Phase II Study of Dual $^{131}$I-labeled Monoclonal Antibody Therapy with Interferon in Patients with Metastatic Colorectal Cancer


Departments of Radiation Oncology [R. F. M., W. E. P.], Medicine [M. B. K., T. L., R. H. W., A. F. L.], Pathology [W. E. G.], and Nuclear Medicine [C. D. R.], University of Alabama at Birmingham, Birmingham, Alabama 35294, and National Cancer Institute, Bethesda, Maryland 20892 [J. S.]

ABSTRACT

The combination of COL-1 (anti-CEA) and CC49 (anti-TAG-72) has shown an increase in binding and distribution in colon cancer by immunoperoxidase staining compared to either antibody alone. To overcome tumor heterogeneity and allow delivery of higher radiation dose, $^{131}$I-labeled COL-1 and CC49 at a total dose of 75 mCi/m$^2$ (2775 MBq/m$^2$) were simultaneously administered to 14 patients with metastatic colon cancer. α-IFN (3 × 10$^6$ IU) was given s.c. on days −5 to +3 to increase carcinoembryonic antigen and TAG-72 antigen expression. Most patients had mild symptoms during IFN therapy, including mild neutropenia, fever, and malaise, which rapidly subsided after IFN cessation. No acute allergic reactions occurred with radioimmunotherapy; two patients experienced transient, delayed grade 2 arthralgias. Transient neutropenia and/or thrombocytopenia, which was maximal at 4–6 weeks, were consistent side effects without adverse events. All patients had tumor localization, and 13 of 14 patients achieved 4+ (highest grade) localization readings to at least one known site of disease. No objective responses occurred; 4 patients were stable and 10 progressed. Tumor dose estimates varied from 393 to 1327 cGy, including liver and extrahepatic sites. Combining two complementary antibodies and IFN administration appeared to increase localization intensity and radiation doses at tumor sites as compared to historical controls. The amount of radiation delivered to tumor sites was still below that required to cause tumor regressions in metastatic colorectal cancer.

INTRODUCTION

Heterogeneity of tumor antigen expression is a well-recognized phenomenon that hampers effective use of monoclonal antibodies. Using mixtures of monoclonal antibody "cocktails" that are "complementary" in that they recognize different antigens expressed by a tumor may provide a more homogeneous distribution of radiolabel throughout the tumor site. Simultaneous administration of complementary antibodies may also increase the total amount of antibody localization and allow delivery of a larger amount of radionuclide to the tumor.

The murine antibodies COL-1 and CC49 both react against adenocarcinoma of the colon (1–3). These antibodies have different but complementary specificities, with COL-1 reacting against CEA and CC49 reacting against a TAG-72, which has mucin characteristics (2, 3). The combination of COL-1 and CC49 has shown an increase in antibody binding and distribution in colon cancer tumor specimens by immunoperoxidase staining as compared to either antibody alone (4). The therapeutic efficacy of combining two antibodies that react against different epitopes on the same tumor has been tested in animal models. In mice, Blumenthal et al. (5) have reported that the combination of two $^{131}$I-labeled antibodies reactive with tumor xenografts produced greater tumor growth inhibition than either antibody alone using the same amount of radionuclide (5).

A second strategy to enhance antibody localization is to alter tumor antigen density. Recent mathematical model studies of the effects of antigen density and antibody affinity on the uptake of monoclonal antibody in solid tumors emphasize the importance of abundantly expressed antigen on tumor cells for maximizing uptake in high-dose administrations (6). Both IFN-γ and IFN-α have been reported to up-regulate TAG-72 and CEA expression in tumor cells (7–10). In animal tumor models, IFN was shown to increase tumor antigen expression, enhance radiolabeled antibody localization, and improve antitumor efficacy of radioimmunotherapy (11). Greiner et al. (12) have demonstrated similar enhancement of TAG-72 antigen expression in human patient sera and ascites tumor cells (13). Subsequently, Murray et al. (14) have reported increased localization of $^{131}$I-labeled CC49 and therapeutic response in patients with breast cancer when adjuvant IFN-α was used (14).

In this study, we combined both of these strategies, i.e., IFN enhancement of antigen expression and dual radiolabeled antibody administration in an attempt to enhance antibody localization and efficacy as compared to prior trials with $^{131}$I-labeled CC49 alone (15–17) or combined with interleukin 1 (18).

Received 1/12/96; revised 7/25/96; accepted 8/19/96.

1 Supported in part by National Cancer Institute Grant CM 87215 and NIH Grant MO1-RR-00032.
2 To whom requests for reprints should be addressed, at Department of Radiation Oncology, University of Alabama at Birmingham, L. B. Wallace Tumor Institute, 1824 Sixth Avenue South, Birmingham, AL 35294-3300. Phone: (205) 934-2760; Fax: (205) 975-6161.

