), renal (creatinine ≤2 mg/dl), and hematological (absolute granulocytes ≥2000/mm3; platelets ≥100,000/mm3) function was required for entry into the study. To determine whether rIFN-α was capable of modulating TAG-72 expression in vivo, all patients were required to have one or more tumors accessible for punch or excisional biopsy (i.e., skin or lymph nodes). This protocol was approved by the National Cancer Institute and the Surveillance Committee at the University of Texas M. D. Anderson Cancer Center. All patients gave written informed consent to participate in the study.

Radiolabeled Antibody Preparation. CC49 was produced using conventional hybridoma techniques and purified under good manufacturing practice conditions by Dow Chemical Company (Cincinnati, OH). The MoAb was provided by the National Cancer Institute and the Surveillance Committee at the University of Texas M. D. Anderson Cancer Center. All patients gave written informed consent to participate in the study.

Radiolabeled Antibody Preparation. CC49 was produced using conventional hybridoma techniques and purified under good manufacturing practice conditions by Dow Chemical Company (Cincinnati, OH). The MoAb was provided by the Cancer Treatment Evaluation Program, National Cancer Institute, in sterile vials containing PBS at a total mass of 15.9 mg/vial (10.6 mg/ml). CC49 was radiolabeled with 60–75 mCi/m2 of high specific activity 131I (500–700 mCi/ml; DuPont-NEN, Boston, MA) at a specific activity of 60 μg of protein per mCi of 131I to be used via the iodogen technique and purified as described previously (16). Purified, radiolabeled CC49 was collected through a 0.2-μm sterile filter. Labeling yield was estimated, and aliquots were taken for quality control tests. Unlabeled MoAb then was added to the preparation so that the immunoreactivity of the labeled MoAb then was added to the preparation so that the immunoreactivity of the labeled antibody was greater than 95%. The immunoreactivity of the labeled antibody was greater than 95%. The bound to antigen-coated beads, was greater than 75% (Rhochek, Rhomed Inc., Albuquerque, NM). The injected radiopharmaceutical was always sterile and free of pyrogens as assayed using Bectec Sterility Kits (Johnson Laboratories, a subsidiary of Becton Dickinson, Baltimore, MD) and the Limulus assay (Whitaker Bioproducts, Walkersville, MD), respectively.

IFN-α. Roferon-A (Hoffman-La Roche) was supplied for this trial by the National Cancer Institute as sterile lyophi-
WBRT, which are the integral of the %IA versus time (area under the curve) were also calculated for the tracer and therapy doses. Patients were discharged from the hospital when the total body activity of 131I was estimated to be ≤30 mCi, equivalent to a dose rate of 5 mRem/h at 1 meter. Following discharge, all patients had an additional total body image acquired as described above.

**Red Marrow Dose Estimation.** The red marrow dose for each patient was calculated from WBT and blood retention data measured for both tracer and therapy activities administered to each patient, as described previously (19), using the Tulane table to estimate blood volume from the height and weight of each patient (20, 21).

**Tumor Dosimetry.** Radiation absorbed dose estimates were calculated for all tumor sites that were visualized in serial Anger camera images from the number of counts detected after background subtraction. For chest wall tumors, a ROI was drawn around the perimeter of the uptake site, and a background region was selected in the area adjacent. For axilla uptake sites, a contralateral region was selected for background subtraction. The net counts after background subtraction were converted to activity using the sensitivity measured from the image of a standard 10-ml source of 131I (1% of the administered 131I) that was included in every Anger camera image and placed alongside the boundary of the body. The sensitivity of the Anger camera whole body image is derived from the number of counts detected from this source in air and used with no attenuation correction because lesions with demonstrated uptake were soft tissue lesions on the chest wall or axillary lymph nodes. The tumor activity measured on each imaging day was corrected for physical decay of 131I to the time of administration of the radiopharmaceutical and then converted to %IA using the following equation:

\[
%IA_{(tumor)} = \frac{[CROI - Cbgd]}{S \times A_{(\mu Ci)}} \times 100
\]

where \([CROI - Cbgd]\) represents the counts detected at time \(t\) after administration in a ROI and a background region of the same size, \(S\) is the sensitivity of the camera/collimator system cps/\(\mu Ci\), and \(A\) is the administered 131I in \(\mu Ci\).

