Comparison of Equitoxic Radioimmunotherapy and Chemotherapy in the Treatment of Human Colonic Cancer Xenografts

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ABSTRACT

The therapeutic efficacy of 5-fluorouracil (5-FUra; 0.6 mg/day × 5 days) + leucovorin (LV; 1.8 mg/day × 5 days) and of 131I-labeled MN-14 anti-carcinoembryonic antigen IgG (275 μCi single dose) was evaluated in size-matched (0.3–0.7 cm³) s.c. LoVo, HT-29, DLD-1, HCT-15, LS174T, and MOSER, GW-39, and WidR human colonic tumors. These lines express varying amounts of carcinoembryonic antigen and exhibit varying degrees of in vitro responsiveness to 5-FUra. Unlike radioimmunotherapy (RAIT), multiple cycles of chemotherapy were feasible over a 3-week period. However, no therapeutic advantage to a second cycle of 5-FUra/LV administration was found. Therefore, it is reasonable to compare single cycles of both treatment modalities. RAIT was statistically more effective in 5 of 8 tumor lines (LoVo, LS174T, MOSER, WidR, and GW-39). In 1 other line (DLD-1), RAIT was marginally more efficacious, but tumors responded well to both therapies. The lack of a statistical difference between the 2 modalities of treatment may indicate that the efficacy of the 2 treatments is equivalent, or the relatively large variability within the treatment groups may have prevented significance given the number of animals evaluated. RAIT and 5-FUra/LV were equally efficacious in the HT-29 and the HCT-15 tumor lines. Of the 5 xenografts that responded better to RAIT, 3 lines (LS174T, GW-39, and WidR) demonstrated a greater percentage of tumors responding over a 5- to 6-week period. The other 3 lines (LoVo, MOSER, and DLD-1) exhibited a similar percent of tumors responding to both therapies, but a greater growth inhibition in those RAIT-treated tumors that responded. In vivo responsiveness to 5-FUra/LV did not directly correlate with in vitro responsiveness (r² = 0.664), since LS174T and LoVo tumors, with rapid growth rates (0.05–0.36 cm³/day), were not highly responsive to therapy. Growth inhibition from RAIT also did not correlate with total tumor carcinoembryonic antigen content (r² = 0.003), an observation that may be due to additional variables, such as accessibility of antigen and innate radiosensitivity of the tumor. RAIT was most effective in the fastest growing tumor lines (LS174T, GW-39, MOSER, WidR, and LoVo). These preclinical results suggest an advantage to radioimmunotherapy over one of the most commonly used forms of chemotherapy to treat colorectal cancer. These studies also highlight the need to establish criteria that will enable the selection of therapeutic modalities in patients.

INTRODUCTION

Colorectal cancer is the third most common cancer in the United States (1). The fluorinated pyrimidine, 5-FUra, is the antitumor agent of choice for single-agent therapy of advanced colon cancer or in an adjuvant setting (2). The drug is cytotoxic as a result of its ability to be metabolized to fluorodeoxyuridine monophosphate, which can decrease thymidine production by irreversibly blocking thymidylate synthase, or by being activated to fluorouridine triphosphate and incorporated into RNA. To date, 5-FUra has served as the standard reference for potential alternative colorectal cancer therapeutics. However, the overall response rate to 5-FUra is <25%, and has not significantly improved patient survival (3). The antitumor activity can be potentiated by modulating the metabolism of this drug with reduced folates (LV). LV acts by increasing and prolonging the inhibition of the target enzyme, thymidylate synthase, by forming a stable ternary complex between the enzyme, the folate coenzyme, and the fluoropyrimidine inhibitor (4). Phase III clinical trials have shown that 5-FUra/LV provides a significantly higher response rate than 5-FUra alone, and in some trials of Dukes’ C colonic cancer patients median survival was increased by 3–4 months (5–7). Even under these improved conditions, the response rate is only 35% and median survival is 14 months. Therefore, new therapeutic approaches are needed to treat this type of cancer. Wessels et al. (8) have reviewed several aspects of cancer therapy and have concluded that colorectal cancer is a prime candidate for antibody-directed radiotherapy.