The abbreviations used are: CEA, carcinoembryonic antigen; TAG-72, "pancarcinoma" glycoprotein-72; SPECT, single photon emission computed tomography; MIRD, medical internal radiation dosimetry; HAMA, human antimouse antibody; SSRI, saturated solution of potassium iodide.
MATERIALS AND METHODS

Clinical Protocol. Eligible patients had histologically confirmed adenocarcinoma of the colon or rectum and unresectable metastatic disease. Eligibility criteria included TAG-72 and CEA-positive tumor on immunoperoxidase staining (19), Karnofsky performance status >60, and adequate hematological, renal, and liver function. Patients were excluded for iodine allergy, a history of cytotoxic chemotherapy that included mitomycin, radiation to >20% of the active marrow, other invasive malignancies, or infection with hepatitis B or human immunodeficiency virus. Concurrent chemotherapy, radiation, or immunotherapy were not allowed. This study was reviewed and approved by the University of Alabama at Birmingham Institutional Review Board, and all patients gave informed consent.

The trial design was an open-label Phase II study coordinated through the National Cancer Institute (CM87215). After satisfactory pretherapy evaluation, patients received daily s.c. injections of human recombinant IFN-alfa (3 X 10^6 IU) for 8 days, beginning 5 days before radiolabeled antibody. This dose was selected from previous clinical trials (20, 21). Synthroid (50 μg daily) and 10 drops of a saturated solution of potassium iodide (SSKI, 1 g/ml), were initiated 2 days prior to radiolabeled antibody therapy and continued for 14 days thereafter. Patients were treated in lead-lined hospital rooms at the University of Alabama at Birmingham General Clinical Research Center and were confined in isolation until their exposure rate at 1 meter was <5 mRem/h. Prior to the radiolabeled dose, patients received a test dose of unlabeled CC49 and COL-1 (100 μg), with vital signs monitored every 10 min times three. If no signs of allergic or other adverse reactions were observed, the radioimmunoconjugate (75 mCi/m2) was infused over 1 h, with vital signs monitored every 15 min during administration and for 1 h postinfusion. This dose of radioisotope was determined by prior individual Phase I studies with 131I-labeled CC49 or 131I-labeled COL-1 (17, 22).

Subsequent to therapy, patients had serial gamma camera imaging, whole-body gamma counts, and blood sampling for determination of immune response to the administered antibody. Follow-up evaluation included history and physical exam, blood counts, and liver, renal, and thyroid function studies. Tumor response was assessed 6 weeks posttherapy. Toxicity was scored according to the Cancer Clinical Trials Common Toxicity Criteria.

Response criteria required >50% reduction in the product of the longest diameters of all known lesions and the appearance of no new lesions for a partial response. “Stable” was defined as <25% change in the product dimensions of known lesions and no detection of new lesions. “Progression” required >25% increase in the product dimensions of known lesions or the appearance of new lesions.

Dosimetry and Imaging Techniques. Dosimetry data collection by gamma camera imaging and whole-body gamma counts was as described previously (23). Whole-body scans were performed on at least three time points after radioimmunotherapy, and SPECT imaging was done for at least one region of interest. Whole-body views were acquired on a 128 X 128 Matrix using a high energy, general purpose collimator. SPECT images were obtained with a 64 X 64 Matrix using step-and-shoot tomography. To obtain 40–90 K counts per step, 10–13-s stops were required. Gamma camera images of localization sites were reported as positive for a score of 2–4 according to the following grading scale: 0, within normal limits; 1, probably negative; 2, suspicious; 3, positive; 4, strongly positive.

Radiation doses to total body, red marrow, and tumor masses were calculated according to the MIRD formalism (24) with absorbed dose per unit cumulated activity (5 values) taken from MIRD pamphlet no. 11 (25), or, in the case of tumors, derived from emission parameters and absorbed fractions in MIRD pamphlet nos. 10 (26) and 8 (27), respectively. Total body clearance was obtained from a mono-exponential fit of whole-body count data.

Red marrow doses include the marrow-to-marrow self dose based on plasma radioactivity and dose from the remainder of the body based on whole-body counts. Activity in the red marrow was related to measured blood plasma activity concentrations through a red marrow-to-blood activity concentration ratio (28). Since activity measurements were made on plasma rather than on whole blood, a constant factor of 0.19, the nominal value of the extracellular fluid fraction of the marrow space, was used to relate the specific activity of the plasma to that of the red marrow. The S value used to compute the red marrow dose from activity in the remainder of the body was adjusted to account for nonuniformity of distribution in the body (29). Red marrow and total body masses for standard man (30) were used for all patients. Scaling of marrow activity based on estimates of the marrow volumes of individual patients was not included, since reciprocal scaling factors would have then been required for customizing the corresponding S values.

