A Phase II Study of Adjuvant Therapy with Anti-B4-blocked Ricin after Autologous Bone Marrow Transplantation for Patients with Relapsed B-Cell Non-Hodgkin’s Lymphoma

Michael L. Grossbard, Pratik S. Multani, Arnold S. Freedman, Steven O’Day, John G. Gribben, Catherine Rhuda, Donna Neuberg, and Lee M. Nadler

Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, Massachusetts 02115 [A. S. F., S. O., J. G. G., C. R., D. N., L. M. N.]

ABSTRACT

This Phase II trial was undertaken to determine the safety, toxicity, and potential efficacy of the B-cell restricted immunotoxin anti-B4-blocked ricin (Anti-B4-bR) when administered as adjuvant therapy to patients in complete remission (CR) after autologous bone marrow transplantation (ABMT) for B-cell non-Hodgkin’s lymphoma (NHL). Forty-nine patients with B-cell NHL in CR 46–202 days (median, 112 days) post-ABMT received Anti-B4-bR at a dose of 30 μg/kg lean body weight/day for 7 days by continuous i.v. infusion. Patients were eligible for up to two additional courses of therapy at 14-day intervals. A total of 83 courses of Anti-B4-bR were administered, with 31 patients receiving two or more courses of therapy. The mean serum level on day 7 of the first course was 0.77 ± 0.41 nM. Reversible toxicities included hepatic transaminase elevations, thrombocytopenia, myalgias, fatigue, nausea, hypoalbuminemia, and dyspnea. Human antimouse antibody (HAMA) and/or human antiricin antibody (HARA) responses occurred in 23 patients at a median of 22 days from the initiation of Anti-B4-bR therapy (range, 11–100 days). The 4-year disease-free survival and overall survival are estimated at 56 and 72%, respectively. Twenty-six patients remain in CR after a median follow-up of 54.5 months. This study demonstrates that residual lymphoma cells persisting within the patient or within autologous reinfused marrow can contribute to relapse (3, 4). New modalities of therapy that are non-cross-resistant with standard chemotherapy and radiation therapy may be required to eliminate these residual cells and improve outcome. Ideally, such agents should have nonoverlapping toxicities with chemotherapy and radiation therapy to allow them to be administered either concurrent with or in close proximity to high-dose therapy.

INTRODUCTION

High-dose myeloablative therapy followed by ABMT1 can induce CRs in most patients with chemotherapy-sensitive relapsed NHL (1, 2). Unfortunately, disease recurs in more than 50% of patients within 2 years of completing therapy. Recent studies demonstrate that residual lymphoma cells persisting within the patient or within autologous reinfused marrow can contribute to relapse (3, 4). New modalities of therapy that are non-cross-resistant with standard chemotherapy and radiation therapy may be required to eliminate these residual cells and improve outcome. Ideally, such agents should have nonoverlapping toxicities with chemotherapy and radiation therapy to allow them to be administered either concurrent with or in close proximity to high-dose therapy.

We have reported previously the use of Anti-B4-bR as a therapy for patients with relapsed B-cell neoplasms (5, 6). This immunotoxin combines the specificity of the Anti-B4 (CD19) antibody with the cytotoxicity of blocked-ricin toxin (7). Unfortunately, formidable obstacles arise in treating patients with large tumor burdens, including the rapid clearance of immunotoxin due to antigen excess and the impaired diffusion of these sizable molecules into tumor masses (8, 9). Moreover, immunotoxin penetration into lymphomatous nodes may be hampered by poor capillary permeability and the presence of tight junctions in the vasculature (10, 11).

In an effort to circumvent the problem of immunotoxin delivery and provide a non-cross-resistant therapy for patients with B-cell NHL, we conducted a Phase I trial using Anti-B4-bR as adjuvant therapy for patients in CR after ABMT (12). We demonstrated that Anti-B4-bR could be administered safely to patients as early as 61 days after ABMT with a MTD of 40 μg/kg/d × 7 days by continuous i.v. infusion. The DLT was defined by transient, reversible grade 4 thrombocytopenia and hepatic transaminase elevations. Serum levels above 0.5 nM, which were predicted to be therapeutic based on in vitro studies, could be achieved and sustained for up to 5 days. Although patients were eligible to receive repeat courses of therapy at 28-day intervals, the development of HAMA and HARA within

1 Supported by NIH Grants CA66996 and CA55207. M. L. G. was a recipient of a National Cancer Institute Clinical Oncology Research Career Development Award (1K2CA01730).