A trapezoidal method was used to calculate the area under the curve of %IA versus time for each source in the body. The area under the curve of %IA versus time (residence time) was calculated for each tumor site using a trapezoidal integration scheme that was implemented in a spreadsheet. An extrapolation scheme was used from the last measured point to 0 assuming that the activity is 0 at 10 times the biological half-time of the uptake site. The volume of each tumor site was estimated from a set of contiguous transverse section images that were reconstructed from a SPECT study that was acquired at day 4 after the patient received radionuclide therapy.

The SPECT images indicated that the uptake sites were usually highly irregular and followed the contour of the chest wall. Because no S values were available for these irregularly shaped source regions, radiation absorbed dose to each tumor was calculated by assuming the dose to each site was a combination of energy deposited by the β particles emitted by 131I and a photon dose contribution equivalent to the penetrating fraction from activity in the total body. This was defined as follows:

\[
D_{(tumor)} = D_{(np)} + D_{(p)}(whole\ body)
\]

where \(D_{(np)}\) is the tumor self dose from the β particles emitted by the 131I in the tumor and \(D_{(p)}\) is the photon dose from all of the 131I in the whole body. This equation applies only to superficial lesions in the chest wall and axilla, and the sensitivity measured from the image of the 10-ml source in air was used for all of the activity calculations described in this study. It is also based on the assumptions that all of the β particles from 131I in the tumors are absorbed locally and the penetrating fraction of the total 131I flux in the whole body has no appreciable gradient across these small tumor volumes. The reproducibility of this technique for an individual patient is greater than 90%.

**Radiometric and Immunohistochemical Analyses of TAG-72 and CC49 in Tumor Biopsies.** Tumor biopsies were weighed and analyzed per gram for this %IA, as well as TAG-72 expression and CC49 uptake, in 13 of the 15 patients (2 patients had insufficient tumor samples for analysis) using quantitative immunohistochemistry as described previously using a SAMBA 4000 image analyzer (22) as reported previously (23). Data were expressed as MA index, which is equivalent to a mean labeling concentration (22).

**Measurement of HAMA Response.** Blood for HAMA assay was drawn from each patient 1 week before the study and at 6–8 weeks. Serum was analyzed for the presence of HAMA using an Immunostrip® ELISA according to the specification of the manufacturer (Immunomedics, Warren, NJ; Ref. 16). Values above 74 ng/ml were considered significant. This value represents 2 SDs above the mean value of HAMA obtained in sera of 25 healthy individuals.

**Response Criteria.** Patients were followed at appropriate intervals to assess toxicity and response. Appropriate radiographic and other studies were repeated at 8 weeks after initiating therapy. Responses were assessed using standard criteria (24).

**Statistical Analyses.** Comparison of mean ± SD pharmacokinetic parameters between group 1 and group 2 patients was analyzed using the t test for independent means. Differences in TAG-72 expression as determined by binding of CC49 and CC49 uptake in tumor by quantitative immunohistochemistry pre- and post-IFN-α treatment or in patients not receiving IFN-α were analyzed using the paired t test. This test was also used to compare differences in BT and WBT and residence times between the tracer and therapy doses of 131I-CC49.

**RESULTS**

**Patient Characteristics.** Fifteen women with a mean age of 51 years were studied. Mean Karnofsky performance status was 90%. Metastatic sites were primarily skin and soft tissue (14 of 15 patients), lung (6 of 15), liver (3 of 15), and bone (3 of 15). Fourteen of the 15 patients had received more than one course of combination chemotherapy, and 10 had received local radi-
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**Fig. 2** Hematological toxicity. Significant decreases in WBC and platelet counts occurred at 4–6 weeks posttreatment; the counts recovered by 8–10 weeks.