Dosimetry estimates for tumors include the self dose derived from gamma camera scintigraphy along with a total body contribution. For liver lesions, a liver background term to account for the hepatic activity outside the tumor was also included. Tumor masses in excess of 25 g were determined quantitatively from SPECT studies by means of a threshold technique. Smaller masses were estimated from computed tomography and gamma camera images. For the purpose of assigning S values for self dose, all tumors were assumed to be spherical (31). Tumor activities for at least three time points were derived from geometric means of background and attenuation-corrected count rates in regions of interest circumscribing the tumors on serial, planar scintigraphs (32). A monoexponential fit to these data points gave the tumor clearance rate. Since the peak tumor uptake is expected to have occurred relatively early (33), maximum tumor uptake was estimated by projecting the clearance curve to the time of infusion. Since the count rates on which tumor activities were calculated were corrected for background activity in the patient’s body, integration of the tumor clearance curve was assumed to represent cumulated activity for the tumor in excess of that due to background activity distributed uniformly in the total body. Consequently, the measured cumulated activity in the patient was regarded as that due to activity uniformly distributed in the total body plus that associated with excess activity in the tumor, and the value of S used for calculating the total body-to-tumor dose contribution was not adjusted for nonuniformity of the associated cumulated activity (29). Considering the insensitivity with respect to organ mass of the 131I values of S for total body dose...
to provide equal amounts of radioactivity from each antibody preparation.

**Antigen Expression.** Immunoperoxidase staining for assessment of reactivity with COL-1 and CC49 antibodies was performed on 5-μm sections of paraffin-embedded tissue samples, as described previously (19). Each specimen was scored for the percentage of positive cells and 0–4+ strength of reactivity for each of the antibodies. Fig. 1 presents this data in two forms. The percentage of positive-staining cells is presented in the lower portion of the figure (0–100% of cells), whereas a staining index is provided in the upper portion (0–400). The staining index is the product of the percentage of cells positive and the strengths of reactivity (0–4+). Thus, if 100% of cells were immunoperoxidase positive and strength of reactivity was 4+, the staining index would be 200. The serum CEA and TAG-72 levels were measured using standard kits (CEA, Hybritch; TAG-72, Centocor, Inc.).

**Pharmacokinetics.** Blood samples for pharmacokinetics were obtained preinfusion, immediately postinfusion, at 24, 48, and 72 h, and at 7 and 14 days postinfusion. Samples were allowed to clot and were then spun; the fluid was removed for quantitative assay of radioactivity and antibody content.

**HAMA.** Assays for HAMA were performed with the double antigen assay, as described previously (36) for blood samples pretreatment at 1, 2, 4, and 6 weeks posttreatment and at 6-week intervals subsequently. A positive assay was defined as a posttreatment binding value at least twice the pretreatment value and >12 ng/ml.

**IFN.** Human recombinant IFN α-2b (NSC 377523) was manufactured by Schering and supplied by the Division of Cancer Treatment, National Cancer Institute.

**Statistical Analysis.** Descriptive statistics were calculated for several variables, such as serum CEA and radioactivity half-lives. A one-compartment model was fitted to data to estimate half-life values (37). Pearson correlation coefficients were calculated to quantitate the linear relationship between clinical and pharmacokinetic parameters with toxicity. Spear-
man rank correlation coefficients were calculated to characterize the relationship between baseline serum CEA level and the plasma $T_{1/2}$ as well as toxicity (38).

**RESULTS**

Table 1 provides the demographic data on the 14 patients in this trial along with serum levels of circulating antigen (CEA and TAG-72). The age range was 35–76 years (median, 52.5), including 8 women and 6 men, with 2 of 14 previously untreated following surgical resection of the primary. Fig. 1 provides a display of these patients’ tumor specimen reactivity with CC49 and COL-1. COL-1 reactivity was greater than CC49, with 9 of 14 patients demonstrated to have 100% COL-1 reactive cells as compared to 4 of 14 patients with 100% CC49-reactive cells. The staining index for COL-1 was significantly higher than for CC49 ($P = 0.016$), with mean scores of 269 and 185, respectively. However, individual patients did have more CC49 than COL-1 reactivity (e.g., patient no. 11).

As regards toxicity, most patients had mild symptoms associated with IFN, commonly fever and malaise. Such effects were most common in conjunction with the initial treatment and subsided with repeat injections. IFN also produced transient reductions in leukocyte counts, including some grade 1–2 neutropenia (pretherapy values of 4,000–13,000/mm$^3$, changing to 2,300–7,300/mm$^3$ pre-3T112 antibody infusion), with return toward original values by week 2 of the protocol. This is prior to leucopenia secondary to the radioactivity that usually occurred in weeks 4–6. No acute allergic reactions were noted. Overall, six patients had nonserious nonhematological toxicity, including nausea/vomiting, weakness, and fatigue, and two patients experienced transient grade 2 arthralgias two weeks after therapy. Since some of the complaints of nausea were among patients who had similar ongoing complaints of at least an intermittent nature, their relation to the treatment and/or treatment-related agents such as SSKI remains questionable. In some instances, nausea resolved with discontinuation of the SSKI.