An antibody’s ability to target tumors in vivo has been well documented (9, 10), and there are many reports demonstrating RAIT’s ability to control tumor growth in preclinical studies (11–14). Radioimmunotherapy is now being viewed as a promising adjuvant approach for small primary sites or for treating micrometastatic disease (15, 16). In our experience with experimental radioimmunotherapy (17), minimal tumor burden can be treated with a single course of RAIT. As tumor burden increases, surviving populations arise from single treatments of RAIT. In clinical studies, Press et al. (18) demonstrated complete remission in 4 patients for 4–7 months with 131I-labeled MB-1 (delivered dose = 850–4260 CGy to the tumor site). Another pan B-cell antibody, LYM-1, has been utilized by DeNardo et al. (19) initially in 5 patients and then in 18 patients. In the first study, 3 of 5 patients exhibited objective responses and in the second study, 2 complete responses and 7 partial responses (>70% tumor reduction) were observed, lasting greater than 6 months. In another preliminary clinical study, 9 patients (7 with advanced disease) with biopsy-proven non-Hodgkin’s lymphoma were injected with monoclonal antibody LL2 radiolabeled with 131I for therapeutic purposes. All patients had been heavily pretreated with chemotherapy and/or radiation therapy and were refractory to further therapy. Three clinical antitumor responses were seen using low protein and radionuclide dose (from 6.2 to 58 mCi of 131I on 0.2 to 3.8 mg intact IgG MAb). Two of 4 patients given injections of approximately 50 mCi for therapeutic intent have had partial remissions. In addition, one patient given 2 injections of only 6 mCi of 131I had biopsy-proven durable partial responses for 1 year. The patient has subsequently been treated with 10 mCi of 131I (0.6 mg IgG), and a complete response was achieved (20). With more energetic radionuclides (e.g., Re-188 and Y-90), humanized antibodies, and the potential ability to reduce host toxicity and thereby dose-escalate the tolerated dose of radioimmunotherapy, the prospects for improved therapy with RAIT are promising (9, 21).

The relative efficacy of each modality of treatment in the same model system has only recently been addressed in an experimental adjuvant setting using lung micrometastases of the GW-39 colonic carcinoma xenograft (16). In that study, RAIT was shown to be more efficacious at prolonging survival than 5-FUra/LV. In the current studies, we are expanding on this observation by evaluating the therapeutic potential of 5-FUra/LV and RAIT in size-matched xenografts.

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3 The abbreviations used are: 5-FUra, 5-fluorouracil; CEA, carcinoembryonic antigen; GI, growth inhibition; LV, leucovorin; MAb, monoclonal antibody; MTD, maximal tolerated dose; RAIT, radioimmunotherapy; IFN, interferon; NS, not significant.

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grown s.c. in nude mice. The predictive validity of this animal model has been shown for assessing the antitumor activity of various chemotherapeutic agents and for screening the specificity of radiolabeled antibodies, evaluating dosimetry and toxicity, and optimizing treatment regimens (22, 23). We have selected 8 colonic tumor lines of differing histopathology, growth kinetics, CEA content, and in vitro responsiveness to 5-FUra for these studies. Our results demonstrate a greater therapeutic response to RAIT in 5 of 8 lines, a marginal improvement in growth-inhibitory effects by RAIT in 1 of 8 lines, and no statistical significance between the effect of RAIT and 5-FUra/LV in 2 of 8 lines.

**Materials and Methods**

**Radioantibody Synthesis and Quality Assurance.** The MN-14 anti-CEA intact MAB (24) was purified from mouse ascites using protein A and ion-exchange chromatography over S-Sepharose (Pharmacia, Piscataway, NJ). 131I-labeled MN-14 (specific activity of 10–15 μCi/μg) was prepared by the chloramine-T method (25). Free radiiodine was separated from antibody-bound iodine by passage over a PD-10 column (Pharmacia) equilibrated with 0.04 M phosphate-buffered saline (0.04 M phosphate, 0.15 M NaCl, 0.02% NaN3), pH 7.4, containing 1% human serum albumin. Routine quality assurance of radiolabeled antibody revealed no detectable aggregates and 2–4% free radiiodine by size exclusion high-performance liquid chromatography using a Zorbax GF-250 (DuPont, Wilmington, DE) column; and 75–85% of the radiolabeled anti-CEA antibody bound to a CEA-Affigel immunoabsorbent.

