Multimodality Therapy: Potentiation of High Linear Energy Transfer Radiation with Paclitaxel for the Treatment of Disseminated Peritoneal Disease

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Abstract

Purpose: Studies herein explore paclitaxel enhancement of the therapeutic efficacy of α-particle-targeted radiation therapy.

Experimental Design: Athymic mice bearing 3 day i.p. LS-174T xenografts were treated with 300 or 600 μg paclitaxel at 24 h before, concurrently, or 24 h after [213Bi] or [212Pb]trastuzumab.

Results: Paclitaxel (300 or 600 μg) followed 24 h later with [213Bi]trastuzumab (500 μCi) provided no therapeutic enhancement. Paclitaxel (300 μg) administered concurrently with [213Bi]trastuzumab or [213Bi]HuIgG resulted in median survival of 93 and 37 days, respectively; no difference was observed with 600 μg paclitaxel. Mice receiving just [213Bi]trastuzumab or [213Bi]HuIgG or left untreated had a median survival of 31, 21, and 15 days, respectively, 23 days for just either paclitaxel dose alone. Paclitaxel (300 or 600 μg) given 24 h after [213Bi]trastuzumab increased median survival to 100 and 135 days, respectively. The greatest improvement in median survival (198 days) was obtained with two weekly doses of paclitaxel (600 μg) followed by [213Bi]trastuzumab. Studies were also conducted investigating paclitaxel administered 24 h before, concurrently, or 24 h after [212Pb]trastuzumab (10 μCi). The 300 μg paclitaxel 24 h before radioimmunotherapy (RIT) failed to provide benefit, whereas 600 μg extended the median survival from 44 to 171 days.

Conclusions: These results suggest that regimens combining chemotherapeutics and high linear energy transfer (LET) RIT may have tremendous potential in the management and treatment of cancer patients. Dose dependency and administration order appear to be critical factors requiring careful investigation.

In the past year in the United States, it was estimated that ~37,170 people would be diagnosed with pancreatic adenocarcinoma and ~33,370 will die (1). The 5-year relative survival rate is only 5% for all stages of pancreatic cancer. The outlook for patients with ovarian cancer is not appreciably better. In 2007, it was estimated that ~22,430 women were diagnosed with ovarian cancer, 67% to 69% of whom presented with distant, metastatic disease. The overall 5-year relative survival rate is 40% to 45%. Over the years, there has been a trend toward improvement in this situation by a few percentage points, although that seems imperceptible in the 5-year relative survival rates for both diseases. Clearly, development of new tactics for the treatment and management of patients with pancreatic or ovarian cancer remains a high priority (1).

Targeted radiation therapy is one such strategy that has been reinvigorated in recent years with the Food and Drug Administration approval of Zevalin and Bexxar. In fact, numerous preclinical and clinical studies have appeared in the literature that focus on the application of radioimmunotherapy (RIT) as a treatment modality for i.p. disease (2–9). The majority of these studies have used monoclonal antibodies (mAb) conjugated with β-emitting radionuclides (e.g., 131I, 90Y, and 177Lu). The strategy followed by this laboratory has been to exploit the superb cytotoxicity of α-particle radiation using a mAb as the targeting vehicle of s.c. and i.p. xenografts (6–8, 10). Only three to six transversals of a cell’s nucleus by α-particles delivers a dose of 70 to 100 cGy; α-particle radiation is cytotoxic at a dose rate as low as 1 cGy/h (11–13). The characteristic short path lengths of α-particles render this radiation ideal for the treatment of small tumor burdens, disseminated disease, and micrometastatic disease and for the elimination of malignant single cells. The short path length is also hypothesized to limit normal tissue toxicity. This laboratory has recently reported on the therapeutic potential of two α-emitting radionuclides, 213Bi and 212Pb (the latter as...
Translational Relevance

These investigations reported herein show the potential of combining chemotherapeutics with high-LET RIT for the management and treatment of cancer patients who present with disseminated peritoneal disease at the time of their diagnosis. These studies are a natural progression to prior studies that established the efficacy of paclitaxel administered in conjunction with β-radiation RIT for the treatment of ovarian patients. Chemotherapy in conjunction with α-particle RIT using the appropriate targeting vehicle would be an effective adjuvant therapy following procedures such as cytoreductive surgery or peritoneal external beam radiation therapy. The intent of developing a treatment regimen using α-targeted radiation is to expand the repertoire to patient populations. Such a strategy would be potentially beneficial for not only those with pancreatic or ovarian cancer but also those with cancers of the colon, stomach, and small intestine, which result in peritoneal carcinomatosis as well as those with peritoneal mesothelioma. In general, the results obtained define a potentiating interaction between paclitaxel and the high-LET α-radiation-labeled trastuzumab. The studies also illustrate the necessity of establishing the optimal administration sequence of the treatment components and that dose dependency and administration order are critical factors that require careful investigation.