Fig. 2 provides the platelet and WBC toxicity grades for each patient. The dose selected was intended to produce at least grade 2 marrow suppression in the majority of patients. Two of 14 patients had less than grade 2 toxicity (patient nos. 8 and 10 had grade 1 neutropenia and grade 1 thrombocytopenia). Three of 14 patients had grade 4 thrombocytopenia with transient nadir values of 9,000, 14,000, and 12,000. All three received platelet transfusions and had no serious bleeding episodes. Two patients had grade 4 neutropenia and received granulocyte colony-stimulating factor at their nadir counts of 460 and 300. No serious infections occurred.

Table 2 provides pharmacokinetics and dosimetry data for the combined radiolabeled cocktail. The intended dose administered was close to projected (74 ± 5 mCi/m$^2$), with total mean dose of 145 ± 17 mCi. The pharmacokinetic values of the combination were quite similar to that seen with 3T112-labeled CC49 alone (15–17), with a $T_{1/2}$ of 48.8 h. Similarly, the whole-body and bone marrow dose (estimates of 98 ± 20 and 177 ± 68 cGy) were similar to prior studies with the same dose of 3T112-labeled CC49. Table 2 also provides the biological half-times of whole-body radioactivity for each patient, along with the calculated radiation doses to whole body and the red marrow.

We examined the correlation of pharmacokinetic and dosimetry estimates with the degree of marrow suppression, *i.e.*, grade of leucopenia, thrombocytopenia, or sum of the two grades. There was a significant correlation of plasma $\beta T_{1/2}$ with degree of leucopenia ($r = 0.54; P = 0.05$) and sum of toxicities ($r = 0.54; P = 0.04$), with borderline significance with thrombocytopenia ($r = 0.47; P = 0.09$). Estimates of whole-body dose did not significantly correlate, whereas bone marrow dose produced marginal correlations with leucopenia ($r = 0.48; P = 0.09$).