**Human Tumor Xenograft Models.** The following serially propagated human colonic xenografts were used for these studies: (a) LoVo; (b) HT-29; (c) DLD-1; (d) HCT-15; (e) LS174T; (f) MOSER; (g) WidR; and (h) GW-39. Lines 1–7 are available from the American Type Culture Collection (Rockville, MD). Table 1 summarizes the histopathology, in vivo growth rate, in vitro sensitivity to 5-FUra, and in vitro CEA levels for all tumor lines. For in vivo studies, tumors were first established by s.c. injection of 105 cells s.c. Tumors were serially propagated by passage through a 40-mesh screen, and rinsed with 0.9% sterile NaCl to yield the desired cell suspension. Nude mouse s.c. tumors were initiated with 0.2 ml of a 20% suspension into 6–8-week-old female nu/nu mice (Harlan Sprague Dawley, Indianapolis, IN). All studies were done on 0–3.7-g tumors.

**Tumor Therapy.** Tumor size was determined by caliper measurement in 3 dimensions immediately before therapy and at weekly intervals thereafter. Tumors were either left untreated, treated with a single dose of 275 μCi 131I-labeled MN-14, or treated with a fractionated schedule of 5-FUra (0.6 mg/day/mouse × 5 days i.v.) + a 2-h pretreatment with LV (1.8 mg/day/mouse × 5 days i.p.). The MTD for each radiolabeled antibody, the highest possible dose resulting in no animal deaths, was determined empirically in tumor-bearing animals. For RAIT, the μCi administered was increased by 25 μCi increments from 0.4 to 0.1 μCi/day/mouse by 0.1-mg/day increments. A dose of 0.6 mg/day/mouse 5-FUra × 5 days was the highest possible dose resulting in no deaths. If 0.7 mg/day/mouse was administered, 80% of mice died. For multiple cycle chemotherapy, a 3-week spacing between the dosing permitted both doses to be administered without additional toxicity/lethality. Tumor size was monitored by caliper measurement in 3 dimensions on the day that radio-antibody was administered. Subsequent measurements of tumor size were compared to the initial measurement, and the average change in size was recorded over time. Data from an individual tumor line were aggregated for the growth curve analysis. Within a tumor line, the tumor growth pattern of any one of the 3 comparison groups, namely, control, RAIT, and 5-FUra/LV groups, was either linear or exponential. When the growth pattern was linear, linear regression was used for the statistical analysis. When the growth pattern was exponential, nonlinear regression based on asymptotic approximation was used in the analysis. In comparing a linear growth pattern with an exponential one, area under the curve was the endpoint of comparison rather than the slope, which is not constant over time in exponential growth. In comparing 2 linear growth patterns, the slope was the endpoint of comparison provided the intercepts were not significantly different in the 2 groups; otherwise, area under the curve was the appropriate endpoint. “Substantial Tumor Growth Inhibition” is defined as the percent of tumors exhibiting less than a 2.5-fold increase in size. Growth inhibition (GI) is defined as follows:

$$GI = \frac{(growth\ untreated\ tumor\ group - growth\ treated\ tumor\ group)}{(growth\ untreated\ tumor\ group)} \times 100$$

**In Vivo Chemosensitivity.** Cells were grown in RPMI 1640 (Gibco) with 10% fetal calf serum, 1% penicillin-streptomycin, 1% glucose, 1% nonessential amino acids, and 1% sodium pyruvate. Cell harvesting was done with 1× trypsin (no EDTA), and the cells were dispensed at a concentration of 30,000/μl into 96-well polyurethane microtiter plates. After a 4-h incubation at 37°C, 5-FUra was added at various concentrations (0.01–4.0 μg/ml) in RPMI in triplicate with 50 mg/ml. After a 24-h incubation, the cells were treated with 0.1 μCi/well of 14C-labeled formic acid (Amersham) and incubated for another 16 h. The cells were harvested and washed using a semiautomatic cell harvester (Skatron, Sterling, VA), and the radioactivity incorporated by the cells was counted in Ecolume scintillant (ICN Biomedical). Cytoxicity in 5-FUra-treated wells is expressed as the percent inhibition of 14C incorporation by comparison to cells treated with culture media alone or cells treated with 0.5 μM cycloheximide (cx). The cx group represents a positive cytotoxic agent and the associated counts found in these cells are nonspecific. The following formula is used to calculate inhibition (INH) of 14C uptake:

$$INH = 1 - \frac{(cpm_{cx} - cpm_{cx})}{(cpm_{control} - cpm_{cx})} \times 100$$

The ID50 was determined from linear regression analysis between the percent INH and the dose of 5-FUra.