Materials and Methods

Cell lines. All therapy studies were conducted using the LS-174T, a human colon carcinoma cell line. SKOV-3, a human ovarian carcinoma cell line that expresses ~1 × 10^5 HER-2 molecules per cell, was used for in vitro analysis (32). LS-174T was grown in supplemented DMEM as described previously (33). SKOV-3 cells were maintained in McCoy's 5a medium supplemented with 10% fetal bovine serum and 1 mmol/L nonessential amino acids. Media and supplements were obtained from Quality Biologicals, Invitrogen, or Lonza.

Chelate synthesis and mAb conjugation. The synthesis, characterization, and purification of the bifunctional ligands, TCVM and CHX-A''-DTPA, have been described previously (34, 35). Conjugation of trastuzumab with either CHX-A''-DTPA or TCVM has been described previously (34, 35). The final concentration of trastuzumab was quantified by the method of Lowry (36). The number of CHX-A''-DTPA or TCVM molecules linked to the mAb was determined using spectrophotometric assays based on the titration of either yttrium or lead-Arsenazo (III) complex, respectively (37, 38).

Radiolabeling. A 5 to 10 mCi 224Ra/212Pb generator (AlphaMed) was washed with 2 mol/L HCl (3 mL) to remove impurities, unbound 224Ra, daughter isotopes, and any damaged resin or organic residue. On the following day, 212Pb was eluted from the generator with 1 mol/L HCl (3 mL). The eluate was heated to dryness; the residue was dissolved in 0.1 mol/L HNO₃ (2 × 0.2 mL) and used for radiolabeling of mAb as detailed elsewhere (8). The radiolabeled mAb was purified using a desalting column (PD-10; GE Healthcare) with PBS as the eluant. HulgG (ICN) was similarly conjugated with TCVM and radiolabeled with 212Pb as described above to serve as a negative control in these studies. A calibrated Ge(Li) detector (model GEM10185-P; EG&G Ortec) coupled to a multichannel analyzer Gamma Vision version 5.2 software (EG&G Ortec) was used for radioactivity measurements. 212Pb activity was determined by measurement of the 238.6 keV γ-ray (43.6%). 212Bi was obtained from a 225Ac/212Bi generator (Oak Ridge National Laboratories or Actinium Pharmaceuticals). Elution of the 212Bi and radiolabeling of trastuzumab-CHX-A'' was done as described previously (10).

Radioimmunoassay. Immunoreactivities of the radiolabeled preparations were assessed in a radioimmunoassay using methanol-fixed cells. Briefly, SKOV-3 cells were trypsinized, pelleted, and resuspended in PBS (pH 7.2) containing 1% bovine serum albumin. Serial dilutions of radiolabeled trastuzumab (~140 to 8 nCi) were added in duplicate to cells (1 × 10⁶) in 50 μL of 1% bovine serum albumin in PBS. The cells were washed with 4 mL of 1% bovine serum albumin in PBS following a 2 h (212Bi) or 18 h (212Pb) incubation at room temperature, pelleted at 1,000 g for 5 min, and the supernatant was decanted. The

causes BCL2 dysfunction, and activates apoptosis (20–22). Paclitaxel has activity against ovarian and pancreatic cancer and is a recognized radiosensitizer, which has been reported to sensitize ovarian and pancreatic cancer cells/tumors to the cytotoxic effects of radiation (23–25). The drug has been evaluated in combination with radiotherapy and radiolabeled antibodies in animal models and in patients and has been shown to provide additional therapeutic benefit (9, 26–31).

The mechanism of action by paclitaxel distinctly differs from that of gemcitabine and as such would add another class of chemotherapeutics to the armamentarium to be combined with radiolabeled antibodies. The studies described herein are an evaluation of the ability of paclitaxel to potentiate the therapeutic efficacy of HER-2 targeting α-emitting high-LET 213Bi- and 212Pb-labeled trastuzumab in a multimodality regimen for the management of disseminated i.p. disease.
Table 1. Effect of paclitaxel administration schedule on the therapeutic response to $^{213}$Bi RIT with trastuzumab: median survival

<table>
<thead>
<tr>
<th>RIT</th>
<th>Paclitaxel</th>
<th>0 Concurrent</th>
<th>24 h after RIT</th>
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<tbody>
<tr>
<td>None</td>
<td></td>
<td>300 µg</td>
<td>600 µg</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 µg</td>
<td>600 µg</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>15 (1.0)</td>
<td>23 (1.5)</td>
<td>23 (1.5)</td>
</tr>
<tr>
<td>HuIgG</td>
<td>21 (1.4)</td>
<td>37 (2.5)</td>
<td>53 (3.5)</td>
</tr>
</tbody>
</table>

NOTE: Median survival in days. Mice bearing 3-d tumor burden were injected i.p. with 300 or 600 µg paclitaxel concurrent with or 24 h after i.p. injection with 500 µCi $^{213}$Bi RIT. Therapeutic index, treatment group median survival divided by the untreated group median survival.