**Table 2** Radiolabeled antibodies, pharmacokinetics, and dosimetry estimates

<table>
<thead>
<tr>
<th>Patient</th>
<th>Patient dose$^a$</th>
<th>Dose/kg</th>
<th>Dose/m$^2$</th>
<th>$C_0$</th>
<th>$T_{1/2}$</th>
<th>AUC$^c$</th>
<th>$Cl'$</th>
<th>Body $T_{1/2}$</th>
<th>Body dose$^d$</th>
<th>Marrow dose$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>156.0</td>
<td>1.74</td>
<td>78.9</td>
<td>38.0</td>
<td>56.4</td>
<td>3092</td>
<td>0.56</td>
<td>58.3</td>
<td>130</td>
<td>234</td>
</tr>
<tr>
<td>2</td>
<td>142.9</td>
<td>2.28</td>
<td>81.7</td>
<td>45.2</td>
<td>54.4</td>
<td>3548</td>
<td>0.64</td>
<td>54.1</td>
<td>110</td>
<td>247</td>
</tr>
<tr>
<td>3</td>
<td>134.7</td>
<td>2.16</td>
<td>78.3</td>
<td>34.2</td>
<td>37.0</td>
<td>1729</td>
<td>1.25</td>
<td>42.1</td>
<td>81</td>
<td>143</td>
</tr>
<tr>
<td>4</td>
<td>136.4</td>
<td>2.04</td>
<td>76.2</td>
<td>16.7</td>
<td>51.2</td>
<td>1232</td>
<td>1.66</td>
<td>54.9</td>
<td>107</td>
<td>129</td>
</tr>
<tr>
<td>5</td>
<td>120.0</td>
<td>1.21</td>
<td>62.5</td>
<td>13.9</td>
<td>49.3</td>
<td>989</td>
<td>1.22</td>
<td>49.9</td>
<td>86</td>
<td>104</td>
</tr>
<tr>
<td>6</td>
<td>121.9</td>
<td>2.05</td>
<td>76.2</td>
<td>34.8</td>
<td>31.8</td>
<td>1597</td>
<td>1.28</td>
<td>43.7</td>
<td>76</td>
<td>115</td>
</tr>
<tr>
<td>7</td>
<td>124.9</td>
<td>2.05</td>
<td>73.9</td>
<td>22.7</td>
<td>36.7</td>
<td>1205</td>
<td>1.71</td>
<td>45.8</td>
<td>82</td>
<td>116</td>
</tr>
<tr>
<td>8</td>
<td>138.6</td>
<td>1.68</td>
<td>73.7</td>
<td>23.9</td>
<td>35.5</td>
<td>1228</td>
<td>1.36</td>
<td>39.3</td>
<td>78</td>
<td>115</td>
</tr>
<tr>
<td>9</td>
<td>166.4</td>
<td>1.46</td>
<td>74.0</td>
<td>31.1</td>
<td>36.9</td>
<td>1658</td>
<td>0.88</td>
<td>37.5</td>
<td>89</td>
<td>145</td>
</tr>
<tr>
<td>10</td>
<td>172.8</td>
<td>1.59</td>
<td>73.5</td>
<td>27.3</td>
<td>50.1</td>
<td>1973</td>
<td>0.80</td>
<td>45.3</td>
<td>112</td>
<td>170</td>
</tr>
<tr>
<td>11</td>
<td>144.3</td>
<td>1.60</td>
<td>71.4</td>
<td>35.3</td>
<td>64.7</td>
<td>3296</td>
<td>0.49</td>
<td>51.1</td>
<td>105</td>
<td>224</td>
</tr>
<tr>
<td>12</td>
<td>142.4</td>
<td>1.78</td>
<td>74.9</td>
<td>40.7</td>
<td>44.5</td>
<td>2616</td>
<td>0.68</td>
<td>44.0</td>
<td>90</td>
<td>192</td>
</tr>
<tr>
<td>13</td>
<td>160.0</td>
<td>1.48</td>
<td>68.1</td>
<td>31.4</td>
<td>54.5</td>
<td>2468</td>
<td>0.60</td>
<td>36.4</td>
<td>83</td>
<td>175</td>
</tr>
<tr>
<td>14</td>
<td>168.2</td>
<td>1.82</td>
<td>76.8</td>
<td>50.2</td>
<td>79.6</td>
<td>5773</td>
<td>0.31</td>
<td>58.7</td>
<td>141</td>
<td>349</td>
</tr>
<tr>
<td>$x$</td>
<td>145.0</td>
<td>1.78</td>
<td>74.3</td>
<td>31.7</td>
<td>48.8</td>
<td>2315</td>
<td>0.96</td>
<td>47.2</td>
<td>98</td>
<td>177</td>
</tr>
<tr>
<td>$\pm SD$</td>
<td>17.3</td>
<td>0.31</td>
<td>4.7</td>
<td>10.7</td>
<td>13.1</td>
<td>1298</td>
<td>0.45</td>
<td>7.4</td>
<td>20.2</td>
<td>67.6</td>
</tr>
</tbody>
</table>

$^a$ Patient dose, administered dose in mCi; Dose/kg and Dose/m$^2$ (mCi).
$^b$ $C_0$, initial serum concentration of radioactivity (µCi/ml).
$^c$ $T_{1/2}$, serum half-life of radioactivity (hours).
$^d$ AUC, area-under-the-curve of serum radioactivity (h µCi/ml).
$^e$ $Cl'$, clearance rate (ml/h/kg).
$^f$ Body $T_{1/2}$, whole-body radioactivity half-life (hours).
$^g$ Calculated doses in cGy.
Table 3  Radioimmunodetection of metastatic lesions

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Imaging Known disease</th>
<th>Sites</th>
<th>Grade</th>
<th>Sites</th>
<th>Size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liver, multiple</td>
<td>Lf. liver</td>
<td>2</td>
<td>2.2 × 2.0, 2.8 × 2.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Liver, multiple</td>
<td>Rt. liver anterior</td>
<td>4</td>
<td>2.0 × 2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver dome</td>
<td></td>
<td>2.2 × 2.5</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Liver, multiple</td>
<td>Lf. liver</td>
<td>4</td>
<td>2.0 × 2.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Liver</td>
<td>Rt. liver</td>
<td>4</td>
<td>4.8 × 5.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Liver, multiple</td>
<td>Rt. liver</td>
<td>4</td>
<td>2.5 × 3.4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Lf. lung</td>
<td>Lf. lung</td>
<td>4</td>
<td>5.0 × 6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rt. lung</td>
<td></td>
<td>&lt;2.0</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Liver</td>
<td>Liver dome</td>
<td>3</td>
<td>5.5 × 4.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lf. liver, lateral</td>
<td>4</td>
<td>5.0 × 4.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Liver, multiple</td>
<td>Rt. liver</td>
<td>4</td>
<td>5.0 × 5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver dome</td>
<td></td>
<td>3.5 × 2.2</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Porta</td>
<td>Liver dome</td>
<td></td>
<td>10.0 × 6.0</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Liver, multiple</td>
<td>Lf. lung</td>
<td>4</td>
<td>5.0 × 5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rt. lung</td>
<td></td>
<td>3.5 × 3.0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Abdominal wall</td>
<td>Abdominal wall</td>
<td>4</td>
<td>15.0 × 9.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Renal</td>
<td></td>
<td>5.0 × 5.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rt. inguinal nodes</td>
<td>4</td>
<td>4.0 × 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gastrohepatic ligament mass</td>
<td>4</td>
<td>3.0 × 3.0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Liver, multiple</td>
<td>Rt. liver</td>
<td>4</td>
<td>5.0 × 4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posterior liver</td>
<td></td>
<td>7.5 × 6.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lateral liver</td>
<td></td>
<td>5.0 × 6.0</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Liver</td>
<td>Liver, caudate</td>
<td>4</td>
<td>4.0 × 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung, rt. lower</td>
<td>4</td>
<td>2.0 × 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lung, left upper</td>
<td>4</td>
<td>No disease known</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Abdomen</td>
<td>LLQ mass</td>
<td>4</td>
<td>2.8 × 3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mesenteric mass</td>
<td>4</td>
<td>3.5 × 3.0</td>
<td></td>
</tr>
</tbody>
</table>