2 To whom requests for reprints should be addressed, at Hematology/Oncology Unit Cox 2, Massachusetts General Hospital, 100 Blossom Street, Boston, MA 02114. Phone: (617) 724-1134; Fax: (617) 724-1137; E-mail: mgrossbard@partners.org.

3 The abbreviations used are: ABMT, autologous bone marrow transplant; CR, complete remission; NHL, non-Hodgkin’s lymphoma; Anti-B4-bR, anti-B4-blocked ricin; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; HAMA, human antimouse antibody; HARA, human antiricin antibody; ECOG, Eastern Cooperative Oncology Group; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; HUS, hemolytic-uremic syndrome; DFS, disease-free survival.
therapy administered at 14-day intervals.

3 day

PATIENTS AND METHODS

Anti-B4-bR. Anti-B4-bR was manufactured and supplied by ImmunoGen, Inc. (Cambridge, MA) as described previously (5–7, 13). Anti-B4-bR was formulated as a sterile injectable solution containing 100 µg/ml Anti-B4-bR dissolved in PBS (pH 7.3), with 1 mg/ml human serum albumin (Baxter Hyland, Glendale, CA) added as a carrier. Anti-B4-bR was stored at 2–8°C before administration.

Patient Selection. Patients eligible for this study underwent ABMT for B-cell NHL at the Dana-Farber Cancer Institute between 7/1/91 and 8/30/93. Tumor cells obtained from all of the patients before ABMT were required to demonstrate reactivity with the anti-B1 (CD20) or anti-B4 (CD19) monoclonal antibodies. Before ABMT, all of the patients had disease that had relapsed after one or more primary or salvage chemotherapy regimens. All of the patients had chemotherapy-sensitive disease, as defined by the ability after salvage chemotherapy to achieve a minimal disease state (12). At the time of bone marrow harvest, marrow from the first 31 patients was purged with a cocktail of three monoclonal antibodies (anti-B1, anti-B5, and J5) as described previously (14). For the last 18 patients on the study, the anti-B4 monoclonal antibody was added as a fourth antibody used in purging.

All of the patients received myeloablative therapy with cyclophosphamide (60 mg/kg of body weight/day), infused on each of 2 consecutive days. After completing chemotherapy, all of the patients received total body irradiation in fractionated doses, 200 cGy twice daily on 3 consecutive days (total dose, 12 Gy). Within 18 h after the completion of total body irradiation, all of the patients received an infusion of purged autologous marrow.

Patients were eligible for treatment with Anti-B4-bR if they were in CR at least 30 days and if it had been no longer than 210 days since the reinfusion of autologous marrow. A CR was documented in all of the patients by physical examination, computerized tomographic scans of previous sites of disease, gallium scans in patients with prior documentation of gallium-avid disease, chest radiographs, and bone marrow biopsies. Patients were required to have an ECOG performance status of ≤ 2 at the time of therapy. At protocol entry, all of the patients were required to have adequate hematopoietic engraftment, as defined by an absolute neutrophil count ≥ 500/µL, hemoglobin ≥ 90 g/dL, platelet count ≥ 100,000/µL, and leukocyte count ≥ 4,000/µL. Patients had no prior history of hepatic veno-occlusive disease or hepatitis B or C infection. In addition, patients were excluded from therapy if their SGOT or SGPT rose to greater than five times the upper limit of normal at any time during ABMT. The clinical protocol was approved by the Institutional Review Board of the Dana-Farber Cancer Institute, and all of the patients signed an informed consent form approved by that committee.

Study Design. Within 14 days after the documentation of CR, patients began a continuous i.v. infusion of Anti-B4-bR via a central venous line. All of the patients received a dose of 30 µg/kg lean body weight/day × 7 consecutive days. Lean body weight (LBW) was calculated using the following formulas:

\[
\text{LBW}_{\text{Male}} = 50 \text{ kg} + 2.3 \times (\text{height in inches above 60 inches})
\]

\[
\text{LBW}_{\text{Female}} = 45.5 \text{ kg} + 2.3 \times (\text{height in inches above 60 inches})
\]

Patients received the infusion as outpatients via a Pharmacia CADD-1 infusion pump and returned to the clinic on Day 4 ± 24 h and Day 7 for laboratory studies, physical examination, and to receive a new supply of Anti-B4-bR for the pump cartridge. Patients were monitored for the development of toxicities during the course of the infusion.

