Assessment of Combined Radioimmunotherapy and Chemotherapy for Treatment of Medullary Thyroid Cancer

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Abstract

We have shown previously significant antitumor effects using $^{90}$Y-MN-14 anti-CEA monoclonal antibody (MAb) for radioimmunotherapy (RAIT) of human medullary thyroid cancer (MTC) xenografts using the TT cell line. The purpose of this investigation was to determine the effect of combining chemotherapy and RAIT with $^{90}$Y-MN-14 in MTC. In particular, the toxicity and efficacy of various dose schedules of RAIT and doxorubicin were examined and compared with that at the maximum tolerated dose (MTD) of each single modality treatment. The MTD of RAIT of 105 $\mu$Ci of $^{90}$Y-MN-14 was given alone and combined with 100 and 75% of the MTD of doxorubicin (60 mg/m$^2$); and the MTD of doxorubicin was given alone and combined with 100 and 75% of the MTD of RAIT. In addition, 75% of each agent was also administered in combination. The MTD of RAIT was also evaluated in combination with 58 and 78% of the MTD of Taxol. Whereas $^{90}$Y-MN-14 (105 $\mu$Ci) led to significant antitumor effects ($P < 0.0001$), doxorubicin at 60 mg/m$^2$ or Taxol at 225 mg/m$^2$ yielded only a slight tumor growth delay. The combinations of 100% of the MTD of RAIT and 75% of the MTD of doxorubicin and 100% of the MTD of doxorubicin and 75% of the MTD of RAIT were equitoxic to the MTD of RAIT alone and appear to result in improved efficacy compared with either RAIT or doxorubicin alone. For the 100% RAIT and 75% doxorubicin combination, the therapeutic efficacy was similar when doxorubicin was administered on the same day or 1 day after RAIT, but the treatment was less effective when doxorubicin was administered 2 days after RAIT ($P < 0.03$). Prolonged retardation of tumor progression was also observed in animals treated with the MTD of RAIT combined with 175 mg/m$^2$ of Taxol, without increases in toxicity above that observed with RAIT alone. In conclusion, the combination of RAIT and chemotherapy appears to augment the antitumor effects of either treatment alone without a significant increase in toxicity. In addition, the timing of drug administration relative to RAIT in the combined therapy appears to be important.

Introduction

Approximately 1200 new cases of MTC are diagnosed annually in the United States, representing ~10% of the total new cases of thyroid cancer (1). Patients with lymph node metastases or extrathyroidal disease are not cured by surgery alone and are left with treatment modalities of limited effectiveness (2). Low response rates combined with the toxicity associated with chemotherapy have led to diminished interest in the utilization of chemotherapy for the management of MTC. Doxorubicin has been the most widely used single agent and, therefore, the one associated with the most reported responses. The response rate in the trials using doxorubicin alone was 36% (9 of 25 patients; Refs. 3–8). The doses given usually ranged from 45 to 75 mg/m$^2$ given in 3–4 week cycles with a cumulative dose of up to 550 mg/m$^2$. Cardiac toxicity was dose-limiting beyond this threshold.

We have shown previously that RAIT using $^{131}$I- and $^{90}$Y-labeled MN-14 anti-CEA MAb yields significant antitumor effects for the TT human MTC xenograft (9). In this system, $^{90}$Y-MN-14 was also demonstrated to have a therapeutic advantage compared with the same MAb labeled with $^{131}$I. Although these results demonstrated the significant antitumor effect of RAIT in MTC, they also demonstrated that a single administration of MN-14 labeled with either isotope is not sufficient for a high percentage of cures in this MTC model. This corresponds with the observations made in clinical studies (10, 11) and suggests that more aggressive treatment strategies will be required, including combined modality or myeloablative approaches.

Using various tumor xenograft models, it has been shown that combining chemotherapy with RAIT can augment the antitumor effects of RAIT (12–14). The concept of combining chemotherapy with radiation or targeted radiation such as RAIT is attractive because the two modalities may have independent activity against tumor cell subpopulations; cells that are intrinsically resistant to one modality may be sensitive to the other. For example, doxorubicin possesses the property of selective cytotoxicity against radioresistant hypoxic tumor cells, particularly at low dose levels (15, 16). Indeed, doxorubicin was first used in combination with radiation in the early 1970s and was found to enhance radiation effects in vivo (17). A major concern of combining the two modalities is that an increase in toxicity

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3 The abbreviations used are: MTC, medullary thyroid cancer; CEA, carcinoembryonic antigen; MAb, monoclonal antibody; MTD, maximum tolerated dose; %ID, percentage of injected dose; RAIT, radioimmunotherapy; AUC, area under the curve.
may result. However, except for myelotoxicity, chemotherapy and RAIT exhibit different toxicity profiles.