**CEA Quantitation.** Cells (106) were sonicated for 15 s on ice, and CEA was extracted by incubation with 0.1 IU/ml phosphatidylinositol-specific phospholipase C for 30 min at 37°C (26). Fragmented cells were pelleted with a 40,000 × g centrifugation for 30 min, and CEA content was measured in diluted samples of the supernatant. CEA quantitation was performed using a “sandwich”-type enzyme immunoassay involving two monoclonal antibodies against CEA (27). The microtiter plate was sensitized with the first monoclonal antibody (NP-1). Dilutions of sample or CEA standard (Ciba) were added and incubated for 90 min at 37°C, and then peroxidase-conjugated NP-3 antibody was added, followed by substrate prepared from o-phenylenediamine. After 30 min, the reaction was stopped with H2SO4 and the absorbance read at 490 nm. Each plate contained 2 blank wells (buffer only) and 2 background wells (all additions except antigen). The assay was linear in the range of 2.2 to 58 ng/ml.

**Results**

A total of 8 human colon cancer xenografts were used to assess therapeutic efficacy following a single dose of RAIT or a single cycle of 5-FUra/LV. The growth of individual tumors is depicted in Figs. 1–8, and the growth rate of treated tumors is summarized in Table 2, so that variability in responsiveness between tumors given the same treatment can be readily appreciated. Fig. 1 shows results for the LoVo xenograft, a rapidly dividing, well-differentiated tumor that has 610 ng CEA/105 cells and an ID50 of 0.285 μg/ml to 5-FUra. RAIT decreased the growth rate between days 0 and 28 from 0.194 ± 0.1511 (SD) cm3/day to 0.019 ± 0.004 cm3/day, while the growth rate of 5-FUra/LV-treated tumors was 0.068 ± 0.011 cm3/day (P < 0.001 between treatment groups; P < 0.001 between untreated and RAIT treated tumors). Table 1 summarizes the histopathology, growth rate, CEA content, and in vitro responsiveness to 5-FUra for these studies. Our results demonstrate a greater therapeutic response to RAIT in 5 of 8 lines, a marginal improvement in growth-inhibitory effects by RAIT in 1 of 8 lines, and no statistical significance between the effect of RAIT and 5-FUra/LV in 2 of 8 lines.
Table 2 Growth rate in response to therapy

<table>
<thead>
<tr>
<th>Tumor Line</th>
<th>RAFT</th>
<th>5FU/LV</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LoVo</td>
<td>0.019 ± 0.004 (14)²⁻</td>
<td>0.068 ± 0.011 (10)</td>
<td>P &lt; 0.001 (A)²⁻</td>
</tr>
<tr>
<td>HT-29</td>
<td>0.044 ± 0.006 (9)</td>
<td>0.033 ± 0.005 (9)</td>
<td>P = NS (A)</td>
</tr>
<tr>
<td>DLD-1</td>
<td>0.031 ± 0.013 (8)²⁻</td>
<td>0.047 ± 0.016 (8)²⁻</td>
<td>P = NS (A)</td>
</tr>
<tr>
<td>HCT-15</td>
<td>0.039 ± 0.014 (5)²⁻</td>
<td>0.033 ± 0.005 (7)</td>
<td>P = NS</td>
</tr>
<tr>
<td>LS174T</td>
<td>0.018 ± 0.007 (8)</td>
<td>0.230 ± 0.030 (10)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>MOSER</td>
<td>0.038 ± 0.014 (9)²⁻</td>
<td>0.082 ± 0.011 (15)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>WiDr</td>
<td>0.022 ± 0.003 (6)</td>
<td>0.056 ± 0.007 (6)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>GW-39</td>
<td>0.015 ± 0.003 (13)</td>
<td>0.061 ± 0.011 (6)</td>
<td>P &lt; 0.001 (A)</td>
</tr>
</tbody>
</table>

² Mean ± SD (Number of tumors evaluated).
²⁻ (A), area under the growth curve considered.
²⁻ Log of tumor growth considered.
²⁻ Growth rate not significantly different from control.

Fig. 1. Growth curves for individual LoVo tumors grown s.c. in nude mice. All tumors were 0.3—0.7 cm³ at the start of the study. Animals in the radioimmunotherapy group were treated with a single dose of 275 μCi 131I-labeled MN-14 anti-CEA on day 0. Animals in the chemotherapy group were treated with leucovorin i.p. (1.8 mg/day/mouse × 5 days) 2 h prior to 5-FUra, which was administered i.v. (0.6 mg/day/mouse × 5 days). Caliper measurements of tumor size were made on day 0 and at weekly intervals thereafter. The change in tumor size is recorded during an 8-week period postinitiation of therapy. If tumors grew beyond 4.0 cm³ or became necrotic at a smaller size, the animals were sacrificed.