All of the patients were eligible for retreatment at the same dose every 14 days up to a maximum of three courses of therapy if they continued to meet protocol eligibility requirements, had recovered from all of the toxicities of grade 3 or greater incurred during the prior course of therapy, failed to develop HAMA or HARA after their prior course of therapy, and agreed to continue on the protocol.

Blood samples were obtained from patients at each scheduled visit to examine hepatic transaminases, serum albumin, hematological parameters, and creatine phosphokinase, as well as for pharmacological analysis. Follow-up exams and laboratory studies were required 28 days after completing therapy to ascertain that all of the side effects considered related to Anti-B4-bR infusion had resolved. Formal restaging of all of the patients, including computerized tomographic scans, gallium scans in patients with prior evidence of gallium-avid disease, and bone marrow biopsies, was conducted at 6-month intervals post-ABMT. Median 4.5-year follow-up data are included in this report.

Pharmacology. Blood samples were obtained for the determination of serum levels of Anti-B4-bR just before immuno-toxin infusion and at day 4 ± 24 h, and on day 7 at the conclusion of the infusion. Anti-B4-bR concentration in serum was determined using two independent ELISA methods described previously (6).

HAMA/HARA Detection. HAMA and HARA were measured by established ELISA techniques as described previously (5). HAMA was considered positive if the value was >0.468 µg/ml. HARA was considered positive if the value was >20% above the pretreatment value in the ELISA. All of the patients had HAMA/HARA assays done at baseline, within 72 h before receiving a repeat course of therapy, and again at the initiation of each course of therapy.

PCR Amplification. Nested oligonucleotide amplification of genomic DNA was performed, as described previously (14), at both the major breakpoint region and the minor cluster region of the bcl-2/IgH hybrid gene, in bone marrow samples obtained from 33 patients before bone marrow harvest, after...
Phase II Adjuvant Anti-B4-bR Post-ABMT in B-Cell NHL

One course of therapy, 29 patients receiving two courses, and 3 of Anti-B4-bR were administered, with 17 patients receiving

had received three or more chemotherapy regimens (excluding patients had low-grade NHL, and nearly one-third of the patients ranged from 24 to 59, with a median age of 46 years. Thirty-four 49 patients.

This report documents the observations made on the remaining cause he received syngeneic instead of autologous bone marrow. Finally, 1 of the 50 treated patients was excluded from the analysis because he received syngeneic instead of autologous bone marrow.

Patient Selection and Characteristics. Between 7/1/91 and 8/30/93, 123 patients underwent ABMT for NHL, of which 50 patients were treated with Anti-B4-bR. There were several reasons why patients did not receive Anti-B4-bR, although we were unable to record how many patients were not enrolled for reasons why patients did not receive Anti-B4-bR, although we

were unable to record how many patients were not enrolled for any given reason. These reasons included failure to achieve CR after ABMT, inability to follow-up because of living at a distance from the Dana-Farber Cancer Institute, patient refusal of further therapy, and inadequate platelet engraftment. Finally, 1 of the 50 treated patients was excluded from the analysis because he received syngeneic instead of autologous bone marrow. This report documents the observations made on the remaining 49 patients.

Patient characteristics are listed in Table 1. Patient age ranged from 24 to 59, with a median age of 46 years. Thirty-four patients had low-grade NHL, and nearly one-third of the patients had received three or more chemotherapy regimens (excluding the conditioning regimen during ABMT). A total of 79 courses of Anti-B4-bR were administered, with 17 patients receiving one course of therapy, 29 patients receiving two courses, and 3 patients receiving three courses of therapy. Reasons for discontinuation of therapy were patient refusal in 18 cases, development of HAMA/HARA in 17 cases, serious toxicity in 9 cases, progressive disease in 1 case, and poor compliance with follow-up in 1 case.

Pharmacology. Fig. 1 demonstrates the serum levels of Anti-B4-bR measured in one patient during three consecutive courses of therapy. Serum levels above 0.25 nM could be achieved by day 4 in 43 of the 46 patients sampled during this time period. The mean serum level obtained on the final day of infusion (day 7) during the first course of therapy was 0.77 ± 0.41 nM.

