

# Combination Radioimmunotherapy and Chemoimmunotherapy Involving Different or the Same Targets Improves Therapy of Human Pancreatic Carcinoma Xenograft Models

Robert M. Sharkey<sup>1</sup>, Habibe Karacay<sup>1</sup>, Serengulam V. Govindan<sup>2</sup>, and David M. Goldenberg<sup>1</sup>

## Abstract

Chemoimmunotherapy with antibody–drug conjugates (ADC) is emerging as a promising therapy for solid tumors, whereas radioimmunotherapy (RAIT) of solid tumors has been relatively ineffective because of their resistance to radiation. We developed antibody–SN-38 conjugates that have significant antitumor activity in xenograft models at nontoxic doses. The goal of this study was to determine if an ADC could be combined with RAIT to enhance efficacy without a commensurate increase in host toxicity. Nude mice bearing human pancreatic cancer xenografts (Capan-1 and BxPC-3) were treated with a single dose of <sup>90</sup>Y-labeled antimucin antibody (hPAM4; clivatuzumab tetraxetan) alone or in combination with an anti-Trop-2–SN-38 conjugate, typically administered twice weekly over 4 weeks. The combination, even at RAIT's maximum tolerated dose, controlled tumor progression and cured established xenografts significantly better than the individual treatments without appreciable toxicity. The ADC could be started 1 week after or up to 2 weeks before RAIT with similar enhanced responses, but delaying RAIT for 2 weeks after the ADC was less effective. A nonspecific ADC provided additional benefit over using free drug (irinotecan), but the response was enhanced with the specific ADC. When targeting Capan-1 with ample mucin, hPAM4 could be used as the RAIT and the ADC agent without losing effectiveness, but in BxPC-3 with less mucin, targeting of different antigens was preferred. These studies show the feasibility of combining ADC and RAIT for improved efficacy without increased toxicity. *Mol Cancer Ther*; 10(6); 1072–81. ©2011 AACR.

## Introduction

Radioimmunotherapy (RAIT) is an effective treatment for non-Hodgkin lymphoma, but its effectiveness in solid tumors has been elusive (1). Difficulties in achieving meaningful antitumor responses in solid tumors is most likely related to their increased resistance to radiation, as there is no evidence that monoclonal antibodies (mAb) used to target lymphoma have higher uptake or greater retention than those used for solid tumors (1–3). However, lymphoma therapy is undeniably aided by the therapeutic action of the unlabeled anti-CD20 antibody, including signaling events that enhance the cell's sensitivity to radiation (4–7). Similarly, current efforts at developing antibody–drug conjugates (ADC) are promis-

ing in hematological malignancies, but also in solid tumors, such as breast cancer (8–10).

A number of anticancer drugs can enhance a tumor's sensitivity to radiation, such as 5-fluorouracil, taxol and paclitaxel, and gemcitabine (GEM; refs. 11–16). Most preclinical studies have combined the chemotherapeutic agent at a subtherapeutic dose when given with RAIT because if both treatments were given at therapeutically active doses, overlapping toxicities would likely require a reduction in the radiation dose, which could compromise the therapeutic response to RAIT. Although improved responses have been noted in animal models, achieving substantial additional benefit in patients has been lacking (16–19).

Preclinical studies have indicated potent therapeutic responses when treating human pancreatic cancer xenografts with an antimucin antibody (PAM4 or clivatuzumab tetraxetan) labeled with <sup>90</sup>Y, alone or in combination with GEM (20, 21). More recently, this antibody or an antibody to EGP-1 (also known as Trop-2) conjugated with SN-38, the active moiety of the prodrug, irinotecan, a topoisomerase-I inhibitor (22), was also found to be active against pancreatic cancer (23). <sup>90</sup>Y-clivatuzumab tetraxetan has now advanced to clinical studies, where it is being administered in combination with radiosensitizing doses of GEM (24–26). Promising antitumor activity has been observed, and therefore we undertook this investigation

**Authors' Affiliations:** <sup>1</sup>Garden State Cancer Center at the Center for Molecular Medicine and Immunology; and <sup>2</sup>Immunomedics, Inc., Morris Plains, New Jersey

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**Corresponding Author:** Robert M. Sharkey, Center for Molecular Medicine and Immunology, 300 American Way, Morris Plains, NJ 07950. Phone: 973-844-7121; Fax: 973-844-7020. E-mail: [rmsharkey@gscancer.org](mailto:rmsharkey@gscancer.org)

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to determine if an effective dose of an antibody-targeted drug could be combined with RAIT at its effective dose without increasing toxicity, but with improved efficacy. We were particularly concerned whether this combination can function best against 2 different antigen targets or if the same could be exploited. Also of interest was the temporal relationship in the administration of these 2 modalities. Our results show that 2 different immunoconjugates against the same or a different antigen target can have improved efficacy compared with the single-agent modalities, without increased host toxicity.

## Materials and Methods

### Antibodies, antibody conjugates, and cell lines

Capan-1 and BxPC-3 were purchased from the American Tissue Culture Collection. Capan-1 highly expresses the PAM4-defined mucin, whereas BxPC-3 expresses less of this mucin. For example, immunohistology revealed more than 50% of Capan-1 xenografts stained intensely with the PAM4 antibody, with 5.6 mg/g of extractable antigen, whereas less than 5% of BxPC-3 xenografts stained weakly with PAM4, with a total extractable antigen of just 36  $\mu$ g/g (27). Based on a cell-binding assay, where cell lines in culture plates were incubated with hRS7, which was then revealed with an antihuman IgG-horseradish peroxidase conjugate, both cell lines expressed similarly high levels of Trop-2 (i.e., ~20–30-fold higher than cell incubated with an irrelevant IgG).