$^{212}$Pb was detected using the Ge(Li) detector, whereas the $^{213}$Bi was measured in a γ-counter (WizardOne; Perkin-Elmer). The binding percentage was calculated for each dilution. The specificity of the radiolabeled trastuzumab was confirmed by incubating one set of cells with ~200,000 counts/min of radiolabeled trastuzumab with an excess (5 µg) of unlabeled trastuzumab.

Therapy studies. Therapy studies were done using 19 to 21 g female athymic (nu/nu) mice (Charles River Laboratories). The mice were injected i.p. with $1 \times 10^6$ cells LS-174T in 1 mL medium or PBS as reported previously (39). Radiolabeled trastuzumab, 500 µCi $^{213}$Bi-chX-4A-trastuzumab, or 10 µCi $^{212}$PbTCMC-trastuzumab was administered to the mice ($n = 8-10$) 3 days after implantation of tumor in 0.5 mL PBS. HuIgG labeled with $^{213}$Bi or $^{212}$Pb served as a nonspecific control. In all of the experiments outlined below, radiation therapy doses were administered 3 days after tumor implantation. The choice of 500 and 10 µCi for $^{213}$Bi and $^{212}$Pb trastuzumab, respectively, is based on previous studies that determined these doses were the lower range of the maximum effective therapeutic dose (10, 11, 16). When combining α-particle RIT with chemotherapeutics, it was considered that alterations in tumor sensitivity to radiation would be more discernible if the lower effective doses of $^{213}$Bi or $^{212}$Pb trastuzumab were administered and that any incipient toxicity that might be incurred by doing these studies at higher doses would not obscure observation of enhanced therapy.

In study 1, mice ($n = 9-10$) with 2 days i.p. LS-174T xenografts were injected (i.p.) with 300 or 600 µg paclitaxel. On the following day (24-30 h), mice received $^{213}$Bi-labeled trastuzumab or HuIgG (500 µCi). Additional groups of mice received paclitaxel, $^{213}$Bi-trastuzumab, or $^{213}$Bi-HuIgG or were left untreated.

Study 2 involved reevaluating the sequence of administration of paclitaxel and $^{213}$Bi RIT. In this experiment, mice ($n = 10$) received an i.p. injection of paclitaxel (300 or 600 µg) concurrently or 24 to 30 h after the $^{213}$Bi RIT. Control groups were the same as outlined in study 1.

In study 3, multiple doses of paclitaxel (600 µg/week for up to 3 weeks) were given to mice ($n = 8-10$) bearing LS-174T i.p. xenografts following a single treatment with either 500 µCi $^{213}$Bi-trastuzumab or $^{213}$Bi-HuIgG. Additional groups of mice included those injected with one, two, or three doses of paclitaxel at weekly intervals, with either $^{213}$Bi-trastuzumab or $^{213}$Bi-HuIgG, or no radiation treatment.

Study 4 assessed the combination of paclitaxel with $^{212}$Pb RIT. Tumor-bearing mice ($n = 10$) were injected with paclitaxel (300 or 600 µg) 24 h before, concurrently, or 24 h after treatment with 10 µCi $^{212}$Pb-trastuzumab or $^{212}$Pb-HuIgG. These treatment groups were compared with sets of mice that received only paclitaxel, $^{212}$Pb-trastuzumab, or $^{212}$Pb-HuIgG or those without any treatment.

Progression of disease, especially during the first weeks of the experiment, was observed as an extension of the abdomen, development of ascites, and with noticeable, palpable nodules in the abdomen. Weight loss would occur as an acute response following treatment or much later as the experiments progressed. Mice were monitored and euthanized if found to be in distress, moribund, or cachectic. Mice were also euthanized when 10% to 20% weight loss occurred or if bloating or tumor nodules were apparent. All animal protocols were approved by the National Cancer Institute Animal Care and Use Committee.