Toxicity. Systemic toxicities were observed frequently in patients treated with Anti-B4-bR, although it is difficult to distinguish between toxicities related to the post-ABMT setting versus definite drug-related toxicity (see Table 2). Most adverse reactions were never more severe than grade 1 or grade 2 and were reversible in all of the cases. Myalgias were a particularly prominent complaint (i.e., grade 2 or greater) in 13 patients and more frequently involved the lower extremities than upper extremities. In contrast to reports with other immunotoxins, no creatine phosphokinase elevations were detected, and there was no evidence of rhabdomyolysis.

In Phase I trials of Anti-B4-bR, both in patients with relapsed NHL and in post-ABMT patients, the DLT was defined by grade 3 and 4 hepatic transaminase elevations and thrombocytopenia. With the lower dose of Anti-B4-bR administered to patients on this trial, only two cases of grade 3 elevations of SGOT and SGPT were seen, and there were no cases of grade 4 toxicity. In no case was there evidence of impaired synthetic function of the liver as manifested by an elevated prothrombin time. Furthermore, despite the fact that nine patients began protocol therapy with platelet counts below 75,000, there were only three cases of grade 4 thrombocytopenia and only one case of grade 1 bleeding, manifested as self-limited epistaxis.

Manifestations of capillary leak syndrome occurred frequently but did not prove dose limiting. Six patients had a reduction of 20% or more in serum albumin from baseline. Edema and dyspnea occurred frequently but were not dose limiting and did not require therapy. Three patients developed evidence of grade 3 or 4 HUS within 6 months of completing Anti-B4-bR therapy. This syndrome occurs frequently in the post-ABMT setting (15), although in one of these patients, severe HUS developed during a second course of Anti-B4-bR therapy, making it impossible to exclude a causal relationship.

An acute hypersensitivity reaction was observed in one patient 2 h after the initiation of a second course of Anti-B4-bR. The patient had tolerated the first course without complication. Symptoms included pruritis with erythema, dyspnea, chest tightness, and dizziness, which resolved promptly with a single dose of diphenhydramine. Seven other patients developed macular rashes while on therapy with Anti-B4-bR. Because many of these patients were on other therapies including prophylactic trimethoprim/sulfamethoxazole therapy, it is difficult to determine whether these allergic manifestations were directly related to Anti-B4-bR therapy.

HAMA/HARA. Although these patients were immunosuppressed secondary to both their underlying disease and recent ABMT, antibody response to the immunotoxin occurred

### Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>No. of patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27</td>
</tr>
<tr>
<td>Female</td>
<td>22</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>&lt; 40</td>
<td>16</td>
</tr>
<tr>
<td>40–50</td>
<td>18</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>15</td>
</tr>
<tr>
<td>Lymphoma grade (IWF)</td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>34</td>
</tr>
<tr>
<td>WDLL</td>
<td>2</td>
</tr>
<tr>
<td>FSCCL</td>
<td>21</td>
</tr>
<tr>
<td>FML</td>
<td>10</td>
</tr>
<tr>
<td>Mantle zone</td>
<td>1</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>15</td>
</tr>
<tr>
<td>DSCCL</td>
<td>3</td>
</tr>
<tr>
<td>DML</td>
<td>3</td>
</tr>
<tr>
<td>DLCL</td>
<td>7</td>
</tr>
<tr>
<td>Other*</td>
<td>2</td>
</tr>
<tr>
<td>Initial sites of disease</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>46</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>31</td>
</tr>
<tr>
<td>Extramedial</td>
<td>20</td>
</tr>
<tr>
<td>No. of prior regimens*</td>
<td></td>
</tr>
<tr>
<td>≤ 2</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>&gt; 3</td>
<td>4</td>
</tr>
</tbody>
</table>

* IWF, International Working Formulation; WDLL, well-differentiated lymphocytic lymphoma; FSCCL, follicular small cleaved cell lymphoma; FML, follicular mixed lymphoma; DSCCL, diffuse small cleaved cell lymphoma; DML, diffuse mixed lymphoma; DLCL, diffuse large cell lymphoma.
* One case each of: (a) follicular large cell lymphoma; and (b) follicular and diffuse large cell lymphoma.
* Excluding ABMT conditioning regimen.

marrow purging with monoclonal antibodies and complement, before therapy with Anti-B4-bR, and after therapy with Anti-B4-bR.