**Table 1** HAMA response (ng/ml)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pretreatment</th>
<th>3 wk</th>
<th>6 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;74</td>
<td>&lt;74</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>2</td>
<td>&lt;74</td>
<td>&gt;2000</td>
<td>460</td>
</tr>
<tr>
<td>3</td>
<td>&lt;74</td>
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<td>&gt;2000</td>
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<td>&lt;74</td>
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<td>230</td>
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<tr>
<td>15</td>
<td>&lt;74</td>
<td>380</td>
<td>&lt;74</td>
</tr>
</tbody>
</table>

* NT, not tested.

**Table 2** Total body and blood pharmacokinetics of $^{131}$I-CC49

<table>
<thead>
<tr>
<th>Parameter (10–20 mCi) (60–75 mCi/m$^2$)</th>
<th>Tracer dose</th>
<th>Therapy dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBT 1/2 (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>82 ± 8$^a$</td>
<td>78 ± 6</td>
</tr>
<tr>
<td>Group 2</td>
<td>80 ± 13</td>
<td>63 ± 8</td>
</tr>
<tr>
<td>Total</td>
<td>81 ± 86</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>BT 1/2 (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>48 ± 5</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>Group 2</td>
<td>43 ± 5</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>Total</td>
<td>45 ± 4</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>WBRT (h)</td>
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<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>101.0 ± 5</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>Group 2</td>
<td>102.6 ± 7</td>
<td>79 ± 67</td>
</tr>
<tr>
<td>Total</td>
<td>102 ± 6</td>
<td>86 ± 6</td>
</tr>
<tr>
<td>BRT (h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>43 ± 5</td>
<td>51 ± 5</td>
</tr>
<tr>
<td>Group 2</td>
<td>48.8</td>
<td>46 ± 7</td>
</tr>
<tr>
<td>Total</td>
<td>45 ± 7</td>
<td>49 ± 6</td>
</tr>
</tbody>
</table>

* Mean ± SE.

$^a$ NS, not significant.

$^b$ Groups 1 and 2 combined.

**AT**ation to isolated bone metastases or to chest wall skin recurrences.

**Toxicity.** There were no instances of nonhematological toxicity from $^{131}$I-CC49. Therapeutic doses of $^{131}$I-CC49 ranging from 60 to 75 mCi/m$^2$ of $^{131}$I caused reversible grade 3–4 hematological toxicity in 12 of the 15 patients treated (Fig. 2). The initial four patients who received 75 mCi/m$^2$ of $^{131}$I-CC49 plus rIFN-α developed grade 4 thrombocytopenia with platelets <25,000 at 4–6 weeks posttreatment; hence, subsequent patients received $^{131}$I-CC49 at a reduced activity level of 60 mCi/m$^2$. Overall, WBC count fell from a mean ± SE of 7400 ± 900 cells/mm$^3$ to 2300 ± 760 cells/mm$^3$ by day 26 of therapy (range, 14–43) and recovered by 8 weeks. Absolute granulocytes decreased from 5318 ± 826 to 1083 ± 251 over the same time interval. Mean ± SE platelet count decreased from 288,000 ± 22,000 to 35,000 ± 9,000. There were no differences in mean ± SE platelet count between group 1 (34,000 ± 6,800) and group 2 (37,000 ± 4,500). Similar findings were noted for absolute granulocytes.