Statistical analyses. A Cox proportional hazards model was used to test for a dose-response relationship between the dose of $^{212}$Pb-trastuzumab or $^{213}$Bi-trastuzumab and survival (time to sacrifice or natural death). Groups were compared using a log-rank test. A dose-response relationship was examined by testing the dose level as a linear covariate in the Cox model and tested whether the corresponding regression variable was zero using a likelihood ratio test.

For the animal weight data, the maximum percent reduction from baseline was estimated for each mouse. This was calculated as the ratio of the maximum reduction in weight from baseline during the initial 4-week period divided by the baseline weight of the mouse. Box plots were constructed for each treatment group that show the median, upper, and lower quartiles as well as identifying outliers. Differences between treatment groups were tested using a Kruskal-Wallis test (nonparametric ANOVA) for comparison of multiple groups and the Wilcoxon rank-sum test was applied when comparing two groups. All reported $P$ values correspond to two-sided tests.

Results

Previous studies from this laboratory showed potentiation of high-LET radiation ($^{212}$Pb-trastuzumab) by gemcitabine (7). Induction of G2 arrest via microtubule stabilization by paclitaxel provides for an alternate strategy for enhancing the therapeutic response to α-particle radiation. The first study exploring the combination of paclitaxel with high-LET radiation was done with $^{211}$Bi-trastuzumab in the LS-174T i.p. model (6-8). Athymic mice ($n = 10$) bearing a 2-day tumor burden were treated i.p. with either 300 or 600 µg paclitaxel followed 24 to 30 h thereafter with i.p. injection of $^{213}$Bi-trastuzumab (500 µCi). Control groups included mice given no treatment, treatment with paclitaxel alone, or treatment with $^{213}$Bi-HuIgG. The decision to administer the paclitaxel before RIT was based on the 45.6 min half-life of the $^{213}$Bi. It was reasoned that the $^{213}$Bi would no longer be present and most likely not be effective if the paclitaxel were given afterwards. The data, detailed as follows, effectively illustrate that this line of reasoning was incorrect. Mice injected with the $^{213}$Bi-trastuzumab realized a median survival of 43 days (data not shown), whereas mice that received the $^{213}$Bi-HuIgG had a median survival of 25 days compared with 19 days for the untreated group ($P = 0.09$). The median survival for the mice that were injected with the paclitaxel alone was 31 days for the 300 µg dose and 43 days for the 600 µg dose. In those animals given the combination, no further improvement in the median survival was observed at either 300 µg (42 days) or 600 µg (46 days) dose of paclitaxel ($P = 0.84$). The two groups that were injected with 300 and 600 µg paclitaxel followed by treatment the next day with $^{213}$Bi-HuIgG had a median survival of 42 versus 25 days for the group that received $^{213}$Bi-HuIgG alone. The increase from 25 to 42 days observed in these groups was found to be statistically insignificant ($P = 0.07$).

Based on these results, the timing of the paclitaxel administration was reexamined and a study was conducted in...
A study was then designed to investigate the effectiveness of multiple doses of paclitaxel with \(^{212}\)Pb RIT. Groups of mice were treated with 500 \(^{212}\)Pb \(^{213}\)Bi trastuzumab followed by one, two, or three 600 \(\mu\)g doses of paclitaxel. (Illustrated in Fig. 1A–C, respectively). The first dose was given 24 to 30 h after the \(^{213}\)Bi trastuzumab with subsequent doses being given at 7-day intervals. Additional groups received paclitaxel only, \(^{213}\)Bi HuIgG only, or no treatment. As shown in Table 2, the median survival of the untreated group was 25 days. There was no increase \((P = 0.82)\) in median survival for those mice receiving one, two, or three doses of paclitaxel only; median survival was 45, 63, and 46 days in these three groups, respectively. Mice injected with \(^{213}\)Bi trastuzumab experienced a 5-fold improvement over controls with a median survival of 126 days. When one, two, or three doses of paclitaxel (600 \(\mu\)g) were administered after the \(^{213}\)Bi trastuzumab, the median survivals were 182, 198, or 140 days, respectively. There was an observed but not statistically significant \((P = 0.17)\) shortening of survival following administration of the third dose of paclitaxel. A comparison of the three dose schedules of paclitaxel with \(^{213}\)Bi trastuzumab was not statistically different \((P = 0.17)\). Once again, this therapeutic response was specific to \(^{213}\)Bi trastuzumab. The corresponding groups of mice treated with \(^{213}\)Bi HuIgG and paclitaxel had a median survival of only 36, 38, 43, and 63 days for zero, one, two, or three doses of paclitaxel. The three dose schedules of paclitaxel with HuIgG were not statistically significant \((P = 0.13)\) among themselves.