**RESULTS**

Patient Selection and Characteristics. Between 7/1/91 and 8/30/93, 123 patients underwent ABMT for NHL, of which 50 patients were treated with Anti-B4-bR. There were several reasons why patients did not receive Anti-B4-bR, although we were unable to record how many patients were not enrolled for any given reason. These reasons included failure to achieve CR after ABMT, inability to follow-up because of living at a distance from the Dana-Farber Cancer Institute, patient refusal of further therapy, and inadequate platelet engraftment. Finally, 1 of the 50 treated patients was excluded from the analysis because he received syngeneic instead of autologous bone marrow. This report documents the observations made on the remaining 49 patients.

Patient characteristics are listed in Table 1. Patient age ranged from 24 to 59, with a median age of 46 years. Thirty-four patients had low-grade NHL, and nearly one-third of the patients had received three or more chemotherapy regimens (excluding the conditioning regimen during ABMT). A total of 79 courses of Anti-B4-bR were administered, with 17 patients receiving one course of therapy, 29 patients receiving two courses, and 3 patients receiving three courses of therapy. Reasons for discontinuation of therapy were patient refusal in 18 cases, development of HAMA/HARA in 17 cases, serious toxicity in 9 cases, progressive disease in 1 case, and poor compliance with follow-up in 1 case.

Pharmacology. Fig. 1 demonstrates the serum levels of Anti-B4-bR measured in one patient during three consecutive courses of therapy. Serum levels above 0.25 nM could be achieved by day 4 in 43 of the 46 patients sampled during this time period. The mean serum level obtained on the final day of infusion (day 7) during the first course of therapy was 0.77 ± 0.41 nM.

Toxicity. Systemic toxicities were observed frequently in patients treated with Anti-B4-bR, although it is difficult to distinguish between toxicities related to the post-ABMT setting versus definite drug-related toxicity (see Table 2). Most adverse reactions were never more severe than grade 1 or grade 2 and were reversible in all of the cases. Myalgias were a particularly prominent complaint (i.e., grade 2 or greater) in 13 patients and more frequently involved the lower extremities than upper extremities. In contrast to reports with other immunotoxins, no creatine phosphokinase elevations were detected, and there was no evidence of rhabdomyolysis.

In Phase I trials of Anti-B4-bR, both in patients with relapsed NHL and in post-ABMT patients, the DLT was defined by grade 3 and 4 hepatic transaminase elevations and thrombocytopenia. With the lower dose of Anti-B4-bR administered to patients on this trial, only two cases of grade 3 elevations of SGOT and SGPT were seen, and there were no cases of grade 4 toxicity. In no case was there evidence of impaired synthetic function of the liver as manifested by an elevated prothrombin time. Furthermore, despite the fact that nine patients began protocol therapy with platelet counts below 75,000, there were only three cases of grade 4 thrombocytopenia and only one case of grade 1 bleeding, manifested as self-limited epistaxis.

Manifestations of capillary leak syndrome occurred frequently but did not prove dose limiting. Six patients had a reduction of 20% or more in serum albumin from baseline. Edema and dyspnea occurred frequently but were not dose limiting and did not require therapy. Three patients developed evidence of grade 3 or 4 HUS within 6 months of completing Anti-B4-bR therapy. This syndrome occurs frequently in the post-ABMT setting (15), although in one of these patients, severe HUS developed during a second course of Anti-B4-bR therapy, making it impossible to exclude a causal relationship.

An acute hypersensitivity reaction was observed in one patient 2 h after the initiation of a second course of Anti-B4-bR. The patient had tolerated the first course without complication. Symptoms included pruritis with erythema, dyspnea, chest tightness, and dizziness, which resolved promptly with a single dose of diphenhydramine. Seven other patients developed macular rashes while on therapy with Anti-B4-bR. Because many of these patients were on other therapies including prophylactic trimethoprim/sulfamethoxazole therapy, it is difficult to determine whether these allergic manifestations were directly related to Anti-B4-bR therapy.

HAMA/HARA. Although these patients were immunosuppressed secondary to both their underlying disease and recent ABMT, antibody response to the immunotoxin occurred
intermediate/high-grade NHL (survival curves not shown).