**HAMA Responses.** HAMA responses on all 15 patients are shown in Table 1. No patient had measurable HAMA prior to infusion of $^{131}$I-CC49. Titers became elevated in 9 of 14 patients studied at 3 weeks post-tracer dose. By 6 weeks, four patients who were HAMA negative (<74 ng/ml) at 3 weeks had values greater than 2,000 ng/ml, which is above the limit of detection by the assay. An additional three patients (patients 3, 5, and 9) also had marked increases in HAMA (>2,000 ng/ml) at 6 weeks. Interestingly, six patients who were either HAMA positive or HAMA negative at 3 weeks had no change (patients 8 and 10) or decreases in HAMA by 6 weeks (patients 2, 13, 14, and 15).

**Total Body and Blood Pharmacokinetics of $^{131}$I-CC49.** The disappearance curves or biological half-times ($t_{1/2}$) for $^{131}$I-CC49 in blood and whole body expressed in h are summarized in Table 2. In the first four patients, serial blood samples were not collected during therapy. Hence, BT/2 was calculated for only 11 of 15 patients. In short, there were no significant differences in mean ± SE WBRT between tracer dose (81 ± 8 h) and therapy dose (70 ± 6 h; P > 0.05). Similar findings were observed for BT/2 (tracer dose, 45 ± 4 h; therapy dose, 48 ± 5 h; P > 0.05). There were also no significant differences in WBT/2 and BT/2 between group 1 patients (i.e., those receiving rIFN-α beginning on day 1) and group 2 (those receiving rIFN-α beginning on day 6; Table 2).

In contrast to the above results, in group 1 patients there was a slight yet significant prolongation of mean ± SE WBRT in group 1 (4 ± 0.2 days) compared to group 2 (4 ± 0.1 days; P = 0.05). Two patients had persistent grade 3–4 thrombocytopenia requiring multiple platelet transfusions. One patient did not recover from thrombocytopenia despite platelet transfusions and growth factor support and eventually died of progressive disease.
Fig. 3 Differences in residence times. ⋄, group 1; ○, group 2. A, expected WBRT; area within the dashed lines, region within 10% of the expected WBRT. If the tracer dose were identical to the therapy dose, all points would be expected to fall in the region defined by the dashed lines. As shown, the predicted values for 6 of 7 group 2 patients and 4 of 8 group 1 patients fell below the dashed line, indicating that WBRT values calculated from the tracer dose significantly overestimated the WBRTs for each therapy dose. B, individual points for 4 of 6 group 1 patients fell above the dashed line of identity, indicating that the tracer dose significantly underestimated the BRT for the therapy dose. Two of 5 group 2 patients also fell above the predicted BRT from the diagnostic dose.

92.1 h; $P = 0.07$). The residence time results are presented graphically in Fig. 3.

**Biodistribution of $^{131}$I-CC49.** Mean ± SD whole body %IA as estimated from the tracer study averaged 67.7 ± 12.8% at 48 h after infusion. Mean ± SD %IA in the liver was 6.8 ± 4.9%, and in the blood, 33.5 ± 15%. The %IA in tumor at 48 h was 0.57 ± 0.29 and remained stable or increased slightly in all patients.

A comparison of 48 h %IA for total body, blood, liver and tumor did not differ between groups 1 and 2 (data not shown).

**Red Marrow Activity.** Estimates of red marrow dose in cGy/mCi are shown in Table 3. The cGy/mCi contributed from activity in the blood increased by 28% between tracer and therapy studies in group 1 patients (from 0.64 ± 0.16; mean ± SD to 0.87 ± 0.28; $P < 0.007$, paired $t$ test), whereas the cGy/mCi estimates from whole body activity indicated no significant change (tracer dose, 0.43 ± 0.15; therapy dose, 0.38 ± 0.12; $P > 0.05$). The total red marrow dose from blood plus total body activity also increased significantly in this group (from 1.07 ± 0.26 to 1.25 ± 0.34 cGy/mCi; $P < 0.05$). Similar
Phase II Trial of CC49 and IFN