To provide options for patients whose prior exposure to doxorubicin precludes retreatment with this drug, the combination of other chemotherapeutic agents with RAIT must be evaluated in MTC. This would be of benefit to patients who previously received maximum allowable cumulative doxorubicin doses. DeNardo et al. (12) have reported synergistic enhancement of the therapeutic effect when Taxol (paclitaxel) was added to \(^{90}\)Y-labeled MAb therapy in a human breast cancer xenograft model. The efficacy of the RAIT plus Taxol protocol may be due to enhancement of radiation-induced DNA damage by Taxol and enhancement of Taxol-induced apoptosis by \(^{90}\)Y.

The purpose of this investigation was to determine the effect of combining chemotherapy and RAIT with \(^{90}\)Y-MN-14 in MTC. In particular, the toxicity and efficacy of various dose schedules of RAIT and doxorubicin were examined and compared with that at the maximum tolerated dose of each single modality treatment. Results were also compared with those obtained using combination treatment of the MTC xenografts with Taxol and RAIT.

Materials and Methods

Cell Lines and Reagents. TT (18), a human medullary thyroid cancer cell line, was purchased from the American Type Culture Collection (Rockville, MD). The cells were grown as monolayers in Ham’s F12K medium (Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM L-glutamine. Doxorubicin and Taxol (paclitaxel) were purchased from Florida Infusion (Palm Harbor, FL).

Radiolabeling. The anti-CEA MAb used in these studies was MN-14, which is directed against the class III, CEA-specific epitope (19). \(^{90}\)Y- and \(^{111}\)In-labeled M Abs were prepared as described previously (20) using the \(p\)-isothiocyanatobenzyl derivative of diethylenetriaminepentaacetic acid, provided by Immunomedics, Inc. (Morris Plains, NJ). \(^{90}\)Y was purchased from Pacific Northwest National Laboratory (Richmond, WA), and \(^{111}\)In was purchased from Iso-Tex, Inc. (Friendswood, TX). Specific activity of the \(^{90}\)Y-MN-14 preparations ranged from 4.3 to 4.7 mCi/mg. Specific activity of the \(^{111}\)In-MN-14 preparation used in the biodistribution study was 0.8 mCi/mg. The percentage of unincorporated radioisotope was assayed by ITLC (instant thin layer chromatography) and was 2.8–7.2% for the \(^{90}\)Y-MN-14 preparations and 1.6% for the \(^{111}\)In-MN-14.

In Vivo Studies. Tumors were propagated in female mu/mu mice (Taconic Farms, Germantown, NY) at 6–8 weeks of age by s.c. injection of \(2 \times 10^6\) washed TT cells, which had been propagated in tissue culture. Tumors were allowed to grow for 4–5 weeks before use in experimental studies. Typically, the tumor take rate was \(-85\%\). Exponential growth with a tumor doubling time of \(\sim 6–8\) days was observed in untreated animals. Radiolabeled antibodies were injected i.v. via the lateral tail vein of tumor-bearing mice. Details on the quantities of radioisotope injected and number of mice per group are indicated in “Results” for each study. For biodistribution studies, the mice were sacrificed at the times indicated, and the radioactivity in the tumor, liver, spleen, kidneys, lungs, small and large intestines, muscle, bone (whole femur), heart, and blood was determined after correction for physical decay in a gamma scintillation well counter. For radioimmunotherapy experiments studying the efficacy of various treatments and treatment schedules, tumor size was monitored by weekly measurements of the length, width, and depth of the tumor using a caliper. Tumor volume was calculated as the product of the three measurements. Toxicity was monitored by loss of body weight and blood leukocyte counts (21).

