The anti-CD22 ligand blocking antibody, HB22.7, has independent lymphomacidal properties, and augments the efficacy of $^{90}$Y-DOTA-peptide-Lym-1 in lymphoma xenografts.

Joseph M. Tuscano†, Robert T. O'Donnell†, Laird A. Miers*, Linda A. Kroger*, David L. Kukis*, Kathleen R. Lamborn§, Thomas F. Tedder‡, and Gerald L. DeNardo*

*Department of Internal Medicine, University of California, Davis Medical Center, Sacramento, CA 95817; † Veterans Administration of Northern California Health Care System; ‡ Duke University Medical Center, Durham, NC; § Brain Tumor Research Center, University of California San Francisco, San Francisco, CA 94143.
CD22 is a membrane glycophosphoprotein found on nearly all normal B-lymphocytes and most B-cell lymphomas. Recent *in vitro* studies have identified several anti-CD22 monoclonal antibodies (mAbs) that block the interaction of CD22 with its ligand. One of these mAbs, HB22.7, has been shown to effectively induce apoptosis in several B-cell lymphoma cell lines. Lymphoma xenograft studies with Raji-tumored mice were used to assess the toxicity and efficacy of HB22.7 both alone and with combined modality immunotherapy (CMIT) with $^{90}$Y-DOTA-peptide-Lym-1 radioimmunotherapy (RIT). The effect of the sequence of these agents on the combined treatment was assessed by administering HB22.7 24 hours prior, simultaneously, or 24 hours after RIT. Within the groups treated with RIT alone or RIT and HB22.7 (CMIT), the reduction in tumor volume was the greatest when HB22.7 was administered simultaneously and 24 hours after RIT, and in the RIT treatment groups, this translated into the greatest overall response and survival, respectively. Overall survivals at the end of the 84 day CMIT trial were 67 and 50% in the groups treated with HB22.7 simultaneously and 24 hours after RIT, respectively. This compared favorably with the untreated and RIT alone groups which had 38 and 43% surviving at the end of the trial. Surprisingly, when compared to untreated controls, and all other treatment groups, the greatest cure rate and overall survival was observed in the group treated with HB22.7 alone with 47% cured and 76% surviving at the end of the 84 day trial. RIT clearance was not affected by treatment with HB22.7. When compared to RIT alone there was no significant additional hematologic (WBC, RBC, or platelet
counts) toxicity when HB22.7 was added to RIT. Non-hematologic toxicity (assessed as change in body weight) was also unchanged when HB22.7 was added to RIT. Thus the anti-CD22 ligand blocking antibody, HB22.7, has independent lymphomacidal properties, and augments the efficacy of $^{90}$Y-DOTA-peptide-Lym-1 in lymphoma xenografts, without significant toxicity.
INTRODUCTION

Despite effective chemotherapy for non-Hodgkin’s lymphoma (NHL), more than two-thirds of patients fail to achieve long-term disease free survival. New therapeutic options are needed. RIT is systemic anti-cancer therapy that employs tumor specific, mAb to deliver cytotoxic radionuclides specifically to widespread sites of NHL, thus sparing normal tissue from excessive radiation and the associated toxicity. RIT has proven especially effective for NHL because of the radiosensitivity of NHL, the abundance of target-specific antigens on lymphocyte membranes, and the vascular accessibility of these malignancies (1-7).