For the patients with low-grade NHL were 82 and 68%, respectively, sub-grouped by grade, the four-year overall survival and DFS were assessed. The median follow-up of 54.5 months. The 4-year overall survival for 49 patients treated with Anti-B4-bR remain disease-free after a median follow-up of 35 months. Two patients subsequently converted to negative after Anti-B4-bR therapy and remained negative for at least 8 months. Two of the eight patients subsequently became PCR-positive 21 days and 42 days after initiation of treatment with Anti-B4-bR. The results of this Phase II trial demonstrate that Anti-B4-bR can be administered safely at 14-day intervals to patients in CR. Unfortunately, the use of chemotherapy and radiation therapy in the post-ABMT setting is limited by the impaired marrow reserve of patients early after ABMT. In addition, residual tumor cells that remain after ABMT may be resistant to these conventional therapeutic modalities. Thus, alternative agents, such as IFN-α (16) and interleukin-2 (17–19), have been administered to patients in an effort to prolong the duration of remission. The optimal agent would exert its cytotoxicity through a mechanism of action distinct from traditional chemotherapy but with the potential to target toxicity specifically to residual cancer cells that remain after ABMT. Anti-B4-bR represents one possible, specifically targeted cytotoxic agent.

We have reported previously (12) that Anti-B4-bR can be administered safely as adjuvant therapy for patients in CR after ABMT. In a Phase I trial, Anti-B4-bR could be delivered at a MTD of 40 μg/kg/day × 7 days by continuous i.v. infusion. Patients who had recovered from the toxicities of the initial course of Anti-B4-bR and had failed to develop HAMA and/or HARA were eligible to receive repeat courses of therapy at 28-day intervals, but, unfortunately, 5 of 12 patients developed HAMA and/or HARA after an initial course of therapy, and 2 additional patients developed HAMA/HARA at the initiation of the second course of therapy. Thus, the ability to deliver repeat courses of therapy was compromised by the relatively rapid formation of anti-immunotoxin antibodies. Other patients were unable to receive repeat courses of therapy because of the development of reversible grade 4 hepatotoxicity (1 patient) and thrombocytopenia (4 patients).

A single course of therapy, however, may be insufficient to eradicate all of the residual tumor cells because as many as 10^9 tumor cells may remain in patients in an apparent clinical CR. Thus, more courses of therapy may offer an advantage, provided they could be administered safely. The present Phase II trial was designed, therefore, to decrease the toxicity related to Anti-B4-bR and to use a schedule that allowed repeat dosing. All of the patients received Anti-B4-bR at a dose of 30 μg/kg LBW/day × 7 days, a dose lower than the established MTD from the earlier Phase I trial. Furthermore, dosing was by LBW to account for the fact that Anti-B4-bR is a lipophobic compound and is excluded from body fat. Finally, repeat courses of therapy were administered at 14-day rather than 28-day intervals, with the hope that a shorter dosing interval would permit more courses to be delivered before the development of HAMA/HARA. In addition, because our earlier studies indicated that in vivo administration of Anti-B4-bR is cytotoxic for circulating B-cells, we anticipated that an increased frequency of exposure to the immunotoxin might reduce HAMA and HARA formation.

DISCUSSION

Because many patients relapse after high-dose chemotherapy and radiation therapy for B-cell NHL, investigators have considered providing additional post-ABMT therapy to patients in CR. Unfortunately, the use of chemotherapy and radiation therapy in the post-ABMT setting is limited by the impaired marrow reserve of patients early after ABMT. In addition, residual tumor cells that remain after ABMT may be resistant to these conventional therapeutic modalities. Thus, alternative agents, such as IFN-α (16) and interleukin-2 (17–19), have been administered to patients in an effort to prolong the duration of remission. The optimal agent would exert its cytotoxicity through a mechanism of action distinct from traditional chemotherapy but with the potential to target toxicity specifically to residual cancer cells that remain after ABMT. Anti-B4-bR represents one possible, specifically targeted cytotoxic agent.

We have reported previously (12) that Anti-B4-bR can be administered safely as adjuvant therapy for patients in CR after ABMT. In a Phase I trial, Anti-B4-bR could be delivered at a MTD of 40 μg/kg/day × 7 days by continuous i.v. infusion. Patients who had recovered from the toxicities of the initial course of Anti-B4-bR and had failed to develop HAMA and/or HARA were eligible to receive repeat courses of therapy at 28-day intervals, but, unfortunately, 5 of 12 patients developed HAMA and/or HARA after an initial course of therapy, and 2 additional patients developed HAMA/HARA at the initiation of the second course of therapy. Thus, the ability to deliver repeat courses of therapy was compromised by the relatively rapid formation of anti-immunotoxin antibodies. Other patients were unable to receive repeat courses of therapy because of the development of reversible grade 4 hepatotoxicity (1 patient) and thrombocytopenia (4 patients).