MTD levels of single doses of doxorubicin, Taxol, and \(^{90}\)Y-MN-14, as single agents and in combination, were established in nude mice. MTD is defined as the highest dose that will allow 100% of the animals to survive with no more than 20% loss in body weight. The doxorubicin was administered either on the same day or 24 or 48 h after \(^{90}\)Y-MN-14. In the studies with doxorubicin administered on the same day as RAIT, doxorubicin was given 1 h before MAb to provide time for recovery from possible doxorubicin-induced decrease in tumor blood flow (22, 23). On the basis of the reported experience of DeNardo et al. (12) and our results with doxorubicin, Taxol was administered 24 h after RAIT in the combined studies of Taxol and RAIT.

Radiation dose estimates were determined from the biodistribution data, as described previously (24). Calculations were performed by first integrating the trapezoidal regions defined by the time-activity data (corrected for physical decay). The zero time point was extrapolated from the first two time points. The resulting integral for each organ is converted to cGy/mCi using S-values appropriate for isotope and organ weight. These S-values are calculated for each isotope by assuming uniformly distributed activity in small unit-density spheres (25).

Statistical Analysis. Statistical analyses were performed to compare different treatment groups on the basis of two variables: AUC and survival time. The \(t\) test and nonparametric Wilcoxon rank sum test were used to analyze the first variable, whereas the log-rank test (26) was used to analyze the second one. The end point of the survival time is taken as the time at which the tumor reaches a volume that is double the volume measured at time of treatment. Two-sided tests were used throughout. The statistical analyses were carried out using S-plus.

Results

Toxicity. When doxorubicin was administered alone, nude mice were able to tolerate doses up to 60 mg/m\(^2\) (216 µg/mouse). When \(^{90}\)Y-MN-14 was administered as a single agent, up to 105 µCi/mouse could be tolerated without deaths. Combination treatments were administered using three dose schedules in which the single dose of doxorubicin was administered either on the same day or 24 or 48 h after \(^{90}\)Y-MN-14. Combining the full MTD of both agents was not possible without deaths. \(^{90}\)Y-MN-14 (105 µCi/mouse; 100% of the MTD of RAIT) was tolerated with as high as 75% of the MTD of doxorubicin (45 mg/m\(^2\)), regardless of the timing of doxorubicin relative to RAIT. The full MTD of doxorubicin could only be combined with 75% of the MTD of RAIT when doxorubicin was given 1 or 2 days after RAIT but not on the same day. The combination of 75% of the MTD of each agent was tolerated regardless of the timing of doxorubicin relative to RAIT.
**Clinical Cancer Research**

**Biodistribution.** The effect of doxorubicin on the biodistribution of labeled MAbs MN-14 was evaluated to determine whether MAbs accrue in either tumor or normal organs is affected by doxorubicin administration. In this study, TT-bearing nude mice were given doxorubicin 24 h after labeling with $^{111}$In-MN-14. As shown in Fig. 1, administration of doxorubicin did not significantly affect accrual of radiolabeled MN-14 in tumor or nontumor tissues. Tumor:nontumor ratios at 7 days after administration of $^{111}$In-MN-14 ranged from 10.6 to 16.6 for the heart, kidney, lungs, liver, and spleen and from 39.8 to 66.3 for the small and large intestines and muscle.

Cumulative absorbed radiation doses were calculated for $^{90}$Y-MN-14 from the biodistribution study of $^{111}$In-MN-14 shown in Fig. 1. It is estimated that 5.9% of the total absorbed radiation dose will be delivered to tumor in the first 24 h and another 26.2% between 24 and 48 h in this nude mouse MTC xenograft system. On the other hand, in considering the dose to the blood and normal organs, taking lungs as an example, 36.3 and 21.7% of the total absorbed radiation dose will be delivered in the first 24 h to the blood and lungs, respectively. On the second day, another 18.9 and 19.4% will be delivered to the blood and lungs. Thus, the difference in percentage of total absorbed radiation dose between the tumor and nontumor organs is greatest at 24 h after RAIT, when a relatively small fraction of the radiation dose has been delivered to the tumor.