The weights of the mice were recorded over a 4-week period monitoring the potential toxicity of the treatment regimens. Some weight loss was observed among the various groups in this particular RIT study (Fig. 2). The differences were significant among the groups that were untreated or injected with \(^{213}\)Bi trastuzumab and \(^{213}\)Bi HuIgG \((P = 0.02)\) with those animals treated with \(^{213}\)Bi HuIgG showing the greatest amount of weight reduction. A lack of difference was also noted for the groups that received paclitaxel only \((P = 0.30)\) or those that received paclitaxel and the \(^{213}\)Bi trastuzumab \((P = 0.93)\). A significant difference was found in the groups that were injected with \(^{213}\)Bi HuIgG, with toxicity decreasing with number of weekly doses \((P = 0.05)\).

The potential of combining \(^{212}\)Pb RIT with paclitaxel was also investigated. A therapy study was again conducted in which mice received the paclitaxel at the time of \(^{213}\)Bi RIT or \(~24\) to 30 h afterwards. The resulting data presented in Table 1 were encouraging. The median survival of the group injected with \(^{213}\)Bi trastuzumab was 31 days, a 2-fold increase compared with 15 days for the untreated mice. When 300 \(\mu\)g paclitaxel was administered concurrently with \(^{213}\)Bi trastuzumab, median survival increased to 93 days, which translates to a therapeutic index of 6.2. The response was specific to trastuzumab in that the median survival of mice receiving 300 \(\mu\)g paclitaxel at the time of injection with \(^{213}\)Bi HuIgG was 37 days. The benefit and specificity appeared to be negated when the paclitaxel dosage was increased to 600 \(\mu\)g. The median survival of the mice injected with either \(^{213}\)Bi-labeled trastuzumab or HuIgG was 53 days. Therapeutic efficacy of combining paclitaxel with \(^{213}\)Bi RIT became evident when the paclitaxel, at either 300 or 600 \(\mu\)g, was administered the day after \(^{213}\)Bi-labeled trastuzumab. In this administration scenario, median survival of mice injected with \(^{213}\)Bi trastuzumab or \(^{213}\)Bi HuIgG and 300 \(\mu\)g paclitaxel was 100 or 45 days, respectively. The benefit was even greater when 600 \(\mu\)g paclitaxel was injected after \(^{213}\)Bi trastuzumab. A therapeutic index of 9.0 (135 days) was obtained, whereas only a 1.9 therapeutic index was observed with the combination of \(^{213}\)Bi-HuIgG followed by 600 \(\mu\)g paclitaxel \((P = 0.05)\).

<table>
<thead>
<tr>
<th>RIT</th>
<th>No. paclitaxel treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>25 (1.0)</td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>126 (5.0)</td>
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<tr>
<td>HuIgG</td>
<td>36 (1.4)</td>
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NOTE: Median survival in days. Mice bearing 3-d tumor burden were injected i.p. with 300 or 600 \(\mu\)g paclitaxel. Twenty-four hours later, they were injected i.p. with 500 \(\mu\)Ci \(^{213}\)Bi RIT. The indicated groups then received an additional injection of 600 \(\mu\)g paclitaxel at 7-d intervals for a total of one, two, or three doses administered to the mice. Therapeutic index, treatment group median survival divided by the untreated group median survival.
which paclitaxel was administered at two dose levels (300 and 600 µg) 24 h before, concurrently, or 24 h after the injection of $^{212}\text{Pb}$trastuzumab or $^{212}\text{Pb}$HuIgG. Additional groups of mice were treated with each of the $^{212}$Pb-labeled antibodies alone, at each of the paclitaxel doses alone, and one group was left untreated. Consistent with prior studies (Table 3), a modest but significant therapeutic effect due to the paclitaxel alone was observed. Median survival of the untreated group was 16 days, whereas for those receiving either 300 or 600 µg paclitaxel this was extended to 31 and 29 days, respectively ($P = 0.007$). The 300 µg dose of paclitaxel appears to only have a modest affect on median survival irrespective of the timing of the administration of the $^{212}$Pbtrastuzumab. The median survival of the mice injected with $^{212}$Pbtrastuzumab is 44 days, whereas it is 49, 53, or 51 days when paclitaxel (300 µg) is injected 24 h before, concurrently, or 24 h after the $^{212}$Pbtrastuzumab. At this dose level of paclitaxel, there is a 2-fold increase in the median survival of animals that are injected with the chemotherapeutic 24 h before $^{212}$PbHuIgG. An enhancement of the therapeutic efficacy of $^{212}$Pbtrastuzumab becomes evident in the group that received 600 µg paclitaxel 24 h before the RIT. Under these conditions, there is a 3.9-fold increase in the median survival compared with the group that received $^{212}$Pbtrastuzumab alone resulting in an increase from 44 to 171 days. This response appears to be specific to the $^{212}$Pbtrastuzumab because the median survival of the corresponding group receiving the $^{212}$PbHuIgG remained at 44 days. There is an increase in median survival (86 days) of the mice that were given the paclitaxel the day following the $^{212}$Pbtrastuzumab, which translates to a 1.9-fold increase over the $^{212}$Pbtrastuzumab alone, whereas no effect is observed when the chemotherapeutic is given at the same time as the $^{212}$Pbtrastuzumab.