Groups; P < 0.02 between untreated and 5-FUra/LV groups). There was some variability in the growth of the individual tumors of the untreated and the 5-FUra/LV-treated groups, which could not be explained by the differences in the initial size of the tumor at the start of the study (range, 0.3—0.7 cm³). However, the variability in the RAFT-treated group was minimal. RAFT-treated tumors increased an average of 2.8-fold in size over 5 weeks, while 5-FUra-treated tumors increased an average of 10.9-fold and untreated tumors increased an average of 21.4-fold over the same 5 weeks.

Fig. 2 presents results for the HT-29 xenograft, a tumor with a moderate growth rate, well-differentiated character that has 19.1 ng CEA/10⁷ cells, and an ID₅₀ of 0.260 μg/ml to 5-FUra. RAFT decreased the growth rate between days 0 and 35 from 0.118 ± 0.048 cm³/day to 0.044 ± 0.006 cm³/day, and 5-FUra/LV decreased tumor growth rate to 0.033 ± 0.005 cm³/day (P = NS between the 2 treatment groups; P < 0.01 between untreated and RAFT group; P < 0.01 between untreated and 5-FUra/LV group). There was some variability in the growth of the individual tumors in all 3 groups. RAFT-treated tumors increased an average of 3.1-fold in size over 5 weeks, while 5-FUra-treated tumors increased an average of 6.5-fold and untreated tumors increased an average of 7.7-fold over the same 5 weeks.

Growth curves for the DLD-1 xenograft, a poorly differentiated tumor with a slow growth rate, which produces very small amounts of CEA (0.11 ng/10⁶ cells) and exhibits moderate responsiveness to 5-FUra (ID₅₀ = 0.466 μg/ml), are shown in Fig. 3. RAFT decreased the growth rate between days 0 and 28 from 0.057 ± 0.036 cm³/day to 0.031 ± 0.013 cm³/day, while 5-FUra/LV therapy decreased the growth rate to 0.047 ± 0.016 cm³/day (P = NS between treatment groups; P < 0.01 between untreated and RAFT groups; P > 0.2 between untreated and 5-FUra/LV groups). There was considerable variability in the growth of the individual tumors in both treatment groups, thereby limiting the ability to demonstrate significance between the treatment groups. RAFT-treated tumors increased an average of 3.4-fold in size over 4 weeks, while 5-FUra-treated tumors increased an average of 6.1-fold and untreated tumors increased an average of 10.8-fold over the same 4 weeks. During the next 2 weeks (day 42 posttherapy), the growth of RAFT-treated tumors was still stable at 3.8-fold, while 5-FUra/LV-treated tumors increased 14.0-fold in size.

Fig. 4 provides results for the HCT-15 xenograft, a slow-growing, moderately differentiated tumor, with negligible expression of CEA and a relatively low response to 5-FUra (ID₅₀ = 1.51 μg/ml). RAFT decreased the growth rate between days 0 and 35 from 0.065 to 0.039 ± 0.014 cm³/day, while 5-FUra/LV-therapy decreased the growth rate to...
Radioimmunotherapy versus chemotherapy

Growth curves for the GW-39 xenograft, a rapidly growing, signet-ring cell carcinoma, are depicted in Fig. 7. This colonic line grows poorly in vitro; therefore, CEA and responsiveness to 5-FUra could not be determined for this line. However, in vivo CEA content is similar to that observed in the LS174T model. RAIT reduced the growth rate between days 0 and 28 from $0.151 \pm 0.015 \pm 0.003$ cm$^3$/day, while 5-FUra/LV therapy decreased growth rate to $0.061 \pm 0.011$ cm$^3$/day ($P < 0.001$ between treatment groups; $P < 0.001$ between untreated and RAIT groups; $P < 0.01$ between untreated and 5-FUra/LV groups). RAIT-treated tumors increased an average of 1.85-fold in size over 4 weeks, while 5-FUra-treated tumors increased an average of 4.5-fold and untreated tumors increased an average of 6.5-fold in 4 weeks.

Fig. 8 gives results for the WidR xenograft, a fast-growing, moderately differentiated tumor, that is a low CEA expressor (11.6 ng/10$^7$ cells) and a good in vitro responder to 5-FUra (0.304 µg/ml). RAIT decreased the growth rate between days 0 and 35 from $0.158 \pm 0.073$ to $0.022 \pm 0.003$ cm$^3$/day, while 5-FUra/LV therapy decreased growth rate to $0.056 \pm 0.007$ cm$^3$/day ($P < 0.001$ between treatment groups; $P < 0.01$ between untreated and both treatment groups). Although there is considerable variability in the growth of individual untreated tumors, both treated groups exhibited minimal variability. RAIT-treated tumors increased an average of 2.9-fold in size over 4 weeks, while 5-FUra-treated tumors increased an average of 4.7-fold and untreated tumors increased an average of 12.5-fold in 5 weeks.