**Therapy with Combinations of Doxorubicin and $^{90}$Y-MN-14.** Using the MTD doses described above, doxorubicin and $^{90}$Y-MN-14 were evaluated as single modality therapeutic agents and in combination in nude mice bearing s.c. TT xenografts. Fig. 2A shows the growth curves of TT tumors in mice given various treatment regimens. Combination treatments in which doxorubicin was administered 24 h after $^{90}$Y-MN-14 are compared with untreated mice and mice treated with a single dose of doxorubicin or $^{90}$Y-MN-14 only. Whereas $^{90}$Y-MN-14 (105 µCi) led to significant antitumor effects ($P < 0.0001$, t test comparison with untreated animals), doxorubicin (60 mg/m²) yielded only a marginal tumor growth delay ($P < 0.04$, t test comparison to untreated animals). The mean tumor volume doubling time was 5 weeks in the $^{90}$Y-MN-14 treatment group compared with 11 days in the animals treated with doxorubicin and 6 days in the untreated group. The combinations of 100% of the MTD of RAIT and 75% of the MTD of doxorubicin and 100% of the MTD of doxorubicin and 75% of the MTD of RAIT appear to result in improved efficacy compared with either RAIT or doxorubicin alone. Mean tumor doubling times in these groups were 9 weeks. Treatment groups were composed of six to seven nude mice. Statistical comparisons of the combined modality treatments versus RAIT alone did not reach 95% confidence levels; $P$s were 0.13 and 0.14 for two sided $t$ tests comparing 75% RAIT + 100% doxorubicin and 100% RAIT +

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**Fig. 1** Effects of doxorubicin on the biodistribution of $^{111}$In-labeled MN-14 in nude mice bearing TT tumor xenografts. Mice were either given 45 mg/m² of doxorubicin 24 h after administration of 10 µCi of $^{111}$In-MN-14 (○) or received 10 µCi of $^{111}$In-MN-14 only (■). The mice were sacrificed at the indicated times after $^{111}$In-MN-14 injection, and tumor, organ, and blood radioactivity were counted. The %ID per gram of tissue was calculated from these data. Results are the mean of three to five mice; bars, SD.
therapy, the mean tumor volume was 0.123 ± 0.086 cm³ (range, 0.039–0.438 cm³). Tumor-bearing mice were either left untreated (○, n = 6) or given a single injection of either 60 mg/m² doxorubicin (□, n = 7) or 105 μCi ²⁹⁵Y-MN-14 (□, n = 6), or given 45 mg/m² doxorubicin 24 h after 80 μCi ²⁹⁵Y-MN-14 (▲, n = 7), 60 mg/m² doxorubicin 24 h after 80 μCi ²⁹⁵Y-MN-14 (●, n = 6), or 45 mg/m² doxorubicin 24 h after 105 μCi ²⁹⁵Y-MN-14 (●, n = 7). At the start of therapy, the mean tumor volume was 0.123 ± 0.086 cm³ (range, 0.039–0.438 cm³). A, points represent the mean tumor size of the treatment groups; bars, SD (shown in only one direction for clarity). B, points represent the fraction of mice in which tumors are less than two times the volume at the time of ²⁹⁵Y-MN-14 administration.

Fig. 2 Growth of TT tumors in mice given various RAIT and doxorubicin treatment regimens. Tumor-bearing mice were either left untreated (○, n = 6), given a single injection of either 60 mg/m² doxorubicin (□, n = 7) or 105 μCi ²⁹⁵Y-MN-14 (□, n = 6), or given 45 mg/m² doxorubicin 24 h after 80 μCi ²⁹⁵Y-MN-14 (▲, n = 7), 60 mg/m² doxorubicin 24 h after 80 μCi ²⁹⁵Y-MN-14 (●, n = 6), or 45 mg/m² doxorubicin 24 h after 105 μCi ²⁹⁵Y-MN-14 (●, n = 7). At the start of therapy, the mean tumor volume was 0.123 ± 0.086 cm³ (range, 0.039–0.438 cm³). A, points represent the mean tumor size of the treatment groups; bars, SD (shown in only one direction for clarity). B, points represent the fraction of mice in which tumors are less than two times the volume at the time of ²⁹⁵Y-MN-14 administration.

75% doxorubicin, respectively, to the RAIT-only treatment group. Larger sample sizes may improve the confidence level. In Fig. 2B, the results of this study are presented as the fraction of animals in which the tumors have not yet doubled in volume compared with the time of treatment. This figure emphasizes the significant antitumor effect of the RAIT compared with chemotherapy with doxorubicin, as well as the observation that the combined modality treatments prolong the delay in tumor growth compared with the single-agent therapies.