Humanized PAM4 (hPAM4) and RS7 (hRS7) and their SN-38 conjugates were provided by Immunomedics, Inc. The hPAM4 and an irrelevant anti-CD20 antibody (vel-tuzumab; ref. 28) was conjugated with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and radiolabeled with  $^{90}\text{Y}$  (Perkin Elmer), according to a method published previously (29). Each radiolabeled product was tested for unbound  $^{90}\text{Y}$  ( $\leq 5\%$ ) and immunoreactivity by size-exclusion high-performance liquid chromatography (HPLC), using an anti-hPAM4 idiotype antibody ( $\geq 85\%$  immunoreactive), as described previously (30).

Specific targeting hPAM4 and hRS-7-SN-38 conjugates (IMMU-134 and IMMU-132, respectively) and an irrelevant anti-CD20-SN-38 conjugate (IMMU-133), were prepared using the CL2A-SN-38 derivative as described by Cardillo and colleagues (23). The conjugates contained an average of 5 to 6 SN-38 moieties per IgG, with 1.0 mg of conjugate containing 16  $\mu$ g of SN-38 equivalents. The conjugates were lyophilized and reconstituted in saline on the day of use.

### Animal studies

Female athymic nude mice (Taconic) were implanted subcutaneously at 4 to 5 weeks of age with Capan-1 and BxPC-3 using a mixture of cells from tissue culture ( $1 \times 10^7$ ) and 0.2 mL from a 10% suspension taken from serially transplanted tumors. Tumors reached a size of ~0.3 to 0.6  $\text{cm}^3$  (L  $\times$  W  $\times$  D) in 2 to 4 weeks, at which time animals

were sorted into various groups, each having the same tumor-size range, and treatment was initiated.

The  $^{90}\text{Y}$ -hPAM4 (specific RAIT) or  $^{90}\text{Y}$ -anti-CD20 IgG (irrelevant RAIT) was injected intravenously ( $\leq 0.2$  mL). Because most ADC treatments were given twice weekly for 4 weeks, the ADCs and irinotecan always were given intraperitoneally ( $\leq 0.3$  mL). The specific dosage and treatment regimens of the ADC, RAIT, and GEM are provided for each experiment in the Results section. The standard ADC dosing was 0.5 mg/dose (~25 mg/kg; 0.4 mg/kg SN-38 equivalents). This treatment plan was devised to maintain a high level of the fully loaded ADC in the blood based on the faster blood clearance of the ADC as compared with the unconjugated antibody, and the fact that the SN-38 gradually releases from the IgG (e.g., 50% cleaved within ~1 to 1.5 days; refs. 23, 31). Irinotecan dosing assumed 1 mg of irinotecan yielded 0.575 mg of SN-38 equivalents (i.e., full conversion), as mice convert irinotecan to SN-38 much more efficiently than humans (32). When combination treatments were given on the same day, the ADC was injected 2 hours before the  $^{90}\text{Y}$ -antibody to reduce personnel radiation exposure. GEM was given 2 days after the  $^{90}\text{Y}$ -hPAM4, which allowed time for the radiolabeled antibody to reach maximum levels in the tumor and clear somewhat from the blood and tissues.

Once treatments were initiated, animals were monitored daily, with once or twice weekly 3-dimensional measurements using a caliper and weight. Tumors were allowed to progress to 3.0  $\text{cm}^3$ , at which time the animals were removed from study and euthanized humanely. Animals showing any signs of morbidity or a decrease of more than 20% of their starting weight were also removed (censored).

Radioimmunoconjugate uptake in Capan-1 or BxPC-3 tumors was determined in animals that were necropsied 2 days after receiving  $^{90}\text{Y}$ -labeled DOTA-hPAM4 or DOTA-anti-CD20 IgG. The tissues were digested in a tissue solubilizer, and then counted in a scintillation cocktail, along with standards prepared from the injected product. These data are expressed as percent injected dose (ID) per gram (% ID/g).

### Statistical analysis

Tumor sizes in the individual animals were plotted for each measurement. The time to progression to 3.0  $\text{cm}^3$  (TTP) was considered the endpoint for a survival analysis carried out using GraphPad Prism Software, version 5.0. The software calculated the median survival and statistical differences were based on the log-rank (Mantel-Cox) test using  $P \leq 0.05$ .