*Lf., left; Rt., right; PA, para-aortic; RLQ, right lower quadrant; LLQ, left lower quadrant.

As listed in Table 3, 11 of the 14 patients had liver metastases, 4 patients had extrahepatic intraabdominal metastasis, and five patients had lung metastases in addition to intraabdominal disease. All patients had positive localization of radioimmunoconjugate to at least one known site of disease. Generally, the localization was judged to be strong, with 13 of 14 patients having a +4 scored lesion, which was the highest grade of positive imaging. Thirty-two of 33 known lesions of at least 2 cm diameter were detected, as well as smaller metastases in two patients. An occult lung lesion may have been detected in one patient (no. 13), but this was not confirmed by biopsy.

Table 4 provides estimated tumor doses for six patients with quantifiable tumor characteristics that varied from 393 to 1327 cGy. One-half of the tumor sites were liver metastases, and the other sites included two nonliver abdominal masses and one case of lung metastasis.

As shown in Table 1, the pretreatment serum CEA levels ranged from 3.8 to 13,323 ng/ml, whereas TAG-72 levels varied from <3.0 to 83.9 ng/ml. The correlation of CEA level with pharmacokinetic parameters showed a trend toward a correlation between the baseline CEA and the rate of blood clearance of radiolabeled antibody that was not statistically significant. The correlation coefficients between CEA and grades of leukopenia, thrombocytopenia, and total marrow toxicity were −0.40 (P = 0.157), −0.61 (P = 0.021), and −0.56 (P = 0.038), respectively. The three patients who had CEA levels of >3000 ng/ml were among the four patients with the least toxicity as measured by grade of thrombocytopenia. When the 10 patients with CEA of <100 were compared to the four patients with higher levels using the t test, significantly greater platelet tox-
Table 5  Days of peak human anti-COL-1 and anti-CC49 immune responsea

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Day</th>
<th>Anti-COL-1</th>
<th>Anti-CC49</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>245</td>
<td>932</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>672</td>
<td>1927</td>
</tr>
<tr>
<td>3</td>
<td>14</td>
<td>1338</td>
<td>4018</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>27</td>
<td>35</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>413</td>
<td>221</td>
</tr>
<tr>
<td>7</td>
<td>41</td>
<td>165</td>
<td>245</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>6727</td>
<td>9127</td>
</tr>
<tr>
<td>Week 6</td>
<td>14</td>
<td>10770</td>
<td>1965</td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>5217</td>
<td>1195</td>
</tr>
<tr>
<td>10</td>
<td>73</td>
<td>2595</td>
<td>3125</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>812</td>
<td>1438</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>2812</td>
<td>175</td>
</tr>
<tr>
<td>13</td>
<td>28</td>
<td>3389</td>
<td>5466</td>
</tr>
<tr>
<td>14</td>
<td>28</td>
<td>3557</td>
<td>3249</td>
</tr>
<tr>
<td>15</td>
<td>12</td>
<td>10113</td>
<td>3590</td>
</tr>
<tr>
<td>16</td>
<td>42</td>
<td>8139</td>
<td>5317</td>
</tr>
<tr>
<td>17</td>
<td>42</td>
<td>9747</td>
<td>6633</td>
</tr>
<tr>
<td>18</td>
<td>36</td>
<td>12931</td>
<td>10366</td>
</tr>
</tbody>
</table>

a Only the day of peak response (ng/ml sera) against COL-1 or CC49 is listed. Values $\geq 60$ ng/ml are positive values.

Discussion

Human tumor xenografts in nude mice can be cured by radioimmunotherapy, whereas similar lesions in human patients are more difficult to control. Due to a number of differences between the murine model and the actual human patient, greater concentrations of the radioimmunoconjugate more readily accumulate to deliver higher radiation doses to the mouse xenografts. In an effort to improve antibody delivery of $^{131}$I and provide higher tumor dose, we combined two complementary antibodies and used them in conjunction with IFN, a biological response modifier known to increase tumor antigen expression.