Toxicity evaluations on the mice in this study are shown in Fig. 3. In all groups, body weight and total WBC counts decreased 1 week after therapy, followed by recovery. Doxorubicin alone caused only a 20% decrease in total WBCs, compared with a 40%–60% decrease from RAIT or the combination of RAIT and doxorubicin. When lymphocytes and neutrophils were analyzed separately, it was observed that doxorubicin lowered the neutrophil count only, whereas RAIT had a more severe effect on lymphocytes. Doxorubicin also had less of an effect on body weight than the treatment with 100% RAIT or 100% RAIT in combination with doxorubicin (Fig. 3D). There was no evidence of increased gastrointestinal toxicity caused by the combination of doxorubicin with RAIT, as indicated by the absence of diarrhea or excessive weight loss in the combined modality treatment groups. Thus, combinations of 100% of the MTD of RAIT and 75% of the MTD of doxorubicin or 100% of the MTD of doxorubicin and 75% of the MTD of RAIT were equitoxic to RAIT alone.

Fig. 4 shows the effect of the timing between RAIT and doxorubicin administration on treatment efficacy. For the 100% RAIT and 75% doxorubicin combination, the therapeutic efficacy was similar when doxorubicin was administered on the same day or 1 day after RAIT, but the treatment was less effective when doxorubicin was administered 2 days after RAIT. The difference between zero and 1 day was insignificant, whereas the difference between 2 days and 0/1 day was statistically significant, P < 0.03 in survival analysis. The linear model, AUC = Days + treatment, and the Cox proportional hazards model, survival time = Days + stratified treatment, were used in the analysis, in addition to the pairwise comparisons. Again, the number of days was statistically significant in the survival analysis with P = 0.013. At the 9-week time point, 60% of the tumors in mice given doxorubicin either on the same day or day 1 after RAIT had not yet doubled in volume compared with the time of treatment, whereas in those mice that were given doxorubicin 2 days after RAIT, all tumors had more than doubled (Fig. 4A). This trend was also observed for the 75% RAIT and 100% doxorubicin combination. At the 7-week time point in the mice treated with doxorubicin 1 day after RAIT, 80% of the tumors were <2 times the size at the time of RAIT, compared with only 30% in the group treated with doxorubicin on day 2 after RAIT (Fig. 4B). Thus, the timing of drug administration relative to RAIT in the combined therapy appears to be important.

Therapy with Combinations of Taxol and ²⁹⁵Y-MN-14. Tumors in mice treated with Taxol alone (225 mg/m², 810 μg/mouse) grew rapidly (Fig. 5). The combination of 130 mg/m² Taxol (58% of the MTD) with 100% RAIT yielded an equivalent growth delay to that observed in the 75% doxorubicin + 100% RAIT group. Combining treatment at the MTD of RAIT with a Taxol dose of 175 mg/m² (78% of the MTD) prolonged the suppression of tumor growth. At 16 weeks after RAIT, 67% of the tumors in this group had still not doubled, whereas this value was 36% for the 100% RAIT + 75% doxorubicin group and 27% for 100% RAIT + 58% Taxol group. It was found that the groups receiving RAIT, either in combination with Taxol or doxorubicin, had statistically significantly smaller AUC and longer survival than the Taxol-alone group, with P < 0.005 for the Wilcoxon tests and Ps < 0.0006 for the log-rank tests. However, the difference between the group that received 100% RAIT and 78% Taxol and the group that received either 100% RAIT + 58% Taxol or 100% RAIT + 75% doxorubicin did not reach statistical significance. Toxicity evaluations indicated that the combined Taxol and RAIT therapy caused a similar decrease in blood counts as the doxorubicin and RAIT combined treatment, but body weight loss was less severe than for the doxorubicin and RAIT combined treatment (Fig. 6).
Fig. 3  Toxicity evaluations. Total WBCs, neutrophils, lymphocytes, and body weight were evaluated weekly on the mice shown in Fig. 2. A–C, blood cell counts are presented as percentage of the untreated group. D, body weights are presented are a percentage of body weight at the time of RAIT. □, 60 mg/m² doxorubicin; □, 105 μCi ⁹⁰Y-MN-14; ▲, 45 mg/m² doxorubicin 24 h after 80 μCi ⁹⁰Y-MN-14; ●, 60 mg/m² doxorubicin 24 h after 80 μCi ⁹⁰Y-MN-14; ○, 45 mg/m² doxorubicin 24 h after 105 μCi ⁹⁰Y-MN-14. Data points represent the means of all animals shown in Fig. 2; bars, SD.