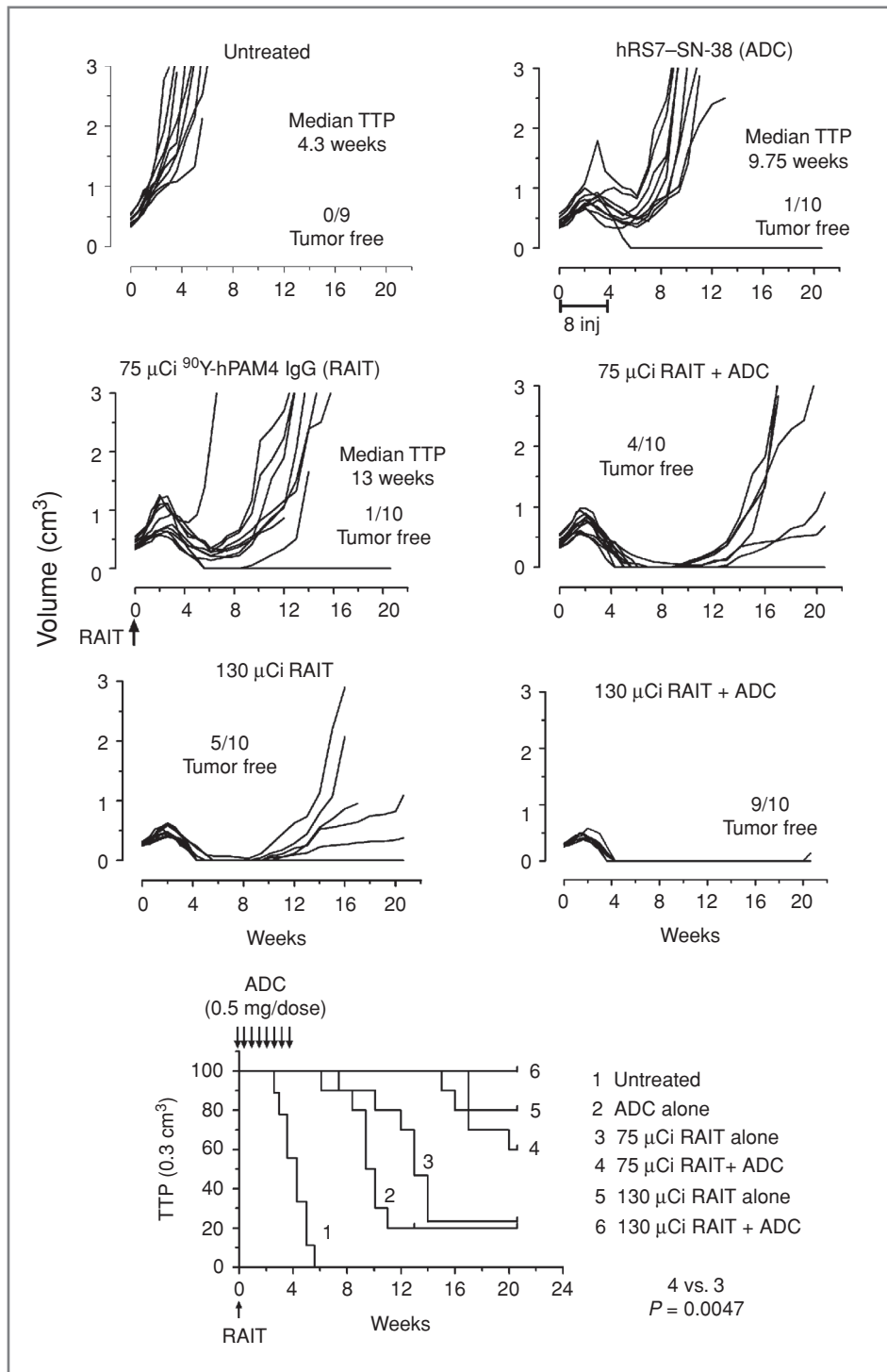
## Results

### $^{90}\text{Y}$ -hPAM4 IgG RAIT with hRS7-SN-38 ADC

Previous studies had shown the hRS7 ADC given twice weekly for 4 weeks at 0.5 mg/dose did not result in any appreciable loss in weight, but significantly inhibited

the growth of Capan-1 xenografts, but was not curative (23). <sup>90</sup>Y-hPAM4 IgG cured most mice at its maximum tolerated dose (MTD) of 130 μCi (30). Therefore, the combination studies focused primarily on a reduced dose of only 75 μCi of <sup>90</sup>Y-hPAM4 (~60% MTD) to assess the potential for therapeutic enhancement, but also

with 130 μCi of <sup>90</sup>Y-hPAM4 IgG to determine whether a therapeutically effective dose of ADC could be tolerated with the MTD of RAIT. Biodistribution studies confirmed that <sup>90</sup>Y-hPAM4 IgG uptake in Capan-1 tumors measured 2 days after its injection was not affected by administering 0.5 mg of the hRS7-SN-38



**Figure 1.** Enhanced therapeutic response by combining RAIT and ADC without substantial toxicity. Nude mice (9–10 animals/group) bearing s.c. Capan-1 tumors averaging ~0.3 cm<sup>3</sup> were given twice weekly injections of 0.5 mg (8 μg of SN-38) of the hRS7-SN-38 conjugate for 4 weeks starting on day 0 or a single injection of <sup>90</sup>Y-hPAM4 IgG at 75 μCi or 130 μCi (50 μg of hPAM4 IgG). Other groups of animals received the ADC treatment 2 hours before the 2 RAIT treatments. Individual animal tumor growth curves are shown as well as a survival curve based on the TTP to 3.0 cm<sup>3</sup>. The median survival times and number of animals having no evidence of tumor at the conclusion of the study (21 weeks) are indicated. RAIT, radioimmunotherapy.

2 hours earlier ( $48.4 \pm 16.4$  vs.  $45.3 \pm 10.8\%$  ID/g, respectively;  $n = 3/\text{group}$ ).

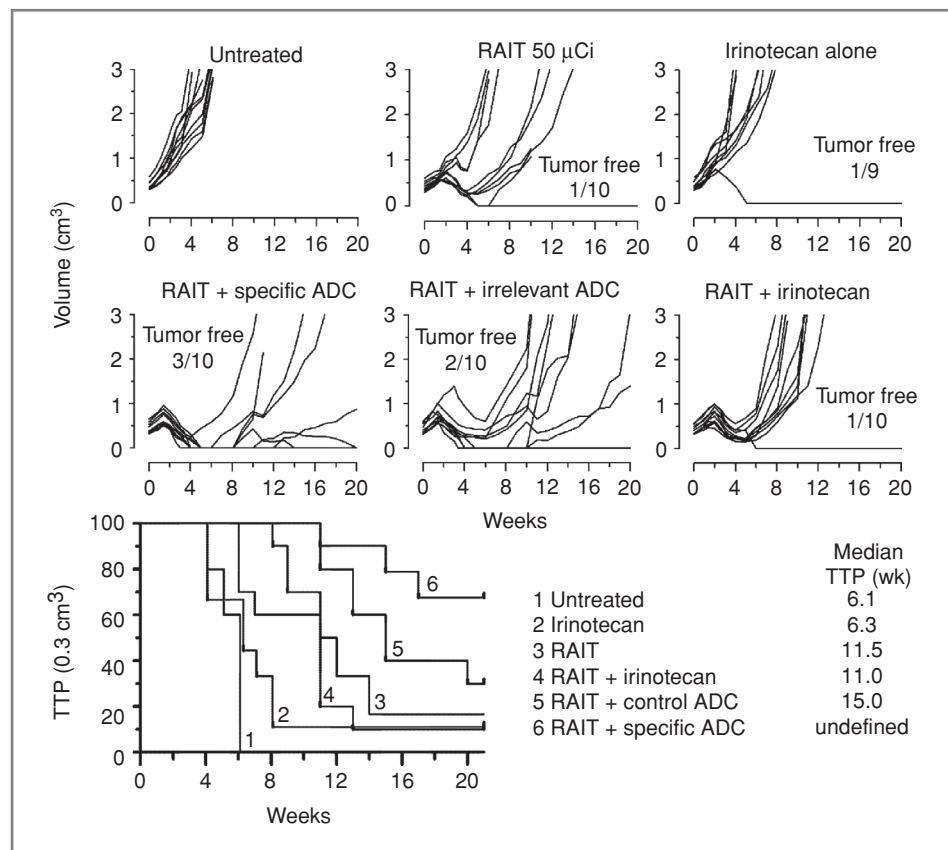
As shown in Fig. 1, ADC alone improved the TTP from a median of 4.3 weeks in the untreated group to 9.75 weeks ( $P < 0.001$ ). The 75- $\mu\text{Ci}$  RAIT treatment alone increased the median survival to 13 weeks, and like the ADC-alone group, 1 animal was tumor free after 21 weeks. In contrast, when ADC was initiated on the same day as 75  $\mu\text{Ci}$  RAIT, only 3 animals had tumors that progressed to 3.0  $\text{cm}^3$  after 21 weeks, and 4 animals had no evidence of tumor, a significant improvement over RAIT alone ( $P = 0.005$ ). The efficacy of this combination with the reduced RAIT dose was similar to the MTD of RAIT alone, but adding ADC to 130  $\mu\text{Ci}$  of RAIT improved efficacy, with 9 of 10 animals being tumor free after 21 weeks. Importantly, the combination at RAIT's MTD was safely tolerated in all animals with minimal additional weight loss over that seen with RAIT alone (Supplementary Fig. S1; no weight loss in the ADC-treated animals, not shown).