Prior reports of combinations of complementary antibodies have mainly involved in vitro and animal model studies. The few human trials have involved imaging or detection of radiolocalization rather than therapeutic intent (39, 40). In general, these studies have suggested a beneficial effect of combining antibodies. In this trial, we noted that 13 of 14 patients had strongly positive radioimaging of at least one lesion, and 32 of 33 lesions of $>2$ cm had positive localization. This is a stronger degree of radiolocalization than reported in prior trials with single antibody infusions with CC49 or COL-1 from our institution (15) as well as others (16, 22). With the high level of reactivity of CC49 and COL-1 in the biopsy specimens tested from these patients, strong radiolocalization would be expected based on the recent study of Buist et al. (41), which showed a correlation between localization and immunoperoxidase-detected antigen expression. In addition, the IFN administration would be expected to enhance antigen density further, as reported by others (11, 13, 14).

Analysis of tumor radiation dosimetry provides another parameter of radiolabeled antibody delivery to tumor. The prior Phase II trial of 75 mCi/m² of $^{131}$I-labeled CC49 in metastatic colorectal cancer reported radiation doses to liver metastases of 164 to 337 cGy and extrahepatic tumor sites of 423 to 667 cGy (16). The range of doses reported in the current report were 592 to 745 cGy and 393 to 1327 cGy, respectively. Direct analyses of tumor tissues suggest that these calculated values are likely to underestimate the dose delivered as well as the therapeutic index (22). We recognize that a definitive conclusion regarding these comparative issues would require direct comparison in the same protocol, but we believe that the observations are encouraging and warrant further study. Unfortunately, the radiation delivery is still below doses that induce tumor regression. A higher dose of unlabeled COL-1 prior to the radiolabeled dose might have bound circulating CEA to allow a greater concentration in tumors. However, our results suggest the effect would be relatively small.

To estimate the impact of this strategy (IFN/dual antibody) on marrow toxicity, we have compared this trial with prior reports using the same radioactive dose (75 mCi/m²) conjugated to murine CC49 in trials without IFN administration in patients with colon (16) or prostate cancer (15). As seen in Fig. 3, the current trial using dual antibodies and IFN produced marrow suppression that was not significantly different from the two single antibody trials and further suggests that administration of radiolabeled antibodies based on maximum tolerated doses established in Phase I trials can produce a consistent pattern of marrow suppression similar to that seen with other marrow-suppressive strategies (e.g., chemotherapy). Thus, IFN did not produce a substantial change in marrow toxicity, as reported previously by Murray et al. (14). The trial reported by Murray et al. used a 14-day course of IFN in patients with extensive prior chemotherapy for their metastatic breast cancer. They also reported that IFN prolonged the plasma half-life of the radiolabeled CC49. This was not the case in our trial where a plasma half-life of the cocktail was 48.8 ± 13.1 h, as compared to prior trials of $^{131}$I-labeled CC49 with $T_1/2$ of 45.4 ± 11.0 h in metastatic colon patients (17) and 50.1 ± 3.8 h in metastatic prostate cancer patients (15) or $^{131}$I-COL-1 trials in metastatic colon cancer patients with a plasma $T_1/2$ of 45.7 ± 16.2 h (22). The prior report of enhanced marrow toxicity with IFN probably reflects the heavily treated patient population and possibly prolonged IFN administration schedule used (14). Although some anti-CEA antibodies cross-react with an antigen expressed on granulocytes, COL-1 is not among them and is free of binding.
effects were seen, reflecting the radiation resistance of colon 
of IFN and dual antibody strategies, no objective antitumor 
in this trial, the combined effects were modest. Despite the use 
versus 
from IFN 

to most normal tissues (1). Thus, we do not anticipate that the 
level of neutropenia was influenced by specific antibody local-
ization to blood or marrow components.

The Phase I trial of Yu et al. (22) using $^{131}$I-labeled COL-1 
reported that patients with CEA levels greater than >500 had 
more rapid plasma clearance of radiolabeled antibody and less 
marrow toxicity. In our trial, less impressive correlates were 
found, presumably reflecting that kinetics and marrow suppres-
sion were less likely to be affected, given that one-half of 
the antibody molecules were not reactive with CEA, i.e., anti-
TAG-72.