Fig. 4  Effect of time elapsed between RAIT and doxorubicin administration. A, tumor-bearing mice were given a single injection of 45 mg/m² doxorubicin either 1 h before (▲, n = 7) or 24 h (●, n = 14) or 48 h (■, n = 9) after injection of 105 μCi ⁹⁰Y-MN-14. B, tumor-bearing mice were given a single injection of 60 mg/m² doxorubicin either 24 h (○, n = 12) or 48 h (□, n = 8) after injection of 80 μCi ⁹⁰Y-MN-14. At the start of therapy, the mean tumor volume was 0.286 ± 0.250 cm³ (range, 0.013–1.292 cm³). Points represent the fraction of mice in which tumors are less than two times the volume at the time of ⁹⁰Y-MN-14 administration.

Discussion

We report here our results on the effects of combining chemotherapy with RAIT in MTC. Combinations of doxorubicin or Taxol and the ⁹⁰Y-labeled anti-CEA MAb MN-14 were evaluated. The RAIT alone led to prolonged antitumor effects compared with only a slight tumor growth delay with these chemotherapeutic agents administered in a single dose. Combining the chemotherapy with RAIT appeared to increase the
Fig. 5 Growth of TT tumors in mice given various RAIT and Taxol treatment regimens. Tumor-bearing mice were either given a single injection of either 225 mg/m² Taxol (Δ, n = 6), 130 mg/m² Taxol 24 h after 105 µCi 90Y-MN-14 (●, n = 14), 175 mg/m² Taxol 24 h after 105 µCi 90Y-MN-14 (○, n = 14), or 45 mg/m² doxorubicin 24 h after 105 µCi 90Y-MN-14 (□, n = 14). At the start of therapy, the mean tumor volume was 0.209 ± 0.128 cm³ (range, 0.038–0.524 cm³). A, points represent the mean tumor size of the treatment groups; bars, SD (shown in only one direction for clarity). B, points represent the fraction of mice in which tumors are less than two times the volume at the time of 90Y-MN-14 administration.

The enhancement of therapeutic efficacy by the drugs may be due to an additive antitumor effect of chemotherapy and RAIT and/or to the ability of both Taxol and doxorubicin to act as radiosensitizing agents. Doxorubicin is among a class of intercalating agents that stabilize the formation of complexes between the protein topoisomerase II and DNA, altering the three-dimensional structure of the DNA and inhibiting the enzymatic repair of radiation-induced single- and double-strand breaks in DNA (17). Doxorubicin also has marked effects on host physiology, which could play a role in its effectiveness in combined modality treatments (22, 23). Specifically, doxorubicin affects tumor oxygenation, an important factor in light of the radioresistance of hypoxic cells. The cytotoxicity of Taxol is caused by its ability to disrupt the microtubule cytoskeleton (27). Cells exposed to Taxol become growth arrested at the G2-M phase of the cell cycle. This alteration in cell cycle distribution causes an increase in the percentage of cells in the radiosensitive phase of the cell cycle.

Radiosensitization by these chemotherapeutic agents has implications for the timing of the combined modality treatments. If the radiosensitizing drug is administered at the same time or before RAIT, all tissues (tumor and nontumor) will become equally sensitized to the effects of radiation. If, on the other hand, the drug is given after RAIT, a substantial quantity of 90Y-MN-14 will already have cleared from the normal organs because of the faster clearance of 90Y-MN-14 from these tissues compared with the tumor. Thus, the effect of radiosensitization will be less pronounced in the nontumor tissues in comparison with the tumor. This can explain our observation that the full MTD of doxorubicin could not be combined with 75% of the MTD of RAIT if both treatments were administered on the same day but was tolerated 1 or 2 days after RAIT.

In assessing how long to delay chemotherapy after RAIT administration, it is important to ensure that a substantial fraction of the radiation dose to tumor will be delivered after the radiosensitizing drug is delivered. Taking into consideration the kinetics of 90Y-MN-14 accretion (derived from the 111In-MN-14 biodistribution study shown in Fig. 1) and the physical properties of 90Y, it is estimated that 5.9% of the total absorbed radiation dose will be delivered to tumor in the first 24 h. In comparison, >36% of the total absorbed radiation dose will be delivered to the blood in the first 24 h. This is especially important, because blood doses are regarded as an index of hematological toxicity (28). Thus, on a theoretical basis, the optimum time to administer a radiosensitizing agent would be 24 h after RAIT, when a relatively small fraction of the radiation dose has been delivered to the tumor, while a greater proportion has been delivered to the blood and normal organs. Indeed, the experimental results reported here show that administration of doxorubicin 24 h after 90Y-MN-14 yielded greater treatment efficacy than if doxorubicin was given 48 h after RAIT. The results reported by DeNardo et al. (12) using combination therapy with Taxol and 90Y-ChL6 in a nude mouse-breast cancer model also indicated that timing is an important variable in multimodality dosing. The cure rate was much better when Taxol was given 6–24 h after 90Y-MAb rather than 24–72 h before RAIT. A longer delay prior to Taxol administration was not evaluated in that study.