Additional studies were conducted to determine if there could be a temporal advantage by administering one treatment before the other. In this study group, the untreated animals had a median TTP of 3.4 weeks. The combination given on the same day again improved the median TTP (undefined after 18 weeks; 6 of 10 tumor free at 18 weeks) significantly compared with RAIT alone (median TTP = 16.1 weeks;  $P = 0.039$ ) or

ADC alone (median TTP = 11.6 weeks;  $P = 0.023$ ), with only 2 animals in the individual treatment groups being tumor free at the conclusion of the study. No statistical difference in TTP was determined when ADC treatment was delayed 1 or 2 weeks after RAIT, and there were 8 animals tumor free in each group. When RAIT was delayed 1 week after ADC, the antitumor effects were similar to when given on the same day ( $P = 0.456$ ). However, when RAIT was delayed for 2 weeks after the start of ADC, only 4 animals were tumor free at the conclusion of the study, with a median survival of 19.4 weeks, yielding significantly shorter TTP than when RAIT and ADC were given on the same day ( $P = 0.042$ ), indicating that a lengthy delay in initiating RAIT after ADC can reduce therapeutic efficacy.

Another set of studies compared animals given RAIT (75  $\mu\text{Ci}$   $^{90}\text{Y}$ -hPAM4) in combination with the twice weekly ADC dosing regimen, giving 0.5 mg/dose for 2, 3, or 4 weeks (i.e., 4, 6, or 8 doses, respectively), and another group that received 2, 2.0-mg doses in the same week as RAIT. In this set, untreated animals had a median TTP of 4.5 weeks and within 5 weeks all animals were censored because of tumor progression. The median TTP was not reached by the end of the study (16 weeks) in any of the other groups, but 2, 0, and 4 tumors were progressing in the groups receiving 1, 2, or 3 weeks of ADC dosing, respectively, at the termination of the study. The RAIT + ADC group that received 4 weekly injections had

**Figure 2.** Selective targeting by the ADC improves the combination response. Nude mice bearing Capan-1 tumors (all groups starting with 10 animals) were given a single treatment of  $^{90}\text{Y}$ -hPAM4 IgG (50  $\mu\text{Ci}$ ) on the same day treatment was initiated with irinotecan (13  $\mu\text{g}$ , 7.5  $\mu\text{g}$  SN-38 equivalents, twice weekly for 4 weeks). The specific (hRS7-SN-38; 0.5 mg/dose) or irrelevant (anti-CD20-SN-38; 0.5 mg/dose) conjugate was given twice weekly for 4 weeks. RAIT, radioimmunotherapy.