A box plot showing the distribution of maximum percent reduction in weight over follow-up across the 17 groups is given in Fig. 3. Toxicity was low in 13 of the groups. Changes in weight were observed in those groups that were injected with paclitaxel, at either 300 or 600 µg dose, 24 h after the $^{212}$Pb RIT was administered. A comparison of paclitaxel after $^{212}$Pbtrastuzumab with trastuzumab alone showed a statistically significant increase in toxicity with paclitaxel at the 300 µg dose ($P = 0.05$). There is a statistical trend of an increase for the 600 µg dose ($P = 0.10$). Similar differences for HuIgG were also noted with a statistically significant increase in toxicity at the 600 µg paclitaxel 24 h after $^{212}$PbHuIgG compared with HuIgG alone ($P = 0.03$).

**Discussion**

Food and Drug Administration approval of “naked” mAbs such as trastuzumab (Herceptin), cetuximab (Erbitux), and panitumumab (Vectibix) and of the radiolabeled mAbs, Zevalin and Bexar, are ample evidence of the burgeoning interest and ongoing efforts to incorporate mAbs into cancer patient treatments. The strategy of molecular targeting, in conjunction with the realization that treatments will likely be tailored to individual patients, provides the impetus for development and refinement of mAB-based therapies.

The efficacy of targeted α-particle radiation has been shown with increasing frequency in both preclinical and clinical settings (2, 5–8). As hypothesized, targeted α-particle radiation is effective in the treatment of low disease burden, especially when administered locoregionally or when applied for

![Fig. 2. Effect of multiple paclitaxel administrations combined with $^{213}$Bi RIT on animal weight. The maximum percent reduction in weight was calculated for each of the treatment groups and presented as box plots. Light line, median; top region, third quartile; bottom region, first quartile; brackets, 15 times the interquartile range; lines outside of the brackets, outlying observations. Alterations in the weights of the mice during and 4 wk following treatment were monitored as an indicator of toxicity.](image)

<table>
<thead>
<tr>
<th>Table 3. Effect of paclitaxel administration schedule on the therapeutic response to $^{212}$Pb RIT with trastuzumab: median survival</th>
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</thead>
<tbody>
<tr>
<td>RIT</td>
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<td></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Trastuzumab</td>
</tr>
<tr>
<td>HuIgG</td>
</tr>
</tbody>
</table>

**NOTE:** Median survival in days. Mice bearing 3-d tumor burden were injected i.p. with 300 or 600 µg paclitaxel either 24 h before RIT, concurrent, or 24 h after RIT with 10 µCi $^{212}$Pb RIT. Therapeutic index, treatment group median survival divided by the untreated group median survival.
Paclitaxel, IFN, and 90Y-CC49 (9). Findings were observed in ovarian cancer patients receiving 2 to 3 weeks after the paclitaxel administration (9). Similar with paclitaxel and IFN with only a transient neutropenia noted. Ovarian cancer patients tolerated 177Lu RIT in combination, particularly in combination with RIT, is not unprecedented. Tumor to the drug and minimizes systemic exposure and circulation is slow, which in turn prolongs the exposure of rate of paclitaxel from the peritoneum into the peripheral administration of the paclitaxel would result a higher local.

Tuzumab targeting HER-2 (7, 8). Paclitaxel as the next treatment of single-cell disease. There remains, however, a great deal to improve upon with this therapeutic mode.

As with other modalities, expectations with RIT are that this would not be a stand-alone treatment but would become incorporated as an element of a multimodality treatment scheme. Chemotherapeutics (e.g., gemcitabine, paclitaxel, cisplatin, 5-fluorouracil, and doxorubicin) in combination with RIT are under evaluation (7, 9, 22, 26–29, 40–42). This laboratory has recently reported on the potentiation of high-LET radiation using gemcitabine in combination with [212Pb]trastuzumab targeting HER-2 (7, 8). Paclitaxel as the next chemotherapeutic for investigation was an attractive candidate based on several factors including established usage and radiosensitization properties. The studies reported here were designed to explore conditions for the i.p. administration of paclitaxel with α-particle RIT with the objective of developing a multicycle treatment scheme for both 213Bi and 212Pb RIT.