The therapeutic potential of RIT in patients with NHL has been shown by a number of investigators utilizing numerous B-cell-specific targets including CD20, CD19, CD22, and HLA-DR10 (Lym-1) (8-15). Lym-1 has high affinity against a discontinuous epitope on the beta chain of the HLA-DR10 antigen on the surface of malignant B-lymphocytes (16). Due to its greater avidity for malignant rather than normal B-cells, Lym-1 preferentially targets malignant lymphocytes. Multiple preclinical and clinical studies with $^{131}$I-, $^{67}$Cu-, and $^{90}$Y-Lym-1 have demonstrated significant efficacy in relapsed and refractory NHL (8,9,17-20). In an MTD trial in which heavily pretreated NHL patients were treated with $^{131}$I-Lym-1, 85% had tumor regression, with 19% achieving a complete response (8).
Combined modality therapy (CMT) consists of the concurrent or sequential use of chemotherapy and external beam radiation. CMT has become an increasingly frequent maneuver for treatment of solid tumors and provides an example applicable to RIT for NHL. At least two concepts are involved in CMT: radiosensitization of cancer cells by drugs and the direct cytotoxic effect of chemotherapy. A randomized study in aggressive, but early stage NHL showed superior results with CHOP plus involved field radiation versus CHOP alone (21). While demonstrating that the combination of external beam radiation and chemotherapy can be beneficial for patients with NHL, it also illustrates the challenge--external beam radiation, although effective, can only be delivered in high doses to a limited region of the body, while NHL most frequently is widespread. While RIT has proven to be an effective strategy for delivery of tumor-specific radiation, to date, the higher response rates with RIT have not translated into longer overall survival rates compared with treatment using the parent naked, mAb. The efficacy of RIT is limited by toxicity, particularly myelosuppression (7, 22-25). We have taken several approaches to improve the therapeutic index of RIT. Newly developed linkers used in the conjugation of the mAb and the radiometal chelator selectively degrade in the liver. One biodegradable linker, DOTA-peptide, has demonstrated a favorable biodistribution profile when utilized with the chimeric L6 mAb in breast xenograft studies (26) and in a phase I clinical trial (27). Another approach to improving efficacy involves the enhancement of tumoricidal effects of RIT by combining RIT with chemotherapeutic agents or as described herein, antibodies (19, 28-30).
Conventional CMT has proven clinically useful for locally advanced malignancies (31-35). Combined modality immunotherapy (CMIT) goes one step further by pairing the specific delivery of systemic radiation (i.e. $^{90}$Y-DOTA-peptide-Lym-1) to NHL with the systemic radiation sensitizing effects of an additional agent (i.e. monoclonal antibody). CMIT is further enhanced by its ability to provide continuous radiation at the site of the malignancy--the ultimate in hyperfractionation. Because the radiation is delivered continuously, cancer cells that are hypoxic are more likely to pass through the radiosensitive G2/M phase of the cell cycle during the course of treatment, making cure more likely. The benefit of CMIT is provided by the specific targeting of NHL by RIT, and by the timing of the radiosensitizing agent. This allows for the radiation sensitizer to potentially synergize only at the sites targeted by RIT, thus maximizing efficacy and minimizing toxicity. In several different previous xenograft studies synergy has been demonstrated when the radiation sensitizer (Taxol) was given 24-48 hours after RIT (20,36).

CD22 is a membrane glycoprophoprotein found on nearly all B-lymphocytes and most B-cell lymphomas. Crosslinking CD22 triggers CD22 tyrosine phosphorylation and assembles a complex of effector proteins that activate the stress-activated protein kinase (SAPK) pathway. In conjunction with interleukins, antigen receptor crosslinking or CD40 crosslinking, CD22 crosslinking provides a co-stimulatory signal in primary B-cells and pro-apoptotic signal in neoplastic B-
cells (37-40). In addition, CD22 is thought to be a B-cell receptor (BCR) modulator, with recent reports demonstrating both positive and negative effects on BCR-mediated signaling (41). Recent studies have revealed that several anti-CD22 mAbs (termed HB22.7, HB22.23, and HB22.33) block the interaction of CD22 with its ligand and have distinct functional properties (42). Crosslinking CD22 on several B cell lymphoma cell lines with these mAbs resulted in a 3 to 5-fold induction of SAPK activity and efficient and effective induction of apoptosis. Based on these finding we have proposed that the blocking mAb, HB22.7, when given in the appropriate sequence will enhance the efficacy of RIT. Presented below are the results of Raji lymphoma xenograft trials that were designed to assess the toxicity and compare the efficacy of RIT (90Y-DOTA-peptide-Lym-1) alone, the combination of RIT and HB22.7 administered in three different sequences (24 hr before, simultaneously, and 24 hr after RIT), HB22.7 alone, and no treatment.

MATERIALS AND METHODS

Reagents. Carrier-free 90Y (Pacific Northwest National Laboratory, Richland, WA) and 111In (Nordion, Kanata, Ontario, Canada) were purchased as chlorides in dilute HCl. Lym-1 (Techniclone, Inc., Tustin, CA) is an IgG2a mAb generated in mice immunized with human Burkitt’s lymphoma cell nuclei. Lym-1 recognizes a cell surface 31-35 kD antigen on malignant B cells, and reacts with greater than
80% of human B cell NHL. Lym-1 purity was assessed according to the specifications that required greater than 95% pure monomeric IgG by polyacrylamide gel electrophoresis. $^{90}$Y-DOTA-peptide-Lym-1 was prepared as previously described (43). Assessment by HPLC, TLC, and cellulose acetate electrophoresis revealed that $^{90}$Y-DOTA-peptide-Lym-1 was prepared to 98% radiochemical purity with less than 5% aggregate content.