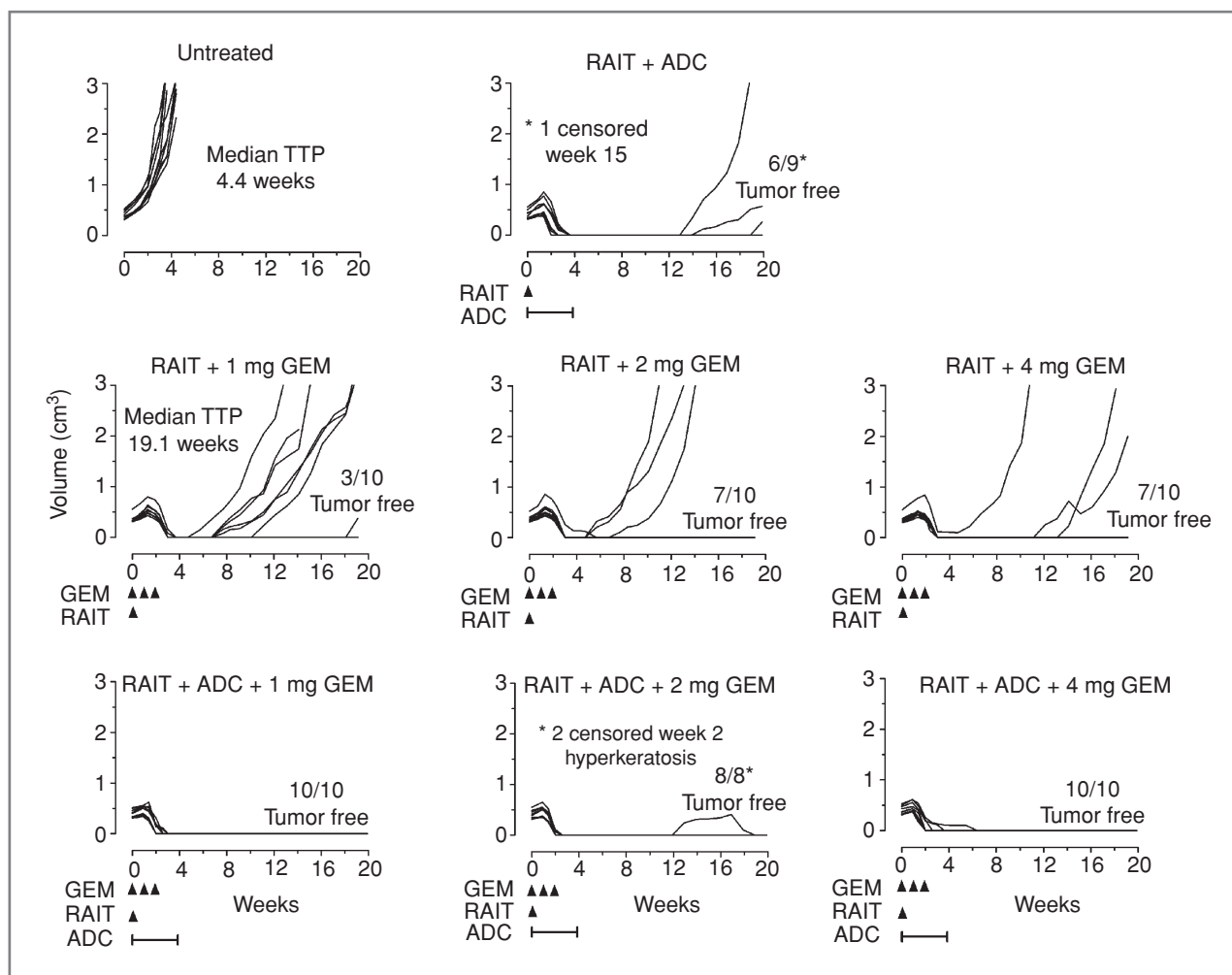


a better response than seen in the previous studies, rendering all animals tumor free in less than 4 weeks, with 1 animal removed at week 12 because of a sudden excessive loss in weight, but it was tumor free at the time. Thus, there was no evidence that shortening the number of ADC doses significantly reduced the response rate, but somewhat more tumors were progressing in those groups, whereas animals receiving 4 weeks of treatment were all tumor free at the conclusion of the study.

The efficacy of irinotecan alone and a control, nontargeted ADC (humanized anti-CD20-SN-38) was examined in comparison to the specific ADC. In this study, the RAIT dose was reduced to 50  $\mu$ Ci (50  $\mu$ g IgG) in an effort to enhance the ability to assess the therapeutic benefits of the combinations. The irinotecan dose was adjusted so the same SN-38 mole equivalents were given as contained in the ADC. At this dose, irinotecan had a similar TTP as the untreated animals (6.3 vs. 6.1 weeks, respectively; Fig. 2). Adding this amount of irinotecan to RAIT provided no additional treatment benefit. Using an

irrelevant IgG-SN-38 conjugate increased the median TTP to 15 weeks, a significant improvement over the combination with irinotecan ( $P = 0.0239$ ). Adding the specific hRS7-SN-38 conjugate not only increased the median TTP (undefined after 21 weeks), but there was a more robust initial complete response in more animals. Thus, the irrelevant conjugate is a more effective SN-38-delivery agent than irinotecan, but the specific conjugate provided additional benefit.

Because clinical studies are currently examining  $^{90}\text{Y}$ -hPAM4 IgG in combination with GEM, another study assessed the therapeutic efficacy of a single RAIT dose (75  $\mu$ Ci/50  $\mu$ g) combined with 3 weekly GEM doses at varying amounts, with or without the hRS7-SN-38 ADC given (0.5 mg twice weekly  $\times$  4 weeks). The ADC treatment was started on the same day of RAIT, but GEM treatment was given 2 days after RAIT. GEM dosing ranged from 1 to 4 mg/dose, which extrapolates to a human equivalent dose of  $\sim$ 4 to 16 mg/kg. As indicated in Fig. 3, the addition of 1 mg of GEM to RAIT was not as



**Figure 3.** RAIT, ADC, and GEM combination therapy. Nude mice bearing Capan-1 tumors (all groups started with 10 animals) were given a single treatment of  $^{90}\text{Y}$ -hPAM4 IgG (75  $\mu$ Ci/50  $\mu$ g) with GEM at the doses indicated once weekly for 3 weeks starting 2 days after the  $^{90}\text{Y}$ -hPAM4 treatment or with GEM plus the hRS7-SN-38 IgG conjugate (0.5 mg/dose, twice weekly for 4 weeks) that was initiated 2 hours before the RAIT treatment. RAIT, radioimmunotherapy.