In vivo studies in tumor-bearing nude mice have also demonstrated that doxorubicin is a vasoactive chemotherapeutic agent. Blood flow was reduced more and longer as the i.v.-administered doxorubicin dose was increased (22, 23), the effect typically lasting <1 h. Doxorubicin caused radioprotection when administered by i.v. injection immediately prior to irradiation, with increased drug concentration leading to progressively greater cell survival. This led to the conclusion that drugs similar to doxorubicin, which decrease tumor oxygenation, should not be administered immediately prior to radiation. It is important to note, however, that because the effect on blood flow was short-lived, doxorubicin can be administered ~1 h before RAIT, without concern over reduced blood flow affecting MAb delivery to tumor.
A major concern of combining the two treatment modalities is that an increase in toxicity may result. However, except for myelotoxicity, chemotherapy and RAIT exhibit different toxicity profiles. This fact allows the combination of relatively high doses of both modalities. The biodistribution studies reported here show that uptake of $^{90}$Y-MN-14 in the heart is low. Thus, the radiation dose delivered to cardiac muscle is expected to be low, and adding RAIT as an adjuvant treatment in the clinical setting is not expected to substantially increase cardiac toxicity, a common toxicity of doxorubicin. Because myelosuppression is virtually the only toxicity of RAIT given at nonmyeloablative doses and because doxorubicin could result in mild to moderate myelotoxicity, a major challenge is to ensure that the myelotoxicity of the combined regimen is not excessive. On the other hand, it is important to choose a combination of both agents, both with respect to dose and dose sequencing, that is likely to result in optimal antitumor effects (i.e., responses that are higher than those seen with any individual therapy). In the animal model used here, these conditions were met by administering the combination of either 100% of the MTD of RAIT with 75% of the MTD of doxorubicin or 75% of the MTD of RAIT with 100% of the MTD of doxorubicin, with the chemotherapy administered 24 h after RAIT. In addition, our results indicate that Taxol is as good a candidate for combined modality treatment of MTC as doxorubicin. This is especially important for the clinical application of this approach, because prior doxorubicin therapy can make it unsuitable for some patients.

The fact that 100% of the MTD of a single dose of doxorubicin or Taxol, two drugs that are sometimes used for treatment of MTC, could be combined with as high as 75% of the MTD of RAIT without increase in toxicity and with clearly better antitumor effects than those associated with the MTD of a single dose of either drug provides a rationale for adding RAIT to treatment with these chemotherapeutic agents. However, because these agents are usually given in multiple cycles (e.g., 3–6 cycles) given at relatively fixed intervals of 3–4 weeks, further studies will be needed to determine how RAIT will be integrated into these chemotherapeutic regimens. For example, 75% of the MTD of RAIT may be given with the last cycle of chemotherapy, so that there is no delay in administering subsequent cycles of chemotherapy through administering a relatively high RAIT dose at the beginning of the treatment regimen. Alternatively, a smaller fraction of the MTD of RAIT may be given with each cycle of chemotherapy so that there is no substantial increase in myelotoxicity and/or nonhematological toxicity, necessitating a delay in administering subsequent chemotherapy cycles. Although animal studies may be useful in assessing all of these combinations, clinical studies will ultimately determine the feasibility of these strategies in patients.

In conclusion, the combination of RAIT and chemotherapy with doxorubicin or Taxol appears to augment the antitumor effects of RAIT without a significant increase in toxicity. The data also show that the timing of drug administration relative to RAIT in the combined therapy is important. The superiority of the combined modality treatment over that of a single dose of chemotherapy argue for the integration of RAIT in existing, albeit infrequently used, chemotherapeutic regimens for MTC treatment. Clinical Phase I and/or feasibility trials are needed to assess these principles in MTC patients.
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