The anti-CD22 mAb, HB22.7, was prepared as previously described (42), using a Protein A Sepharose Fast Flow column (Pharmacia). HB22.7 purity was determined by HPLC and flow cytometry, and found to be >95% pure. Physiologic properties were determined by flow cytometric-based analysis of apoptotic induction (Apo-Tag, Pharmacia) and found to be consistent with previous published results (42). Endotoxin removal was achieved using an ActiClean ETOX column (Sterogene), with final endotoxin levels determined to be < 0.15 Endotoxin Units (EU)/mg mAb (Bio Whitaker). The Lym-1 and HB22.7 mAbs met MAP (mouse antibody production) guidelines for murine, viral, mycoplasma, fungal, and bacterial contamination, as well as endotoxin, pyrogen and DNA content and general safety testing in animals.

**Cell lines and Scatchard Analysis.** Raji and Ramos Burkitt lymphoma cell lines were purchased from American Type Culture Collection (ATCC, Gathersberg, MD). Both cell lines stained for CD22 expression by flow cytometric methods utilizing the HB22.7 mAb, as described previously (42). The cell lines were
maintained in RPMI 1640 supplemented with 10% fetal calf serum at 0.5 x 10^6 cells/ml. A Scatchard analysis using Raji and Ramos cells was performed as described previously (44). Briefly, HB22.7 was labeled with 125I by the chloramine T method (specific activity of 1.1 uCi/ug). A competitive binding assay was performed utilizing serially diluted, unlabeled HB22.7.

**Mouse studies.** Female athymic BALB/c nu/nu mice (Harlan Sprague-Dawley), 7-9 weeks of age were maintained according to University of California, Davis animal care guidelines on a normal diet ad libitum and under pathogen-free conditions. Five mice were housed per cage. Raji or Ramos cells were harvested in logarithmic growth phase; 2.5-5.0 X 10^6 cells were injected subcutaneously into both sides of the abdomen of each mouse. Studies were initiated 3 weeks after implantation, when tumors were 28-328 mm^3. Groups consisted of untreated, 125uCi of RIT alone, 1.4 mg of HB22.7 alone, or the combination of RIT and HB22.7, with HB22.7 being administered 24 hours prior, simultaneously, or 24 hours after RIT. To minimize ambient radiation, bedding was changed daily for 1 week after treatment with 90Y-DOTA-peptide-Lym-1, and twice weekly thereafter.

**Tumoricidal Effect.** Tumor volume was calculated as described by the formula for hemiellipsoids (45). Initial tumor volume was defined as the volume on the day prior to treatment. Mean tumor volume was calculated for each group on each day of measurement; tumors that had completely regressed were
considered to have a volume of zero. Tumor responses were categorized as follows: C, cure (tumor disappeared and did not regrow by the end of the 84 day study); CR, complete regression (tumor disappeared for at least 7 days, but later regrew); PR, partial regression (tumor volume decreased by 50% or more for at least 7 days, then regrew).

**Statistical Analysis.** Differences in response among treatment groups were evaluated using the Kruskal Wallis rank sum test with the response ordered as none, PR, CR, and Cure. Survival time was also evaluated using the Kruskal Wallis test. Tumor volume was compared at 3 time points: month 1 (day 26-29), month 2 (day 55-58), and at the end of the study (day 84). If an animal was sacrificed due to tumor-related causes, the last volume was carried forward and used in the analysis of later time points. Analysis of variance was used to test for differences among treatment groups. P values are two-tailed and represent the nominal p-values. Protection for multiple comparisons is provided by testing only within subsets of groups found to be statistically significantly different.

**RESULTS**

**Scatchard Analysis.**

Scatchard analysis was utilized to assess the binding affinity of HB22.7 and the number of CD22 receptors on Ramos and Raji cells. The cells were assayed for
maximum binding percentage (Bmax), disassociation constant (Ka) and number of antibodies bound per cell. The results are the average of two experiments.