In the design of the experiments, it was felt that i.p. administration of the paclitaxel would result a higher local concentration of the paclitaxel being achieved. The adsorption rate of paclitaxel from the peritoneum into the peritoneal circulation is slow, which in turn prolongs the exposure of tumor to the drug and minimizes systemic exposure and resultant toxicities (43, 44). Paclitaxel given by i.p. administration, particularly in combination with RIT, is not unprecedented. Ovarian cancer patients tolerated 177Lu RIT in combination with paclitaxel and IFN with only a transient neutropenia noted 2 to 3 weeks after the paclitaxel administration (9). Similar findings were observed in ovarian cancer patients receiving paclitaxel, IFN, and 90Y-CC49 (9).

The 500 and 10 μCi doses of [213Bi] and [212Pb]trastuzumab used in these experiments had been determined to be the lower range of the maximum effective therapeutic dose for each radioimmunonoconjugate (6–8, 14). The decision to use these doses in subsequent experiments was based on the reasoning that the lower effective dose ranges were less likely to mask alterations in sensitivity conferred by other modalities such as chemotherapeutics during combination therapy.

The first study evaluating paclitaxel with targeted α-particle was conducted with trastuzumab radiolabeled with 213Bi. Based on the 46 min half-life of the radionuclide, administration of paclitaxel to the mice was executed 24 h before administration of [213Bi]trastuzumab. Paclitaxel has been shown to persist in the peritoneal cavity (t1/2 = 73 ± 18 h), which would allow time for radiosensitization to occur (44). In this situation, the assumption was incorrect. No improvement in therapeutic efficacy of [213Bi]trastuzumab was observed in this scenario with this radionuclide. A subsequent experiment evaluated the effect of administering paclitaxel, at two dose levels, concurrently with [212Pb]trastuzumab as well as on the day after [213Bi]trastuzumab. Consistent with the literature, the greatest therapeutic response was obtained when 600 μg paclitaxel was given ~24 h after RIT, with a therapeutic index of 9 being realized. It should be noted that a therapeutic index of 6.2 was also obtained for the group of animals that were treated with 300 μg paclitaxel given with the [213Bi]trastuzumab.

At the time of designing this first study, there were two areas of experience to draw upon in the literature. One was the combination of paclitaxel with conventional external beam radiotherapy. Paclitaxel had been shown to enhance in vivo the radiation response of murine mammary carcinoma to radiotherapy when given up to 24 h before radiotherapy (45). The combination of paclitaxel and radiotherapy resulted in an increase in mitotically arrested tumor cells and with a corresponding increase in apoptosis. In vivo RIT studies by Supiot et al. showed that paclitaxel added to three different multiple myeloma cell lines 24 h before 213Bi RIT using mAb B-B4 resulted in a decrease in survival in all three cell lines (22). When examined further, the investigators found that only one of the cell lines responded to the combined treatment with a modest increase in apoptosis. In these same studies, the potential of doxorubicin was also evaluated for its ability to sensitize cells to targeted α-particle radiation. In this case, doxorubicin not only inhibited cell growth but also was found to increase the amount of DNA strand breaks and frequency of apoptosis. The first RIT study combining paclitaxel with 90Y-ChL6 therapy from DeNardo et al. showed potentiation of therapy by paclitaxel (29). Scheduling of paclitaxel with the RIT was examined and was found to affect a response rate of 100% when started 24 h before RIT. The greatest number of cures, however, was obtained when paclitaxel was administered 24 h after the 90Y-ChL6 (29). Clinical trials have also been conducted in which the feasibility of i.p. radioimmunochemotherapy was evaluated. Patients with ovarian carcinoma were treated with paclitaxel (i.p.) 2 days before receiving an i.p. injection of 177Lu-Cc49 or 90Y-CC49 (9). It should be noted that all of these studies were conducted using β emitting radionuclides, 90Y, 177Lu, and 131I, which have half-lives that are appreciably longer (2.7, 6.7, and 8 days, respectively) than that of 213Bi (46 min) or 212Pb (10.2 h). One also must note that the β -emitters are...
low-LET radiation as opposed to the high-LET radiation associated with α-emitters.