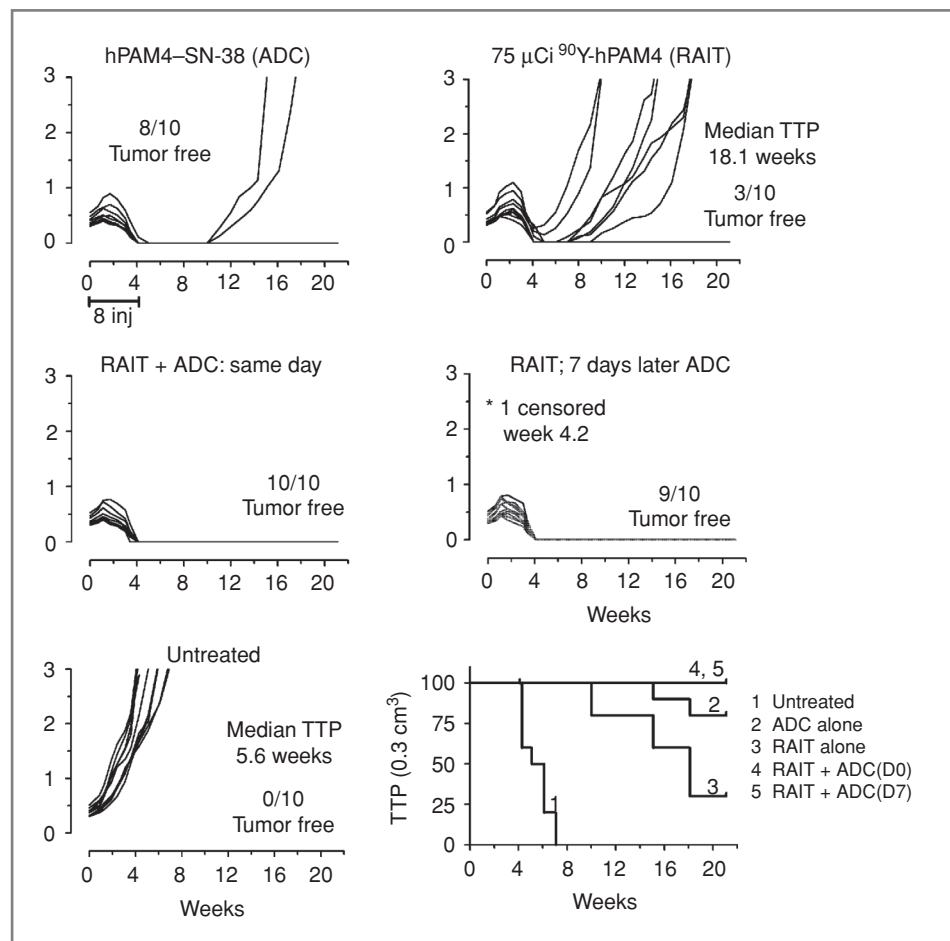
effective in controlling tumor growth as the RAIT + ADC combination, but with the higher doses of GEM, a similar number of animals were tumor free at the study's conclusion (20 weeks). When ADC was added to the combination of RAIT + GEM, irrespective of the GEM dose, all tumors were quickly eradicated, except one that initially progressed in the 2-mg GEM group 8 weeks after the cessation of the ADC treatment, but it eventually regressed. Two animals in this group were also censored just 2 weeks into the study because they developed hyperkeratosis. Animals in the treatment groups lost an average 2% to 6% of their weight 1 week after the treatments began, whereas untreated animals increased ~6% in weight, and while there was a trend for greater weight loss as the GEM dose increased, there were no significant differences among the various treatment groups.

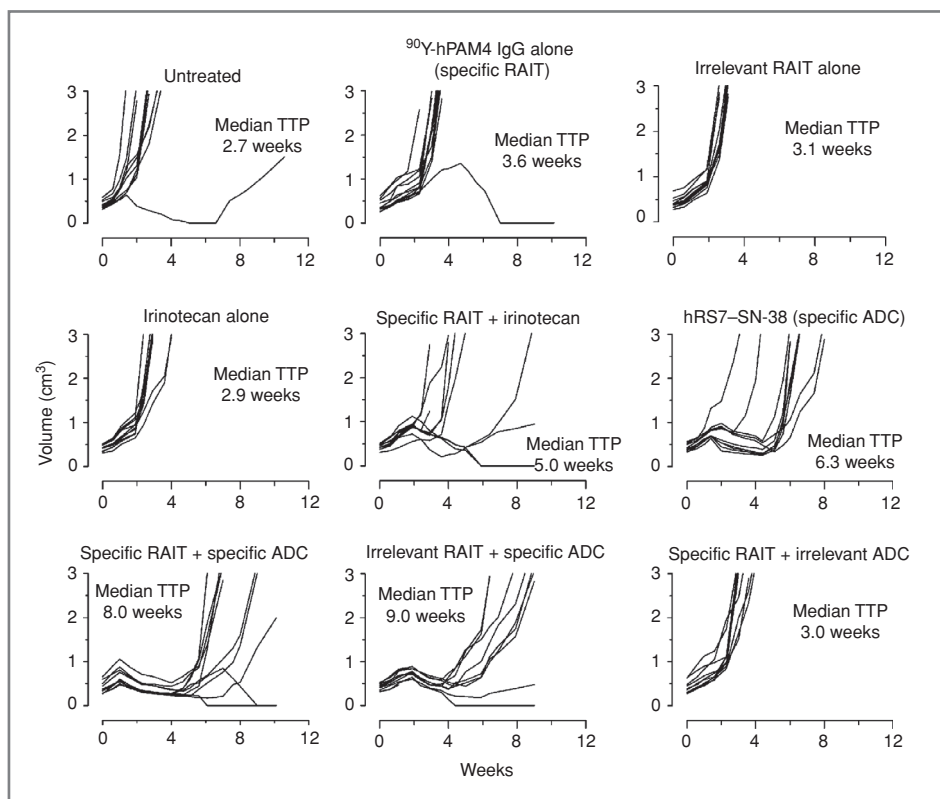
Collectively, these studies provided encouraging data that the combination of RAIT and ADC using antibodies targeting different antigens could enhance the response. We next examined the treatment of Capan-1 using RAIT and ADC targeting only the PAM4-defined mucin. Figure 4 shows the hPAM4-SN-38 conjugate was very effective in controlling tumor progression. In fact, when

compared with all the earlier studies, it appeared to be more effective than the hRS7-SN-38 conjugate in this model. ADC was given either on the same day or 1 week later, with both combination groups providing an equal ability to enhance responses compared with RAIT alone.