The Scatchard analysis (Table 1) revealed a nearly 2.5 fold increase in the number of HB22.7 antibodies bound per cell, and Bmax, and a 2-fold increase in Ka for Raji cells versus Ramos cells, respectively.

### Table 1
Scatchard Analysis

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<tr>
<td>R²</td>
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<tr>
<td>Ka</td>
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Whole Body Autoradiography

In order to assess HB22.7-specific tumor targeting, whole body autoradiography of tumor-bearing nude mice injected with ¹¹¹In-2IT-BAD-anti-CD22 (HB22.7) was performed. Forty-eight hours after injection mice were sacrificed, sectioned and autoradiographed (Figure 1), as previously described (46). Autoradiography revealed intense tumor localization in the Raji-mice and moderate localization in the tumored mice. This study is consistent with...
Scatchard analysis that revealed less HB22.7 bound per Ramos cells as compared to Raji. However the rapid growth of Ramos tumors, and likely central necrosis, may also contribute to the apparent inferior targeting of Ramos.

Efficacy of RIT and CMIT

The initial trial (081500) utilized 125 uCi of $^{90}$Y-DOTA-peptide-Lym-1 alone or in combination with HB22.7 (1.4 mg) given either 24 hours prior, simultaneously, or 24 hours after RIT. In this trial there were 5 mice per group with the exception of the group treated with RIT alone, which had 9 mice and 5 untreated controls (mouse numbers are tabulated in Table 2). As predicted from similar Raji xenograft studies with $^{90}$Y-2IT-BAD-Lym-1, RIT alone resulted in maximal mean tumor volume reduction by day 21, with increasing tumor volume thereafter. Xenografts treated with $^{90}$Y-2IT-BAD-Lym-1(RIT) and HB22.7 (CMIT) demonstrated greater and more sustained mean tumor volume reduction, which was greatest when HB22.7 was administered simultaneously, and 24 hours after RIT. Surprisingly,
HB22.7 administered alone resulted in stabilization of mean tumor volume by 2-3 weeks, then a gradual and sustained tumor volume reduction.

Several additional replicate trials were conducted with highly reproducible results (Table 2). The data from all trials were compiled and revealed results highly consistent with the initial study, (Figure 2).

![Graph showing tumor volume over time for different treatment groups.](image)

**Figure 2.** The temporal assessment of tumor volume in Raji-xenografted mice that were untreated (black ■) or treated with 125 uCi ⁹⁰Y-DOTA-peptide-Lym-1 (RIT) alone (green ▲), anti-CD22 alone (HB22.7) (red ○), or three different sequences of RIT and HB22.7 (CMIT), RIT administered 24 prior (blue △), RIT administered simultaneously (turquoise ●), and RIT administered 24 hr after (fuchsia □). Tumor volume was assessed three times per week. Represents data compiled from all trials (Table 2).

Using analysis of variance, when examining all treatment groups at day 30 the differences were highly significant (p<0.001). While analysis of volume reduction in all treatment groups at day 60 did not demonstrate significant differences
(p=0.39), the differences at day 84 again were significant (p=0.003). The results observed graphically revealed that the difference in volume reduction in the RIT/CMIT groups was highly reproducible and different from HB22.7 alone and untreated control, however, comparison of volume reduction only in only RIT treatment groups (including CMIT) at all time points assessed (day 30, 60, and 84) did not reveal significant differences (p>0.5). Additional CMIT trials were done with HB22.7 being administered 48 and 72 hours after RIT. The extended interval between the administration of RIT and HB22.7 did not result in improved tumor volume reduction when compared to trials in which HB22.7 was given simultaneous and 24 hours after RIT (data not shown).

Response and cure rates were consistent with the effects of treatment on tumor volume, (Figure 3).