Because therapeutic efficacy was observed with paclitaxel administered concurrently with the \[^{219}Bi\]trastuzumab, the experiment combining paclitaxel and \[^{212}Pb\]trastuzumab evaluated the effects of paclitaxel given before, concomitantly, and after \[^{212}Pb\]trastuzumab. Consistent with studies from other laboratories, an enhancement of therapeutic efficacy was observed when tumor-bearing mice were treated with 600 µg paclitaxel 24 h after administration of \[^{212}Pb\]trastuzumab (26–29, 40, 46). In fact, an increase in the median survival of the mice in this group was observed compared with those that received only \[^{212}Pb\]trastuzumab (36 versus 44 days, respectively). However, the therapeutic efficacy of \[^{212}Pb\]trastuzumab was even more impressive when paclitaxel was given 24 h before the \[^{213}Bi\]trastuzumab. The median survival realized was 171 days, a nearly 4-fold increase. This is actually in accordance with the literature (26, 29). In the original study, a 100% response rate and responses that included partial and complete regression with cures were reported when paclitaxel was given before the RIT (29). Paclitaxel (600 µg) given 24 h after RIT generated the highest number of cures (29). Clearly, empirical studies evaluating the scheduling of a chemotherapeutic with RIT is warranted. As shown by the studies described here, the sequence of administration is directly dependent on the radionuclide being targeted. One probably should not be surprised then to discover that the administration sequence might also be dependent on the targeting vehicle and the target as well.

With the objective of developing a multicycle chemo-RIT treatment regimen, studies were done assessing a single injection of \[^{213}Bi\]trastuzumab with paclitaxel being given up to three times at 1-week intervals. Paclitaxel was found to potentiate the therapeutic effectiveness of \[^{213}Bi\]trastuzumab with the greatest benefit being derived from two doses of paclitaxel given after the RIT. To date, one other study has reported results of administering multiple doses of paclitaxel with RIT (28). In this particular study, 300 µg paclitaxel was administered 48 and 72 h after \[^{90}Y\]RIT with two different mAb, one targeting carcinoembryonic antigen (T84.66) and the other targeting HER-2 (trastuzumab). The fractionated paclitaxel dose was found to be effective in inhibiting tumor growth; however, the investigators did not include a direct comparison with a single dose of paclitaxel, nor did they include a nonspecific mAb making it challenging to objectively deconvolute their results versus the therapeutic components.

The overall objective of these studies was to investigate the conditions for a treatment regimen that incorporated α-particle RIT with a chemotherapeutic. Paclitaxel was chosen due to its (a) reported radiosensitization properties, (b) reported synergism with trastuzumab, and (c) proven effectiveness in treating pancreatic cancer patients (6, 47, 48). Interestingly, the LS-174T xenografts used in these studies are modestly responsive to the radiolabeled HulGc combined with paclitaxel. In stark contrast, potentiation of \[^{213}Bi\]and \[^{212}Pb\]trastuzumab was realized, with therapeutic indices reaching values as high as 9.

To understand how paclitaxel enhances the therapeutic response to RIT, a study was conducted by Miers et al. to answer whether there is an effect on the cumulative activity in the tumor. The authors compared patients receiving \[^{90}Y\]-RIT (m170) with \[^{90}Y\]-RIT (m170) with (n = 5) and without (n = 7) paclitaxel (49). When patients were given paclitaxel 2 days after the RIT, an increase in the cumulated tumor activity was observed. No differences were observed in normal tissue. Similar results were obtained when mice bearing breast cancer xenografts were injected with \[^{111}In\]DOTA-Gly3-Phe-ChL6 and then treated with paclitaxel 24 h later (49). Similar studies are planned to test whether the same phenomenon occurs with the LS-174T i.p. xenograft model following paclitaxel therapy.

These investigations suggest that regimens combining chemotherapeutics and high-LET RIT may have tremendous potential in the management and treatment of pancreatic and ovarian cancer patients who present with peritoneal disease at the time of diagnosis. This treatment regimen may also prove beneficial to other patient populations with cancers, such as the colon, stomach, and small intestine, which result in peritoneal carcinomatosis as well as those with peritoneal mesothelioma (50).

Chemotherapy in conjunction with α-particle RIT using the appropriate targeting vehicle would be an effective adjuvant therapy following procedures such as cytoreductive surgery or peritoneal external beam radiation therapy. Although some of the observed effects are difficult to explain and warrant further study, on the whole, the general results obtained pointed in the direction of a potentiating interaction between paclitaxel and the high-LET α-radiation-labeled trastuzumab. The studies also illustrate the necessity of establishing the optimal administration sequence of the treatment components and that dose dependency and administration order are critical factors that require careful investigation.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**

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Multimodality Therapy: Potentiation of High Linear Energy Transfer Radiation with Paclitaxel for the Treatment of Disseminated Peritoneal Disease

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