Because Capan-1 produces exceptionally high levels of the pancreatic mucin than most human pancreatic cancer cell lines (27), a second cell line, BxPC-3, which produced substantially less PAM4-reactive mucin, but had similar levels of Trop-2 as Capan-1, was examined. <sup>90</sup>Y-hPAM4 uptake measured 2 days after injection averaged 18.1 ± 3.8% ID/g (n = 3) in BxPC-3 xenografts (0.42 ± 0.1 g), or ~2.5-fold less than the uptake in Capan-1, but ~2-fold higher than the uptake of the <sup>90</sup>Y-labeled irrelevant anti-CD20 IgG (11.0 ± 4.5% ID/g; n = 3). <sup>90</sup>Y-hPAM4 was less effective in controlling tumor progression in this model than in Capan-1, yet it significantly extended the median TTP to 3.0 cm<sup>3</sup> from 2.7 weeks in the untreated animals to 3.6 weeks (P = 0.023; Fig. 5). Animals given an irrelevant <sup>90</sup>Y-labeled humanized anti-CD20 IgG had a median TTP of 3.1 weeks. Irinotecan alone (dose normalized to same mole equivalents of SN-38 found in the ADC) was similar to the untreated group (2.9 weeks), but the combination of irinotecan and <sup>90</sup>Y-hPAM4 RAIT enhanced the median

**Figure 4.** RAIT and ADC combination therapy targeting the same antigen. Nude mice bearing Capan-1 tumors were treated with <sup>90</sup>Y-hPAM4 IgG alone (75 μCi/50 μg), hPAM4-SN-38 IgG (0.5 mg/dose, twice weekly for 4 weeks) alone, or they were given both treatments either on the same day (ADC given 2 hours before RAIT) or delaying ADC treatment initiation for 1 week after RAIT. RAIT, radioimmunotherapy.





**Figure 5.** Combination therapy in nude mice bearing the BxPC-3 human pancreatic cancer cell line. Animals were given treatments of  $^{90}\text{Y}$ -hPAM4 IgG or the  $^{90}\text{Y}$ -labeled irrelevant anti-CD20 IgG alone (75  $\mu\text{Ci}/50\ \mu\text{g}$ ) or in combination with irinotecan (13  $\mu\text{g}$ , twice weekly for 4 weeks), the specific hRS7-SN-38 IgG or the irrelevant anti-CD20-SN-38 conjugate (ADC given twice weekly for 4 weeks, 0.5 mg/dose). RAIT, radioimmunotherapy.

TTP to 5.0 weeks ( $P = 0.0352$ ). The hRS7-SN-38 conjugate alone had a median TTP of 6.3 weeks (hRS7-SN-38 vs. irinotecan,  $P < 0.0001$ ), and when combined with  $^{90}\text{Y}$ -hPAM4, it increased to 8.0 weeks ( $^{90}\text{Y}$ -hPAM4 + hRS7-SN-38 vs. hRS7-SN-38,  $P = 0.0025$ ). Based on the similarity in response between the  $^{90}\text{Y}$ -anti-CD20 IgG and  $^{90}\text{Y}$ -hPAM4, it was not surprising that the combination of the radiolabeled irrelevant IgG with the hRS7-SN-38 resulted in the same enhanced TTP as with the specific radiolabeled IgG (median TTP = 9.0 weeks), yet the combination of  $^{90}\text{Y}$ -hPAM4 with an irrelevant anti-CD20 IgG-SN-38 conjugate yielded no additional therapeutic benefit, with a median TTP of only 3.0 weeks. Thus, the specific hRS7-SN-38 was the main agent driving the response in this model, but adding a radioimmunoconjugate did improve the response.

## Discussion

Passive immunotherapy of cancer with unconjugated (naked) mAbs has gained an important role in the management of solid tumors with agents such as cetuximab, panitumumab, trastuzumab, and bevacizumab, but most of these mAbs are used to enhance the activity of other anticancer drugs, as alone, these unconjugated antibodies have limited ability to elicit meaningful and durable responses (33). Conjugating drugs, toxins, or radionuclides to antibodies is intended to enhance the selectivity of drug delivery, but often these conjugates are used at

their maximum tolerated dose to elicit an effective response, and thus they usually are not combined with additional cytotoxic modalities unless other supportive measures are taken [e.g.,  $^{131}\text{I}$ -tositumomab and chemotherapy given with autologous stem-cell support (34)], but they could be combined with unconjugated antibodies that are therapeutic, which should enhance the overall effects (35). Whether ADC and radioimmunoconjugates can be combined without increasing host toxicities, and therefore not compromising the therapeutic index of each, was addressed in this study, where we focused on pancreatic cancer because of our development of both ADC and RAIT agents for this disease.

$^{90}\text{Y}$ -labeled hPAM4 IgG is currently being evaluated in patients with advanced pancreatic cancer based on preclinical studies that found this agent was specific and effective in treating well-established pancreatic cancer xenografts (20, 21). The initial phase I trial examining a single dose of the  $^{90}\text{Y}$ -hPAM4 IgG in patients who had received 1 prior therapy found 20 mCi/ $\text{m}^2$  was the MTD, but more importantly, there was evidence of measurable objective responses and disease stabilization in 5 patients (24). When measured against most of the earlier clinical trials in gastrointestinal cancers (19, 36–38), the initial results with radiolabeled PAM4 are encouraging; however, a more comprehensive approach is necessary. In this sense, as preclinical studies indicated that  $^{90}\text{Y}$ -hPAM4's activity is enhanced when combined with GEM (20), clinical studies evaluating this combination

are in progress as a frontline treatment, with initial results reporting an overall disease control rate was 55%, including 6 patients (16%) with partial responses and 15 (39%) with stabilization as best response (26).