By evaluating the growth curves of individual treated tumors, we have observed that RAIT is more efficacious than chemotherapy with 5-FUra/LV in 5 of 8 human colonic tumor xenografts (LoVo, HT-29, WidR, LS174T, and GW-39). This phenomenon can also be appreciated by assessing the percent growth inhibition achieved by each therapeutic modality at various times after administration. Fig. 9 shows that 14 days after therapy, RAIT results in greater growth...
Radioimmunotherapy versus chemotherapy

Inhibition in 2 lines (LoVo and LS174T), while the remaining 6 lines respond equally well to both treatments (less than a 20% difference in growth inhibition between the 2 approaches). By week 3 posttherapy, RAIT continues to be effective at reducing tumor growth in 8 of 8 lines, but the chemotherapy-treated tumors have already begun to resume growth in 5 lines (all except MOSER, DLD-1, and WidR). Similar results are seen at 28 days posttherapy. These findings demonstrate the importance of waiting at least 3 weeks before comparing therapeutic modalities.

Even though 5 of 8 lines are more responsive to RAIT, only 3 of these lines (LS174T, GW-39, and WidR) actually show a greater number of tumors responding for a longer period of time (Fig. 10). The remaining 5 lines (LoVo, HT-29, DLD-1, HCT-15, and MOSER) show the same number of tumors responding to each treatment, but reveal a greater response to RAIT than to chemotherapy. In general, many fewer tumors responded to chemotherapy in the fastest growing lines (e.g., GW-39 and LS174T had 0 tumors responding to 5-FU/VA/LV on day 14 posttherapy), while 70–80% of RAIT-treated tumors responded to treatment. It is important to note the variability in response between size-matched tumors initiated from the same suspension of cells and treated at the same time with either the RAIT or chemotherapy regimen. The difference in responsiveness of “identical” tumors implanted with the same number of cells from the same tumor suspension cannot be explained at this time.

In the final study, we evaluated the growth of tumors treated with a single cycle or 2 cycles of chemotherapy at 3-week intervals (the earliest time that treatment can be repeated without lethality). This study was initiated because we knew that mice could tolerate 2 cycles of chemotherapy in the same timeframe that they can only receive 1 dosing of maximally tolerated RAIT, and we needed to be certain that a comparison of responsiveness from a single dose of each therapeutic modality was appropriate. Fig. 11 shows that no additional growth inhibition is observed in either MOSER or LS174T tumors when they are given a second cycle of chemotherapy. Therefore, the comparison between a single cycle of each treatment is reasonable to make.

In summary, the results of these studies demonstrate that: (a) for single-cycle dosing, RAIT was significantly more effective than chemotherapy at slowing tumor growth in 5 of 8 tumor lines evaluated (LoVo, LS174T, MOSER, WidR, and GW-39). In the DLD-1, the efficacy of the 2 treatments was closer (RAIT was better but the effect was not statistically significant); (b) of the 5 s.c. tumors that respond better to RAIT, only 3 (GW-39, LS174T, and WidR) actually demonstrated a greater percentage of tumors responding. The other lines exhibit an equal percentage of tumors responding to radioimmunotherapy or chemotherapy, but there is a greater growth inhibition in those tumors that do exhibit a response; (c) in vitro responsiveness to 5-FU/VA did not directly correlate well with in vivo responsiveness ($r^2 = -0.664$; Table 3); (d) growth inhibition from RAIT did not correlate well with tumor CEA content, an observation that may be due to additional variables, such as accessibility of antigen and innate radiosensitivity of the tumor; and (e) RAIT was most effective in the fastest growing lines (GW-39, LoVo, LS174T, and MOSER). The relative response of each of the 8 lines to both forms of therapy is summarized in Table 3.

Discussion

To better appreciate the potential antitumor effects of any experimental therapeutic modality, studies aimed at establishing the comparative efficacy of the experimental procedure with established forms of therapy are useful. Over the past decade, radiolabeled antibodies have been used in preclinical and clinical experimental therapy of a...
RADIOIMMUNOTHERAPY VERSUS CHEMOTHERAPY

Fig. 7. Growth curves for individual GW-39 colonic tumors grown s.c. in nude mice during a 7-week period postinitiation of therapy, as described in Fig. 1.