![Figure 3](image-url). The response and cure rate for Raji-xenografted mice that were treated as described in Figure 2. The tumor responses were categorized as follows: C, cure (tumor disappeared and did not regrow by the end of the 84-day study); CR, complete regression (tumor disappeared for at least 7 days but later regrew); PR, partial regression (tumor volume decreased by 50% or more for at least 7 days, then regrew). The data represents results of all independent trials.
Treatment with $^{90}$Y-DOTA-peptide-Lym-1 alone produced 48% PR, 13% CR, and a 13% cure rate. In the CMIT groups, the overall response rate was maximized when HB22.7 and RIT were administered simultaneously generating 45% PR, 15% CR and 25% cure. However in the CMIT groups the cure rate was the greatest (39%) when HB22.7 was administered 24 hours after RIT, which compared favorably to the cure rates observed in the untreated (29%), RIT alone (13%), 24 hours prior (10%) and simultaneous (25%) treatment groups. When examining the degree of response (ranking cure better than CR, better than PR) in all treatment groups using the Kruskal Walis test, the differences were statistically significant ($p=0.01$). Individual comparisons against untreated controls were all statistically significant ($p<0.05$), with the exception of RIT alone ($p=0.06$) and HB22.7 given 24 hours prior to RIT ($p=0.16$). While comparison of only active treatment groups (RIT alone, CMIT, and HB22.7) was not significantly different ($p=0.18$), the CMIT groups treated with HB22.7 simultaneously and after 24 hours had the best observed pattern of response. Interestingly the group treated with HB22.7 alone had the highest cure rate (47%) which was a significant improvement when compared to the untreated controls ($p<0.05$).

Tumor volume regression and cure rates translated into a similar pattern of survival. At the end of the 84 day study period 38 and 42% of the untreated and RIT alone groups were alive respectively, (Figure 4).
In the CMIT treatment groups, survival increased to 67 and 50% when HB22.7 was administered simultaneously and 24 hours after RIT, respectively. Analysis of survival using Kruskal-Wallis was significant (p< 0.05) for comparison of all groups. Similar to the response rate analysis, comparison of survival in the RIT groups only did not reveal significant differences (p=0.41), however the best survival in these groups was consistently observed when HB22.7 was administered either simultaneously or 24 hours after RIT.

The best overall survival, 76%, was observed in the group treated with HB22.7 alone, a significant difference when compared to untreated control (p=0.02).

Toxicity
Hematologic and non-hematologic toxicities were assessed by blood counts (Figure 5 A-C) and mouse weights, respectively.

Figure 5. Hematologic toxicity was assessed by measuring white blood cell (WBC), red blood cell (RBC) and platelet counts twice weekly in the Raji-xenografted mice that were treated as described in Figure 2. When compared to RIT alone there was no difference in hematologic toxicity in the CMIT groups. In addition, there was no hematologic toxicity observed in the mice treated with HB22.7 alone.
WBC and platelet nadirs in the RIT treatment groups were at 14-20, and 10-14 days respectively. WBC and platelet recovery was approximately 28 and 21 days after treatment, respectively. The WBC and platelet nadirs were consistent with observations in previous studies that utilized 150uCi of $^{90}$Y-2IT-BAD-Lym-1. The hematologic toxicity of RIT was not altered by co-administration of HB22.7. No hematologic toxicity was detected in mice treated with HB22.7 alone. Analysis of mononuclear cell counts in all treatment groups revealed that HB22.7 had no effect on RIT-mediated mononuclear cell nadirs (data not shown). Non-hematologic toxicity as assessed by changes in mouse weight, and was found to be equivalent in all treatment groups (data not shown). There were no deaths due to toxicity in any treatment groups.

$^{90}$Y-DOTA-peptide-Lym-1 Pharmacokinetics

Blood and whole body clearances of $^{90}$Y-DOTA-peptide-Lym-1 in Raji-tumored mice with or without HB22.7 were similar (Figure 6).
The blood biological $T_{1/2}$ $\alpha$ was 1.4 hours for RIT alone, and 2.2, 2.4, and 2.0 hours for the 24 hour prior, simultaneous and 24 hour after groups respectively. The blood biological $T_{1/2}$ $\beta$ was 127 hours for the RIT alone group and 133, 87, and 103 hours for the 24 hours prior, simultaneous and 24 hours after groups respectively. The whole body $T_{1/2}$ was 246 hours for RIT alone and 207, 207, and 196 hours for the 24 hours prior, simultaneous and 24 hours after groups respectively. The addition of HB22.7 to RIT did not change the pharmacokinetics of $^{90}$Y-DOTA-peptide-Lym-1.
Discussion

Raji xenograft studies were designed to determine if the anti-CD22 mAb (HB22.7) would generate additive or synergistic effects when combined with RIT to enhance apoptosis and/or DNA damage induced by low dose-rate radiation. The Raji xenograft nude mouse model has proven useful when used to assess toxicity and efficacy of RIT using $^{90}$Y-2IT-BAD-Lym-1 RIT alone (19). Responses in this pre-clinical model translated into significant efficacy in human clinical trials (20,47).