In addition to the radioimmunoconjugate, we are examining the potential of ADCs in solid cancer therapy, focusing on SN-38 conjugated primarily to other anticancer mAbs, such as against CEACAM5 (31) and Trop-2 (23). We selected SN-38 because it is one of the most potent chemotherapeutic agents approved for clinical use in a water-soluble prodrug form, irinotecan (22). Irinotecan's conversion to SN-38 is inefficient, with estimates suggesting that less than 5% is cleaved to the active SN-38 form (39). We reasoned that coupling SN-38 on an antibody would improve SN-38's bioavailability in the tumor. Initial studies examined 6 conjugates prepared using different linkers and an anti-CEACAM5 antibody (31, 40). *In vitro* studies found SN-38 was gradually released from the IgG when incubated in human serum, with half-lives ranging from ~10 to 65 hours. Animal studies revealed the best therapeutic activity occurred with a linkage designated CL2 that retained 50% of the SN-38 over ~35 hours in human serum (31). The therapeutic activity of this conjugate was of particular interest because the CEACAM5 antibody did not internalize, a property generally thought to be essential for an effective ADC. We speculated that SN-38's slow release from the ADC localized in the tumor allowed for locally higher concentrations of the free drug without the requirement for internalization of the intact conjugate. Because SN-38 is given clinically as a prodrug, its slow release from the antibody was not expected to have the same dire side effects that might occur with a more ultratoxic cytotoxic agent, such as calicheamicin, auristatin, or maytansin, as these agents are too toxic to be given alone.

The initial testing of the hRS7 anti-Trop-2 IgG–SN-38 conjugate in mice and monkeys was reported recently (23). Trop-2 is an interesting cancer marker, often being associated with more aggressive forms of various cancers (41, 42); however, it is also expressed in a number of normal tissues, and thus testing in *Cynomolgus* monkeys that also express Trop-2 was imperative. They tolerated 120 mg/kg of hRS7–SN-38 (1.92 mg SN-38 equivalents/kg; human equivalent ~0.64 mg/kg SN-38 equivalents) given in 2 doses, 3 days apart, with minimal toxicity, but 240 mg/kg caused severe GI and hematologic toxicity (23). Mice, which do not express Trop-2, tolerated much higher doses than monkeys (3,000 mg/kg or 48 mg/kg SN-38 equivalents; human equivalent 3.9 mg/kg SN-38 equivalents), but significant therapeutic responses occur at human equivalent dose of 0.26 mg/kg or less SN-38 equivalents (23). Based on toxicity in monkeys and efficacy in mice, a therapeutic index of 3:1 or more is expected. This suggests that the SN-38 conjugate could be given at an effective dose with RAIT, making this an attractive combination for future clinical testing.

Other studies have cautioned that the practice of administering high doses of unlabeled anti-CD20 anti-

body therapy in advance of an anti-CD20 RAIT to ameliorate the CD20 antigen sink could reduce tumor uptake of the radioimmunoconjugate and compromise the therapeutic response in lymphoma (35, 43–45). By targeting with noncompeting antibodies, we ensure that each conjugate can reach its maximum accretion level. However, it is important to emphasize that appreciable amounts of antibody are given in the ADC treatments. As we showed previously (23), it is possible to saturate antigen in small xenografts. Thus, in a repeat-dosing regimen, the later doses might not have the same level of specific localization and retention as the earlier doses, which can diminish specificity. Because less frequent dosing of the hRS7 ADC combined with RAIT also was as effective as using the full 4-week dosing schedule, the standard 4-week treatment regimen selected at the onset was unnecessary. Indeed, the therapeutic effect of the ADC at its MTD cannot be determined reliably in xenograft models because the protein dose would likely far exceed antigen saturation levels. Thus, while the ADC used in these studies appeared to be less effective than RAIT, RAIT was examined at its MTD and about 1.7-fold less than the MTD, whereas the cumulative amount of the ADC given to the mice was 15 times lower than its MTD in mice.

The specificity of the ADC treatment is also affected by the fact the CL2A linker allows SN-38 to be released slowly from the IgG, and therefore even an irrelevant IgG was a more effective means of delivering SN-38 than irinotecan. Indeed, other nontargeted SN-38 conjugates have been very effective in preclinical testing and early clinical trials underway (46–49). Even though the slow-release design reduces specificity, the conjugates are not reliant on internalization to exert an effect (50). Indeed, if internalization were a prerequisite for potency, the hPAM4–SN-38 conjugate would not have been effective, as this antigen is primarily accessible in the extracellular pools of mucin, and not on the cell surface (RMS, unpublished data). However, as hRS7 internalizes, SN-38 could be carried inside the cell on the intact conjugate or as free SN-38 when released locally. The results in the BxPC-3 cell line also point to the value of having a dual targeting system. In this case, where PAM4–reactive mucin levels were much lower than in Capan-1, the <sup>90</sup>Y-hPAM4 was the less effective agent, but overall, the combination provided a better response than the individual agents.

As a topoisomerase I inhibitor, SN-38 exerts its optimal effects when cells are in the S-phase, and thus the 4-week treatment regimen provided sustained ADC exposure. However, because the combination had similar effects when the ADC was given in just 1 week suggests that the enhanced effects did not require an extended exposure. Temporal studies showed that response enhancement for the combination with RAIT could be achieved when the ADC was given as early as 2 weeks before RAIT to as late as 1 week after RAIT, but further delays in RAIT diminished the prospects for improvements to occur. Because GEM is currently being given clinically weekly for 4 weeks in combination with <sup>90</sup>Y-hPAM4 administered



weekly for 3 weeks, the hRS7–SN-38 IgG could either be substituted for the GEM or added with GEM, as even with the lowest dose of GEM, a significantly enhanced response was observed. However, the potential for additive toxicity of this trio combination at RAIT's MTD was not examined.

In conclusion, the combination of RAIT with <sup>90</sup>Y-hPAM4 and a noncompeting SN-38 ADC, such as hRS7–SN-38, provides a complementary platform for improving therapeutic responses in xenografted human pancreatic cancer without substantial increases in toxicity. However, we appreciate that such xenograft models are limited in terms of the effects of undesired targeting by the mAbs in normal human tissues and different pharmacological disposition of the drugs and possibly ADCs in mice versus humans. Nevertheless, just as preclinical studies of radiolabeled PAM4 alone and in combination with GEM appear to have been confirmed clinically, we are encouraged to pursue combination ADC-RAIT clinically.

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## Disclosure of Potential Conflicts of Interest

S.V. Govindan is an employee of and has financial interest in Immunomedics, Inc. D.M. Goldenberg has a financial interest in and is a member of the Board of Directors of Immunomedics, Inc.

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