Enrichment of TAG-72 Expression and CC49 Uptake in Tumor by rIFN-α. Different rIFN-α schedules were designed to compare the %IA/g of CC49 in tumor biopsies from the two groups of patients who were receiving rIFN-α. Results of rIFN-α treatment on TAG-72 modulation and CC49 uptake between pretreatment and 48-h post-tracer dose biopsy specimens from the above patients have been detailed in a separate report (23) and will only be summarized briefly here. Patients who began rIFN-α treatment on day 1 (group 1) had an increase in TAG-72 expression of 46 ± 19% (MA pre-IFN-α, 14.5 ± 4; post-IFN-α, 20 ± 5; P < 0.05) compared to only 1.3 ± 0.95% (MA pre-IFN-α, 12.5 ± 3.7; post-IFN-α, 13.1 ± 3.9; P > 0.05) for group 2 patients not receiving IFN-α on day 1. Likewise, tumor targeting of CC49 in group 1 patients (MA, 8.73 ± 1.8) was 4-fold higher than in group 2 patients (MA, 2.46 ± 0.32; P < 0.01). In contrast, the %IA in tumor between group 1 and group 2 patients was only 2-fold higher (mean %ID/g = 0.008 ± 0.002 versus 0.004 ± 0.002; P = 0.06).

Antitumor Activity. A partial response was observed in 1 of 15 patients studied. A patient with numerous palpable axillary lymph nodes had a 50% decrease in lymphadenopathy following treatment (Fig. 4). The same patient also had complete disappearance of numerous pulmonary nodules <1 cm in size on chest X-ray along with improvement of bone pain and partial ossification of lytic metastases. The response lasted 8 weeks following therapy; subsequently, the patient developed metastases to bone marrow. Minor responses in soft tissue were observed in two patients, and three other patients had mixed responses. Ten patients had tumor progression following one course of treatment. Except for one patient with a persistent MR in s.c. lesions (all <1 cm) lasting for 15 months, all patients had progressive disease by 8 weeks posttreatment.

**DISCUSSION**

This represents one of the first Phase II radioimmuno-therapy trials to analyze the biological effects of IFN-α on the biodistribution pharmacokinetics and tumor uptake of 131I-CC49 in breast cancer patients. A previous study in ovarian cancer patients demonstrated that the i.p. administration of IFN-γ (0.1–100 megaunits/week) could enhance the expression of TAG-72 and CEA in malignant ascites cells (14). In the above-mentioned trial, IFN-γ increased both the percentage of MoAb B72.3 reactive tumor cells and MoAb staining intensity. Greiner et al. (25) have demonstrated elevation of serum CEA and TAG-72 levels in patients given IFN-γ or IFN-β.

Significant nonhematological toxicity was not observed. The majority of patients had significant HAMA responses by 6 weeks. Eight of 15 patients had HAMA greater than 2,000 ng/ml, which is comparable to the results from our previous trial of murine CC49 (16) and a study of chimeric CC49 (26). The hematological toxicity observed with the combination differs substantially from what has been observed with studies of 131I-CC49 alone. In our earlier Phase II trial of 131I-CC49 in colorectal cancer (16), grade 3–4 thrombocytopenia was only observed in 7 of 15 patients, compared to 13 of 15 in this study (P < 0.05, χ²). These findings were also validated in a Phase I trial of 131I-CC49 in colon cancer in which the maximum tolerated dose was 75 MCl/m² (18). Although these findings could be due in part to the fact that the breast cancer patients were more heavily pretreated than the colon cancer patients, it is also possible that rIFN-α added to the hematological toxicity because moderate myelosuppression has been shown to occur with rIFN-α alone. Toxicities at these administered activities have been observed in patients with prostate cancer receiving the combination of 131I-CC49 and rIFN-α (27). Because radiation can cause stem cell toxicity resulting in irreversible marrow damage, caution must be exercised in combining radioimmuno-therapy with drugs/biologics that might enhance this effect.