A single course of therapy, however, may be insufficient to eradicate all of the residual tumor cells because as many as 10^9 tumor cells may remain in patients in an apparent clinical CR. Thus, more courses of therapy may offer an advantage, provided they could be administered safely. The present Phase II trial was designed, therefore, to decrease the toxicity related to Anti-B4-bR and to use a schedule that allowed repeat dosing. All of the patients received Anti-B4-bR at a dose of 30 μg/kg LBW/day × 7 days, a dose lower than the established MTD from the earlier Phase I trial. Furthermore, dosing was by LBW to account for the fact that Anti-B4-bR is a lipophobic compound and is excluded from body fat. Finally, repeat courses of therapy were administered at 14-day rather than 28-day intervals, with the hope that a shorter dosing interval would permit more courses to be delivered before the development of HAMA/HARA. In addition, because our earlier studies indicated that in vivo administration of Anti-B4-bR is cytotoxic for circulating B-cells, we anticipated that an increased frequency of exposure to the immunotoxin might reduce HAMA and HARA formation.

The results of this Phase II trial demonstrate that Anti-B4-bR can be administered safely at 14-day intervals to patients early after ABMT. Although the toxicities seen in this trial were similar to those observed in the earlier Phase I investigation, they occurred with lower frequency and at lower grade. For example, there were no cases of grade 4 hepatotoxicity and only three cases of grade 4 thrombocytopenia. Other grade 4 toxicities included two cases of HUS, which has been well described in the posttransplant setting (15), and one case of sepsis, leading to respiratory failure and death, again a risk commonly associated with ABMT, although a contribution from Anti-B4-bR cannot be ruled out.
Manifestations of capillary leak syndrome, including dyspnea and edema, occurred at higher frequency in this Phase II study than in the earlier Phase I trial, although this observation likely reflects the ambulatory status of the patients on this trial. In the Phase I study, all of the patients received inpatient therapy with Anti-B4-bR, with most spending the majority of their time in bed. Ambulation was minimal, leading to fewer complaints of dyspnea. Likewise, lower extremity edema was seen at higher frequency in the current trial. In further support of the argument that increased reporting of capillary leak syndrome did not reflect greater toxicity, the frequency of hypoalbuminemia was lower in the Phase II trial than in the Phase I trial. In general, although the DLT of the majority of ricin-based immunotoxins is capillary leak syndrome (20–22), this toxicity is not as marked with Anti-B4-bR at this dose and schedule.

Despite the lower dose of Anti-B4-bR administered with each course of therapy compared with the patients treated at the MTD on the Phase I study, 31 patients received a higher total dose of Anti-B4-bR over the initial 4-week interval because they were able to receive two courses of therapy in that time period. Thus, we were able to accomplish our objective of delivering multiple doses of Anti-B4-bR without sacrificing safety. Serum level measurements further demonstrated that despite the lower administered dose of Anti-B4-bR in this trial, the mean serum level obtained at the end of the initial 7-day infusion was 0.77 ± 0.41 nM, which is still above the theoretic cytotoxic threshold. When equivalent concentrations of Anti-B4-bR are incubated with malignant B-cell lines that express the CD19 antigen, four or more logs of cells can be depleted in vitro (23).

Antibodies directed against immunotoxin occurred with surprising frequency. HARA occurred in 23 patients, and HAMA also occurred in 2 of these patients. These frequencies may underestimate the true frequency of antibody formation inasmuch as follow-up samples were not obtained beyond 1 month from many patients. Furthermore, because of a greater assay sensitivity, HARA formation was often detected earlier than HAMA formation, and additional blood samples were not obtained in patients after the detection of HARA, thereby decreasing the reported incidence of HAMA. Although patients who undergo ABMT have low levels of serum immunoglobulins, low numbers of circulating B-cells, and impaired B-cell function for up to one year post-ABMT, these parameters apparently poorly reflect the capacity to mount an immune response to the immunotoxin. Nevertheless, antibody formation frequency in the current trial. In further support of the argument that increased reporting of capillary leak syndrome did not reflect greater toxicity, the frequency of hypoalbuminemia was lower in the Phase II trial than in the Phase I trial. In general, although the DLT of the majority of ricin-based immunotoxins is capillary leak syndrome (20–22), this toxicity is not as marked with Anti-B4-bR at this dose and schedule.