Finally, this trial used a fixed dose of radiolabeled antibody 
based on body surface area determined by prior Phase I trials 
with each antibody (17, 22). The marrow toxicity seen varied 
from modest (total toxicity grades of 1–2) to large (total toxicity 
grades of 7–8) in a relatively homogeneous group of patients. 
This provided an opportunity to correlate standard pharmacoki-
etic and dosimetry parameters with marrow suppression in 
individual patients. There was a moderate level of correlation 
(Table 3) between the pharmacokinetic parameters of plasma 
half-life and area-under-the-curve with the degree of marrow 
suppression, supporting the obvious conclusion that the longer 
the antibody circulates, the greater the radiation exposure of the 
marrow. However, the estimates of whole-body and marrow 
radiation dose exposures provided lower correlation coefficients 
that were generally not statistically significant. This probably 
reflects the ability to accurately measure plasma kinetic param-
eters and the more variable nature of dose estimates. Analysis of 
these correlates in additional trials will allow further clarifica-
tion of these observations. However, the simple use of maxi-

mum tolerated doses determined by Phase I trials appears to 
produce reliable and safe degrees of marrow suppression in 
solid tumor patients.

Although the potentiating effects for antibody localization 
from IFN versus that of mixing antibodies cannot be separated 
in this trial, the combined effects were modest. Despite the use 
of IFN and dual antibody strategies, no objective antitumor 
effects were seen, reflecting the radiation resistance of colon 
cancer and limited doses delivered to tumor sites. Further strat-
egies will need to be explored to enhance antitumor effects.

ACKNOWLEDGMENTS

We thank Pamela Dixon and Carol Daniel for research nursing 
care, Gayle Elliott and Rob Williams for technical assistance, Michael 
Markiewicz for pharmaceutical services, and Sharon Garrison for manu-
script preparation.

REFERENCES

1. Muraro, R., Wunderlich, D., Thor, A., Lundy, J., Noguchi, P., 
Cunningham, R., and Schlom, J. Definition by monoclonal antibodies of 
a repertoire of epitopes on carcinoembryonic antigen differentially ex-
pressed in human colon carcinomas versus normal adult tissues. Cancer 
2. Colcher, D., Hand, P. H., Nati, M., and Schlom, J. A spectrum of 
monoclonal antibodies reactive with human mammary tumor cells. Proc. 
3. Johnson, V. G., Schlom, J., Paterson, A. J., Bennett, J., Magnani, 
J. L., and Colcher, D. Analysis of a human tumor-associated glycopro-
tein (TAG-72) identified by monoclonal antibody B72.3. Cancer Res., 
expression of carcinoembryonic antigen and tumor associated glycopro-
tein-72 (TAG-72) in human colon adenocarcinomas. Intl. J. Biol. Mark-
5. Blumenthal, R. D., Kashi, R., Stephens, R., Sharkey, R. M., and 
Goldenberg, D. M. Improved radioimmunotherapy of colorectal cancer 
xenografts using antibody mixtures against carcinoembryonic antigen and 
M. L., and Dedrick, R. L. Predicted and observed effects of antibody 
affinity and antigen density on monoclonal antibody uptake in solid 
D., Smalley, R., Simpson, J. F., Borden, E. C., Pestka, S., and Greiner, 
J. W. Selective interferon-induced enhancement of tumor-associated 
antigens on a spectrum of freshly isolated human adenocarcinoma cells. 
8. Greiner, J. W., Hand, P. H., Noguchi, P., Fisher, P. B., Pestka, S., and 
Schlom, J. Enhanced expression of surface tumor-associated antigens on 
the antibody with IFN a-2b. Each column represents the 
sum of WBC and platelet toxicity grade for an individual patient. The first trial 
(Colon) represents 14 patients with metastatic colon cancer treated with $^{131}$I-

labeled CC49 alone (16). The third trial 
(Prostate) represents 14 patients with metastatic prostate cancer treated with 
$^{131}$I-labeled CC49 (15). The second trial is the current report of $^{131}$I-CC49 and 
COL-1 with IFN (Colon + Interferon).

Fig. 3  The total marrow toxicity grades 
are compared for three trials of 75 mCi/ 
$m^2$ $^{131}$I-labeled monoclonal antibodies 
with and without human recombinant 
IFN α-2b. Each column represents the 
sum of WBC and platelet toxicity grade 
for an individual patient. The first trial 
(Colon) represents 14 patients with met-
astatic colon cancer treated with $^{131}$I-
labeled CC49 alone (16). The third trial 
(Prostate) represents 14 patients with met-
astatic prostate cancer treated with 
$^{131}$I-labeled CC49 (15). The second trial is the current report of $^{131}$I-CC49 and 
COL-1 with IFN (Colon + Interferon).


Phase II study of dual 131I-labeled monoclonal antibody therapy with interferon in patients with metastatic colorectal cancer.

R F Meredith, M B Khazaeli, W E Plott, et al.


Updated version

Access the most recent version of this article at:
[http://clincancerres.aacrjournals.org/content/2/11/1811](http://clincancerres.aacrjournals.org/content/2/11/1811)

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.