An interesting observation that could also explain the enhanced hematological toxicity was the significant increase in whole blood residence time in patients who began rIFN-α treatment on day 1. We also observed a significant decrease in whole body residence time for patients beginning rIFN-α injections on day 6. A slight decrease was also observed for group 1 (from 101 to 92 h). Although a specific physiological explanation(s) for these findings is lacking, it appears that prolonged administration of rIFN-α resulted in a shift of 131I-CC49 from the extravascular compartment to the blood resulting in a higher BRT:WBRT ratio during the therapy phase (i.e., after at least

**Table 3** Predicted absorbed dose to red marrow (cGy/mCi)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean administered activity (mCi)</td>
<td>18 ± 5.7⁺</td>
<td>15 ± 5.6</td>
</tr>
<tr>
<td>Blood contribution</td>
<td>0.64 ± 0.16</td>
<td>0.81 ± 0.20</td>
</tr>
<tr>
<td>Total body contribution</td>
<td>0.43 ± 0.15</td>
<td>0.41 ± 0.18</td>
</tr>
<tr>
<td>Total</td>
<td>1.07 ± 0.26</td>
<td>1.23 ± 0.30</td>
</tr>
</tbody>
</table>

Therapy dose

| Mean administered activity (mCi) | 100.9 ± 24    | 109.9 ± 18    |
| Blood contribution               | 0.87 ± 0.28ᵇ  | 0.89 ± 0.20   |
| Total body contribution          | 0.38 ± 0.12   | 0.32 ± 0.14   |
| Total                            | 1.25 ± 0.35ᶜ  | 1.23 ± 0.27   |

*Mean ± SD.

⁺Significantly increased (P < 0.001) versus tracer dose.

⁻Significantly increased (P < 0.05) versus tracer dose.

*There was a direct correlation between radiation dose and the %IA/g of CC49 in tumor biopsies from patients who were receiving rIFN-a. Results of rIFN-a schedules were designed to compare the %IA/g of CC49 in tumor biopsies from the two groups of patients who were receiving rIFN-a. Results of rIFN-a treatment on TAG-72 modulation and CC49 uptake between pretreatment and 48-h post-tracer dose biopsy specimens from the above patients have been detailed in a separate report (23) and will only be summarized briefly here. Patients who began rIFN-a treatment on day 1 (group 1) had an increase in TAG-72 expression of 46 ± 19% (MA pre-IFN-a, 14.5 ± 4; post-IFN-a, 20 ± 5; P < 0.05) compared to only 1.3 ± 0.95% (MA pre-IFN-a, 12.5 ± 3.7; post-IFN-a, 13.1 ± 3.9; P > 0.05) for group 2 patients not receiving IFN-a on day 1. Likewise, tumor targeting of CC49 in group 1 patients (MA, 8.73 ± 1.8) was 4-fold higher than in group 2 patients (MA, 2.46 ± 0.32; P < 0.01). In contrast, the %IA in tumor between group 1 and group 2 patients was only 2-fold higher (mean %ID/g = 0.008 ± 0.002 versus 0.004 ± 0.002; P = 0.06).

Antitumor Activity. A partial response was observed in 1 of 15 patients studied. A patient with numerous palpable axillary lymph nodes had a 50% decrease in lymphadenopathy following treatment (Fig. 4). The same patient also had complete disappearance of numerous pulmonary nodules <1 cm in size on chest X-ray along with improvement of bone pain and partial ossification of lytic metastases. The response lasted 8 weeks following therapy; subsequently, the patient developed metastases to bone marrow. Minor responses in soft tissue were observed in two patients, and three other patients had mixed responses. Ten patients had tumor progression following one course of treatment. Except for one patient with a persistent MR in s.c. lesions (all <1 cm) lasting for 15 months, all patients had progressive disease by 8 weeks posttreatment.