Despite the lower dose of Anti-B4-bR administered with each course of therapy compared with the patients treated at the MTD on the Phase I study, 31 patients received a higher total dose of Anti-B4-bR over the initial 4-week interval because they were able to receive two courses of therapy in that time period. Thus, we were able to accomplish our objective of delivering multiple doses of Anti-B4-bR without sacrificing safety. Serum level measurements further demonstrated that despite the lower administered dose of Anti-B4-bR in this trial, the mean serum level obtained at the end of the initial 7-day infusion was 0.77 ± 0.41 nM, which is still above the theoretic cytotoxic threshold. When equivalent concentrations of Anti-B4-bR are incubated with malignant B-cell lines that express the CD19 antigen, four or more logs of cells can be depleted in vitro (23).

Antibodies directed against immunotoxin occurred with surprising frequency. HARA occurred in 23 patients, and HAMA also occurred in 2 of these patients. These frequencies may underestimate the true frequency of antibody formation inasmuch as follow-up samples were not obtained beyond 1 month from many patients. Furthermore, because of a greater assay sensitivity, HARA formation was often detected earlier than HAMA formation, and additional blood samples were not obtained in patients after the detection of HARA, thereby decreasing the reported incidence of HAMA. Although patients who undergo ABMT have low levels of serum immunoglobulins, low numbers of circulating B-cells, and impaired B-cell function for up to one year post-ABMT, these parameters apparently poorly reflect the capacity to mount an immune response to the immunotoxin. Nevertheless, antibody formation

![Overall Survival](image-url)
A small Phase II trial of adjuvant therapy post-ABMT. These patients represent a small and select subset of patients who undergo ABMT for NHL because they were required to achieve a CR before ABMT and continue in CR for 2–5 months afterward before enrollment. Nevertheless, at the initial evaluation of relapse and survival for this cohort of patients, 75% of the patients remained in CR after a median follow-up of 15 months, which was significantly better than would be expected based on historical controls. These data offered hope that adjuvant immunotoxin therapy may lengthen remission duration of patients in CR after ABMT for B-cell NHL and provided the impetus for a randomized, Phase III trial of Anti-B4-bR post-ABMT conducted as an Intergroup study by the Cancer and Leukemia Group B and ECOG (24). The study was terminated early when it was determined that further follow-up would be highly unlikely to demonstrate an advantage to Anti-B4-bR therapy. After a median follow-up of 2 years, the estimated 2-year DFS was 42% for patients treated with Anti-B4-bR compared with 62% for patients receiving observation alone. Thus, what appeared to be a promising initial efficacy of Anti-B4-bR in the Phase II setting could not be borne out in a larger randomized trial. This cautions against drawing conclusions of efficacy from early follow-up data in a pilot study. The longer follow-up data of the Phase II study presented here confirm that DFS progressively decreased with longer follow-up, falling to 56% at 4 years. Despite these discouraging results, other agents are being explored in the minimal residual disease setting. One such agent is Rituxan, which has response rates of about 50% as a single-agent in patients with relapsed low-grade NHL (25, 26). The value of Rituxan as maintenance therapy in low-grade lymphoma is the subject of two cooperative groups trials: (a) a Phase III trial of cyclophosphamide and fludarabine followed by Rituxan, being conducted by ECOG; and (b) a Phase II trial of CHOP followed by Rituxan, with special attention to measurement of minimal residual disease, being conducted by Southwestern Oncology Group. The Cancer and Leukemia Group B is also incorporating maintenance therapy with Rituxan in its Phase III trial of CHOP in elderly patients with aggressive NHL. Thus, although Anti-B4-bR adjuvant therapy appears ineffective, it does not preclude the possibility that other antibody-based approaches may be more active in the adjuvant setting.

ACKNOWLEDGMENTS

We thank Walter A. Blättler for his critical review of the manuscript.

REFERENCES


Blood, tologous and T-cell-depleted allogeneic bone marrow transplantation.


A Phase II Study of Adjuvant Therapy with Anti-B4-blocked Ricin after Autologous Bone Marrow Transplantation for Patients with Relapsed B-Cell Non-Hodgkin's Lymphoma