In the study described herein, the addition of the anti-CD22 mAb HB22.7 to $^{90}$Y-DOTA-peptide-Lym-1(125uCi ) enhanced the efficacy of RIT without any change in toxicity. Previous Raji xenograft studies with 150 and 200uCi of $^{90}$Y-2IT-BAD-Lym-1 generated response and cure rates that were comparable to those observed in the present study (19). The 125 uCi dose of $^{90}$Y-DOTA-peptide-Lym-1 was chosen based on these previous studies with the 2IT-BAD linker. While the previous studies with 2IT-BAD demonstrated greatest efficacy with the 200 uCi dose, the choice of 125 uCi was based on the hypothesis that HB22.7 would be synergistic or additive with RIT and the lower dose would allow for better assessment of these effects. This study utilized a novel linker (DOTA-peptide) that has not been previously examined in lymphoma xenograft models. The DOTA-peptide linker was designed for enhanced hepatic degradation of unbound radiopharmaceutical thereby leading to a more favorable biodistribution. While
tumor-specific uptake was not assessed in detail in this study, the toxicity profile observed with 125 uCi of $^{90}$Y-DOTA-peptide-Lym-1 alone was acceptable with no treatment-related mortality and predictable leukocyte and platelet nadirs.

HB22.7 was chosen based on *in vitro* studies demonstrating pro-apoptotic and signaling effects (42). The treatment dose of HB22.7 utilized was empiric, however it was based on the amount that was shown to be effective at inducing apoptosis *in vitro* and extrapolating this to the mouse model. In addition, when formulating the dose of HB22.7 consideration was given to the equivalent (when adjusted for body surface area differences in humans versus mice) dose of Rituximab® used in human clinical trials. The approximation to the rituximab dose was utilized based on the fact that this is the only naked mAb available that has demonstrated efficacy for the treatment of lymphoma, granted, the optimal dose of rituximab is currently undefined.

The study was designed to assess the efficacy of HB22.7 alone, the combination of RIT and HB22.7 as well as the effect of three different sequence combinations. The tumor volume reduction observed with $^{90}$Y-DOTA-peptide-Lym-1 alone was consistent with previous studies with $^{90}$Y-2IT-BAD-Lym-1 in terms of timing, magnitude, and duration of response (19). RIT alone resulted in approximately 50% reduction in tumor volume 14 days after therapy. When assessing at the approximate point of maximal volume reduction (day 21-30) the addition of HB22.7 to RIT significantly enhanced the magnitude of response in a sequence
specific manner. It appears that the addition of HB22.7 was most effective when administered simultaneously or 24 hours after RIT. The distinctive pattern of volume reduction was highly reproducible. Independent replicate trials demonstrated similar patterns and magnitude of tumor volume reduction. The improved reductions in tumor volume translated into superior response rates and survival. RIT alone generated 13% CR and 13% cures, the addition of HB22.7 increased the cure rate to 25% when administered simultaneously with RIT, and to 39% when HB22.7 was administered 24 hours after RIT.

The mechanism by which HB22.7 augments the tumoricidal effects of RIT is an area of active investigation. The mechanism by which apoptotic signals are transferred from the membrane to the nucleus is only partially known. In some cell types this mechanism has been shown to involve the stress-activated protein kinase (SAPK/JNK) system (42). The SAPK/JNK cascade is a generic signaling system that becomes activated by a variety of stimuli and cellular stress (48). Previous studies have shown that exposure of various cell lines to ionizing and UV radiation activated the SAPK cascade downstream of ceramide (49,50). These data suggest that the SAPK/JNK cascade couples membrane and nuclear elements of the apoptotic pathways which become activated by ionizing radiation and other cellular stresses. Previous studies have demonstrated that the pro-apoptotic effects of the mAb HB22.7 are mediated via the SAPK pathway as well (42), and that the SAPK pathway is a known apoptotic mediator in B cell lymphomas. We have hypothesized that the sequence dependence of RIT and
HB22.7 is likely due to augmented SAPK activation by HB22.7 and subsequent downstream pro-apoptotic effects. SAPK activation mediated by ionizing radiation can occur up to four hours after irradiation. Given the time from administration of RIT to optimal binding and SAPK induction, augmentation of SAPK activation by HB22.7 24 hours after RIT administration would account for the additive tumoricidal and clinical effects observed with this sequence. In addition, while in vitro studies have demonstrated the pro-apoptotic potential of HB22.7 in lymphoma cell lines, there is no direct evidence that this is occurring in vivo in this xenograft model. However the observed lymphomacidal effects are potentially consistent with in vivo pro-apoptotic effects. This is the first time that a second monoclonal antibody has been combined with RIT, and demonstrates the potential of utilizing monoclonal antibodies or other agents with well-defined physiologic properties that may augment efficacy without increasing toxicity.