**DISCUSSION**

This represents one of the first Phase II radioimmuno-therapy trials to analyze the biological effects of IFN-α on the biodistribution pharmacokinetics and tumor uptake of 131I-CC49 in breast cancer patients. A previous study in ovarian cancer patients demonstrated that the i.p. administration of IFN-γ (0.1–100 megaunits/week) could enhance the expression of TAG-72 and CEA in malignant ascites cells (14). In the above-mentioned trial, IFN-γ increased both the percentage of MoAb B72.3 reactive tumor cells and MoAb staining intensity. Greiner et al. (25) have demonstrated elevation of serum CEA and TAG-72 levels in patients given IFN-γ or IFN-β.

Significant nonhematological toxicity was not observed. The majority of patients had significant HAMA responses by 6 weeks. Eight of 15 patients had HAMA greater than 2,000 ng/ml, which is comparable to the results from our previous trial of murine CC49 (16) and a study of chimeric CC49 (26). The hematological toxicity observed with the combination differs substantially from what has been observed with studies of 131I-CC49 alone. In our earlier Phase II trial of 131I-CC49 in colorectal cancer (16), grade 3–4 thrombocytopenia was only observed in 7 of 15 patients, compared to 13 of 15 in this study (P < 0.05, χ²). These findings were also validated in a Phase I trial of 131I-CC49 in colon cancer in which the maximum tolerated dose was 75 MCl/m² (18). Although these findings could be due in part to the fact that the breast cancer patients were more heavily pretreated than the colon cancer patients, it is also possible that rIFN-α added to the hematological toxicity because moderate myelosuppression has been shown to occur with rIFN-α alone. Toxicities at these administered activities have been observed in patients with prostate cancer receiving the combination of 131I-CC49 and rIFN-α (27). Because radiation can cause stem cell toxicity resulting in irreversible marrow damage, caution must be exercised in combining radioimmuno-therapy with drugs/biologics that might enhance this effect.

An interesting observation that could also explain the enhanced hematological toxicity was the significant increase in whole blood residence time in patients who began rIFN-α treatment on day 1. We also observed a significant decrease in whole body residence time for patients beginning rIFN-α injections on day 6. A slight decrease was also observed for group 1 (from 101 to 92 h). Although a specific physiological explanation(s) for these findings is lacking, it appears that prolonged administration of rIFN-α resulted in a shift of 131I-CC49 from the extravascular compartment to the blood resulting in a higher BRT:WBRT ratio during the therapy phase (i.e., after at least
Table 4  Predicted absorbed dose in tumor

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mCi)</th>
<th>Tumor site</th>
<th>Tumor volume cm³</th>
<th>% IA/g</th>
<th>Total cGy</th>
<th>cGy/mCi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>101</td>
<td>Axilla</td>
<td>38</td>
<td>0.014</td>
<td>841</td>
<td>8.3</td>
</tr>
<tr>
<td>2</td>
<td>131</td>
<td>Axilla</td>
<td>61</td>
<td>0.005</td>
<td>438</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>Chest/wall axilla</td>
<td>71</td>
<td>0.013</td>
<td>632</td>
<td>6.6</td>
</tr>
<tr>
<td>4</td>
<td>130</td>
<td>Breast</td>
<td>74</td>
<td>0.011</td>
<td>892</td>
<td>6.2</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>61 ± 16</td>
<td>0.011 ± 0.003</td>
<td>678 ± 184</td>
<td>6.1 ± 1.807</td>
</tr>
</tbody>
</table>

a As estimated from SPECT.
b Direct correlation between radiation dose and %IA/g MoAb; r = 0.820; P < 0.01.

Fig. 4 Clinical response in left axilla from an individual patient. A, tumor in left axillary lymph nodes prior to therapy; B, posttherapy: note decrease in size of tumor masses.
7–10 days of rIFN-α treatment). Because rIFN-α has been shown to alter the metabolism of proteins by affecting cytochrome p450 enzymes in liver (28) it may be feasible that alterations in whole body and blood distribution of 131I-CC49 might reflect changes in antibody metabolism. It is also conceivable that changes in tumor vasculature induced by IFN-α could be responsible for the findings observed (29). Additional in-depth studies are required to provide answers to these questions.