The absolute amount of extractable CEA from the individual cell lines was not a good predictor of the response of the 8 colonic tumors to radiolabeled anti-CEA therapy. The 2 highest responders to RAIT (LS174T and LoVo) also were the 2 highest expressors of CEA (LoVo and LS174T). However, HT-29, the poorest responder to RAIT, was the fourth highest CEA expressor. When CEA expression is very high relative to the antitumor response with anti-CEA MAb therapy, one must begin to address issues such as accessibility/targetability of antigen, as we have done previously with the GS-2 colonic tumor (35), and which Pavez et al. (36) have studied with the HRA-19 tumor. Both intratumor and intracellular distribution of antigen are important factors for antibody targeting. It might be argued that in vitro CEA expression may not correlate well with in vivo CEA expression. However, our experience quantitating CEA from tumor extracts indicates a fairly good relationship between in vitro and in vivo values, with the exception of the MOSER line that has much higher CEA content in vivo than in vitro. The reason for the discrepancy between measurements for MOSER is not clear. However, the close relationship between in vitro and in vivo CEA for the other 6 lines allows us to be reasonably confident in the use of in vitro CEA measurements.

Another factor that correlated well with response to radioantibody therapy was tumor growth rate. The 4 most responsive tumors (LS174T, LoVo, GW-39, and WidR) were the 4 tumors with the fastest growth rates (0.151–0.363 cm³/day). The growth rate of the less responsive tumors ranged from 0.057 to 0.133 cm³/day. This obser-
conditions can limit drug access and reduce the proliferative rate, and equate functional vasculature in tumors will lead to a deficiency of oxygen and will result in an acidic microenvironment (39). These take, cell proliferation, and intracellular drug metabolism, which are the lines that are the least responsive to 5-FUra. Indeed, as the information in Tables 1 and 4 indicate, the 2 lines that are most responsive to 5-FUra/LV are the most responsive in vitro, and 2 of 3 of the lines that are the least responsive in vitro are the most responsive in vivo. There are several possible explanations for this finding, encompassing physiological and biochemical differences between the in vitro and in vivo systems. In vitro cytotoxicity does not take into account those factors that influence efficacy in vivo (e.g., tumor uptake, cell proliferation, and intracellular drug metabolism), which are dependent on intratumor oxygen partial pressure and pH (38). Inadequate functional vasculature in tumors will lead to a deficiency of oxygen and will result in an acidic microenvironment (39). These conditions can limit drug access and reduce the proliferative rate, and thereby also induce a resistance to anticancer agents (40, 41). Variability in the percent hypoxia between colonic tumor lines has been observed, ranging between 4.9% for LoVo tumors and 34.1% for LS174T tumors (42). Similarly, intratumor pH varies in malignant tissue from 5.8 to 7.6, with considerable variation within different regions of the same tumor (43). Alternatively, a number of biochemical factors, which may differ in cultured cells and the same cells grown as a xenograft, have been identified that influence the therapeutic activity of 5-FUra/LV (44). These include: (a) the pools of 5,10-methylenetetrahydrofolate; (b) the activity of methyltetrahydrofolate synthetase, the enzyme responsible for the initial cellular metabolism of LV to 5,10-methylenetetrahydrofolate; and (c) the levels of thymidine available for the salvage pathway.

Since randomized clinical trials using different schedules of 5-FUra and LV have demonstrated only a modest increase in response rate compared with 5-FUra alone, recent strategies to further improve on the efficacy of 5-FUra therapy have attempted to utilize biological modulation with such immune modulating agents as levamisole and IFN-α (45–48). Levamisole has immunorestorative properties and influences a variety of T-cell immune responses, including increased production of a variety of cytokines and increased T-cell responses to mitogens (49). Only 1 of 4 studies has shown an improvement in median survival when 5-FUra is combined with levamisole for the treatment of advanced disease (45). However, significant improvements have been noted in the disease-free survival rate for Dukes’ C (but not B2) colonic cancer patients when 5-FUra/levamisole was used as an adjuvant to surgery. IFN has been shown to inhibit thymidine uptake and thymidine kinase activity, thus reducing the salvage pathway of DNA synthesis. Initial trials with the combination of 5-FUra and IFN resulted in very high response rates but several toxic deaths. As doses were reduced, the response rate for 5-FUra/IFN was 26–39% (46–48), a distinct improvement over 5-FUra alone, but only