Surprisingly the mice treated with HB22.7 alone had impressive tumor volume reduction and superior cure and survival rates when compared to all other treatment groups. Again, several independent trials generated highly consistent results with a delayed initial tumor volume stabilization, and then tumor volume reduction beginning approximately 14 days after treatment. This translated into the best cure and overall survival rates observed in any of the treatment groups. The high cure rates and survival observed with HB22.7 alone are under active investigation. While the possibility exists that the residual natural killer (NK) cell function known to be present in nude mice could account for host immune
response that mediate ADCC, this becomes less likely given that the mice are irradiated prior to tumor implantation. In addition, published xenograft studies that utilize unconjugated or naked murine mAbs have not reported similar findings.

While the data clearly demonstrates that HB22.7 and $^{90}$Y-DOTA-peptide-Lym-1 have independent lymphomacidal effects, the data also shows that the pattern of response to these two agents is different. RIT generates prompt tumor shrinkage followed by re-growth of NHL xenografts, while the anti-tumor response to HB22.7 was slower in onset, but sustained. All groups treated with RIT had tumor volume reductions at day 14, a time when the group treated with HB22.7 alone still had increasing tumor volume similar to the untreated control. However, at day 28 the group treated with HB22.7 alone had begun a pattern of consistent tumor shrinkage, while all of the groups treated with RIT (with or without HB22.7) had increasing tumor volumes. The data also show that administration of HB22.7 concurrent with, or 24 hours after RIT augmented the anti-tumor response from RIT as demonstrated by a longer time to tumor progression compared to RIT alone. However, the addition of RIT to HB22.7 did not improve the overall survival or CR rate, which was best with HB22.7 alone. This suggests that these two antibodies act by invocation of different cellular mechanisms, and may involve both synergistic and antagonistic effects.

The pattern of response to these two agents suggests that HB22.7 required time to work, but continued to exert anti-tumor effects over the 84 day study period. Therefore, mice that had rapid and substantial early tumor shrinkage secondary
to RIT, subsequently also benefited from the later effects of HB22.7. This is similar clinically to “induction” followed by “consolidation” therapy for human hematologic malignancies. This is also a quite useful combination because the late therapeutic effect of HB22.7 does not carry with it additional myelosuppression. Conversely, there was no “long-term” additional efficacy from the addition of RIT to HB22.7 because all of the positive effects of RIT were early, within the first 42 days.

The fact that tumor volume reduction and survival were better for HB22.7 alone than for CMIT groups at the end of the 84 day study, suggests not only that HB22.7 and 90Y-DOTA-peptide-Lym-1 have different mechanisms of anti-tumor efficacy, but that there may be some negative interactions as well. Unlabeled Lym-1 has little if any anti-tumor effect by itself, although there is very limited data on its physiologic effects. Studies examining the effects of co-crosslinking CD22 and class II have revealed that co-crosslinking of these two receptors can attenuate class II-mediated signals (52). Lym-1 binds a class II subtype, HLA-DR10. Based on the difference observed when HB22.7 was added to RIT, and the previously established signaling/pro-apoptotic properties of HB22.7, one could postulate that radiolabeled-Lym-1 inhibits an apoptotic pathway that would otherwise have been activated by HB22.7 while still having independent lymphomacidal activity. Further study is required to elucidate the physiologic mechanisms involved in the interaction of anti-CD22 and anti-HLA DR antibodies.
in lymphoma therapy and validates the need to understand the physiologic properties of mAb binding for rational design of combination therapies.

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