The above findings have important consequences with respect to the predicted radiation absorbed dose to marrow from a tracer dose of 131I-labeled antibody. As detailed above, the calculated radiation dose to marrow from the tracer dose in patients already receiving rIFN-α did not predict radiation dose to marrow for the subsequent treatment dose. In essence, the “tracer principle,” i.e., the central dogma that biodistribution of radiolabeled antibodies from the tracer dose should predict distribution of a subsequent dose of radiolabeled MoAb (30), did not hold true in this study. Because as much as 20% of the activity contribution to red marrow may be derived from blood (31), it is conceivable that the increase in blood residence time reported above could have resulted in a higher radiation dose to marrow, with the result of greater toxicity than predicted from the tracer study.

It is possible that lower doses and/or schedules of rIFN-α other than those used here might up-regulate TAG-72 to a similar extent without undue toxicity to marrow. In a previous study performed in malignant melanoma, we observed an enhancement of tumor uptake of the melanoma tumor antigen 96.5 following 3 days of rIFN-α (32). Mahvi et al. (33) demonstrated that a single i.v. infusion of only 10^6 units of rIFN-α increased TAG-72 and CEA expression in 3 of 6 patients with primary colorectal cancer. In a more recent study, Roselli et al. (34) tested four dose schedules of rIFN-α (2 doses of 3 × 10^6 units, 2 doses of 6 × 10^6 units, 3 doses of 3 × 10^6 units, and 3 doses of 6 × 10^6 units) in colon cancer patients prior to surgical resection of primary tumor. Significant enhancement of TAG-72/CEA on resected tumors occurred with either 3 or 6 × 10^6 units of rIFN-α administered for 3 days, but not 2 days. Biopsies were obtained 1 day after the final rIFN-α injections; hence, the duration of increased antigen expression without rIFN-α is not known. One of the reasons for choosing a 14-day schedule in our study was the desire to maintain constant expression of TAG-72 for both diagnostic and treatment phases of the study. Whether enhanced TAG-72 expression persisted over the duration of the trial is not known and requires further study.

The mean radiation dose estimate to tumor (cGy/mCi) in this study was significantly higher (6.09 ± 1.80) than that calculated for colon cancer patients (2.96 ± 2.30; P < 0.05) in our previous trial (16). It is possible that several factors (e.g., tumor location, tumor site, patient status) might also influence uptake and contribute to the differences observed. Since the majority of patients studied had received local radiation to chest wall tumors, the radiation could have enhanced TAG-72 uptake and binding (35).

Although modest clinical responses in soft tissue were observed, it was not possible to determine whether these results were due to 131I-CC49 or rIFN-α alone or to their combination. In a previous trial of rIFN-α in breast cancer, modest antitumor activity was observed (36). Several other studies in the literature using different MoAbs reactive with breast cancer have demonstrated clinical response rates of 50% when higher activities are administered, and peripheral blood stem cell rescue was used to overcome the increase in marrow ablation (37, 38).

In summary, rIFN-α caused an increase in tumor uptake of 131I-CC49 at the expense of increased toxicity. These findings may have ramifications with respect to the use of radiolabeled antibodies combined with cytokines or other biologicals. Although the modest antitumor activity observed is of interest, a 0.2–2-fold increase in the 96/4A and radiation absorbed dose to tumor induced by rIFN-α may be unlikely to eradicate large tumor burdens in patients with solid tumors. Future clinical studies with radiolabeled MoAbs should focus on high-dose radiotherapy combined with autologous bone marrow or peripheral blood stem cell rescue and extending treatment to a minimal disease or adjuvant setting, combined with additional strategies to further enhance the tumor to normal tissue ratio. These approaches are required to significantly improve the therapeutic index and subsequent patient benefit from radioimmunotherapy in patients with solid tumors.

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