![Graph showing percent growth inhibition for the 8 colonic tumor xenografts postradioimmunotherapy at 14, 21, or 28 days postinitiation of therapy. Growth inhibition is defined as (the growth of the untreated tumor group – the growth of the treated tumor group)/(the growth of the untreated tumor group). The day 28 LS174T is missing because all untreated tumors had to be sacrificed prior to day 28.](image)

Fig. 9. Percent growth inhibition for the 8 colonic tumor xenografts postradioimmunotherapy and chemotherapy at 14, 21, or 28 days postinitiation of therapy. Growth inhibition is defined as (the growth of the untreated tumor group – the growth of the treated tumor group)/(the growth of the untreated tumor group). The day 28 LS174T is missing because all untreated tumors had to be sacrificed prior to day 28.

![Graph showing percent of tumors exhibiting a significant growth inhibition in response to radioimmunotherapy or chemotherapy during a 6-week period posttherapy. Substantial tumor growth inhibition is defined as less than a 2.5-fold increase in tumor size. All groups contained 10 mice.](image)

Fig. 10. Percent of tumors exhibiting a significant growth inhibition in response to radioimmunotherapy or chemotherapy during a 6-week period posttherapy. Substantial tumor growth inhibition is defined as less than a 2.5-fold increase in tumor size. All groups contained 10 mice.

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a marginal improvement over 5-FUra/LV. Studies are now ongoing to assess the efficacy of 5-FUra/LV/IFN together. Although our preclinical results have suggested great promise for radioantibody therapy in both an adjuvant setting (16) and in treating bulky disease, we realize that as modifications in the chemotherapy approach begin to show improvement in efficacy, it will be necessary to continue preclinical comparisons using the modified drug therapy with our radioantibody approach.

An important consideration that needs to be addressed when evaluating the results presented is the inherent limitation in the extrapolation from animal to human studies. For example, differences in tumor volume doubling times, tumor vascular bed effects, and host defense mechanisms to murine antibodies exist between mice and humans. In patients, the accretion of antibody can be 1000-fold lower than in mice. Furthermore, in contrast to tumor lesions in experimental animals, tumors in patients are often large and contain poorly vascular-ized, oxygen-depleted areas. Pharmacology, and as a consequence, therapeutic effects of RAIT, may differ between patients and animals, and animal models may exaggerate the potential antitumor effect of radiolabeled antibodies. However, human tumor models are generally considered useful in predicting the activity of anticancer agents in patients, because transplanted tumors to nude mice appear to retain both the chemosensitivity and the histology of the original tumor (50). Additional support for the validity of the nude mouse xenograft model as a predictive system comes from results of one study that evaluated the response of 42 human tumors that had been heterotransplanted into nude mice to 9 different chemotherapeutic agents, including 5-FUra. Responsiveness of human tumors in patients and heterotransplanted into nude mice showed a close correlation (51). Finally, it has also been observed that the radioreponsiveness of individual tumor lines agreed well with the observed response to radiation of the original tumor in patients (52).

How relevant are the preclinical data presented here to the data accumulated thus far on radioimmunotherapy of solid tumors, and what guidance can this type of preclinical data provide for future clinical trials? Responses to RAIT in patients with metastatic solid tumors have generally been poor (53, 54). Tumor doses achieved are usually below 2000 rad, whereas more than 5000 rad is probably minimal for a therapeutic response (55). For example, a recent phase II trial of $^{131}$I-labeled CC-49 in refractory widely metastatic colon cancer resulted in delivery of 19–667 rads to tumors, which produced stable disease in 3 of 15 patients. These patients experienced a marginal improvement over 5-FUra/LV. Patients with much smaller tumor burdens and with tumors that are radiosensitive must be performed.

The results of the preclinical studies described here with 8 colorectal tumors of varying histopathology, growth rate, CEA expression, and in vitro responsiveness to 5-FUra/LV have clearly demonstrated a notable improvement in antitumor effects using the MTD of radioiodinated anti-CEA antibodies in comparison with the standard 5-FUra/LV chemotherapy for colorectal neoplasia. The definitive comparison will necessitate a clinical trial using optimal dose-sched-
ules of each treatment modality in patients with comparable advanced disease or in a similar adjuvant setting.

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REFERENCES


Comparison of Equitoxic Radioimmunotherapy and Chemotherapy in the Treatment of Human Colonic Cancer Xenografts


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