Phase II Clinical Trial of Bolus Infusion Anti-B4 Blocked Ricin Immunoconjugate in Patients with Relapsed B-Cell Non-Hodgkin’s Lymphoma

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ABSTRACT

Immunotoxins, composed of a monoclonal antibody conjugated to a protein toxin, mediate cell death through novel cytotoxic mechanisms. Anti-B4-blocked ricin (anti-B4-bR) recognizes CD19-positive cells, which includes most B-cell non-Hodgkin’s lymphomas (NHLs). Previous Phase I clinical studies of anti-B4-bR, using both bolus and continuous dosing regimens, demonstrated no safety or efficacy advantage to the continuous infusion regimen. This Phase II trial in 16 patients with relapsed CD19-positive NHL was conducted to evaluate the efficacy of anti-B4-bR when administered at the previously established maximum tolerated dose using a daily bolus for a 5 consecutive day schedule. Serum pharmacokinetics were measured in selected patients. Tissue samples of involved lymph nodes and bone marrow were also obtained from a portion of patients for determination of anti-B4-bR penetration into tissues. Toxicity was similar to what has been described previously for anti-B4-bR and consisted mainly of reversible elevations of hepatic transaminases and mild to moderate thrombocytopenia. No sustained clinical responses were documented. Pharmacokinetic measurements demonstrated that serum levels compatible with 3 logs of cell kill in vitro could be sustained for several hours in most patients. Immunohistochemical analysis of tissue samples provided some insight into the low efficacy. The immunotoxin could be detected in three of the four bone marrow aspirate samples but in only two of the seven lymph node specimens. Thus, anti-B4-bR, using a single daily bolus for a 5 consecutive day schedule, is not an active agent in relapsed NHL. Poor penetration into certain sites of disease may be one explanation for its lack of efficacy.

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INTRODUCTION

The ultimate failure of chemotherapy to cure patients with B-cell NHL can be attributed to a combination of tumor cell resistance and an inability for all patients to tolerate the toxicities associated with current therapies. Ample room remains to develop non-cross-resistant therapies with lower toxicity profiles. MoAb-based therapies may offer the ability to target and kill tumor cells specifically via novel cytotoxic mechanisms. The recent introduction of the IDEC-C2B8 MoAb for the treatment of relapsed low-grade B-cell NHL offers hope that the promise of MoAb therapy is finally being realized.

ITs, consisting of a MoAb conjugated to a potent protein toxin, obviate the need for the MoAb to mediate cytotoxicity (1). One such IT is anti-B4-bR, composed of the murine anti-CD19 MoAb and blocked ricin toxin (2). The CD19 antigen is an attractive target for the treatment of B-cell NHL, because it is present on >95% of cases (3). It is also expressed throughout normal B-cell development, is the earliest detectable pan-B cell antigen, and lacks demonstrable expression on other normal tissues. Furthermore, normal hematopoietic stem cells do not express CD19 and thus would not be expected targets of the anti-B4-bR IT. The toxin moiety itself consists of covalently modified ricin toxin, in which the high-affinity galactose-binding sites of the B-chain are blocked to minimize nonspecific binding while retaining the membrane translocation function (4). The A-chain remains unmodified and serves as the cytotoxic component of the anti-B4-bR conjugate, inactivating the 60S ribosomal subunit and thus disrupting protein synthesis, leading to cell death (5).

Extensive in vitro studies using B-cell NHL cell lines have demonstrated the ability of anti-B4-bR to kill greater than 5 logs of malignant cells, through specific antibody-antigen binding (2, 6). This in vitro cytotoxicity is both time and dose dependent, such that with a 30-min exposure at a concentration of 3 nM, <1 log of cells is killed, whereas a 5-h exposure resulted in equal cytotoxicity at <1 nM (7). Subsequent preclinical severe combined immunodeficient mouse tumor xenograft models confirmed in vivo efficacy, using a single daily bolus for a 5 consecutive day schedule and achieving serum levels ranging from 0.7 to 3.6 nM (8). The first human Phase I study, using an identical 5-day bolus schedule, treated patients with B-cell neoplasms with escalating doses of anti-B4-bR (9). The MTD was reached at a total dose of 250 μg/kg, with a DLT of reversible

The abbreviations used are: NHL, non-Hodgkin’s lymphoma; MoAb, monoclonal antibody; IT, immunotoxin; anti-B4-bR, anti-B4-blocked ricin; MTD, maximum tolerated dose; DLT, dose-limiting toxicity; VLS, vascular leak syndrome; CNS, central nervous system; LBM, lean body mass; HAMA, human anti-mouse antibody; HARA, human anti-rat antibody; FNA, fine-needle aspirate.
grade 3 elevation of hepatic transaminases. Other observed toxicities included thrombocytopenia, fatigue, and a mild VLS. One complete response and two partial responses were observed. A follow-up study used a 7-day continuous infusion in a similar population of patients to determine whether efficacy could be increased and toxicity decreased with this alternate schedule (7). The DLT was defined by grade 4 elevations of hepatic transaminases and grade 4 thrombocytopenia. Additional toxicities included fever, malaise, nausea, and headaches, as well as manifestations of VLS. Despite a higher MTD (total dose, 350 μg/kg), the response rates were similar between the continuous and bolus infusion schedules; thus, there appeared to be no demonstrable benefit to continuous infusion IT serotherapy. Studies suggest that the efficacy of ITs may be limited by impaired penetration of these large molecules into sites of poorly vascularized, bulky disease (10, 11). The present Phase II study was performed to: (a) determine the efficacy of bolus dose anti-B4-bR delivered at the previously established MTD in patients with B-cell NHL; (b) measure the pharmacokinetics of anti-B4-bR; and (c) determine whether anti-B4-bR can penetrate into sites of disease by obtaining tissue samples of bone marrow and accessible lymph nodes after therapy.

MATERIALS AND METHODS

Anti-B4 MoAb, Blocked Ricin, and Anti-B4-bR. The anti-B4 MoAb is a murine IgG1 and was prepared as described previously (3, 12). The synthesis of blocked ricin toxin has been detailed elsewhere (4). The anti-B4-bR IT was prepared as outlined previously (2, 7, 9). Two lots of anti-B4-bR were used in this study (lot nos. P0192F05 and P0192J06).

Patient Selection. Patients were eligible for this study if they were between the ages of 18 and 65 and had a B-cell NHL that had relapsed from conventional primary or salvage chemotherapy regimens. Patients with low-grade histology must have failed at least one chemotherapy regimen, and patients with intermediate- or high-grade histology must have failed at least two prior chemotherapy regimens. Tumor cells from all patients were required to demonstrate reactivity to either the anti-B4 MoAb (by flow cytometry or immunoperoxidase staining), the anti-B1 antibody, or the L26 antibody, because it had been demonstrated previously that tumor cells that express the B1 antigen also express the B4 antigen (3). Tissue for antigen analysis could come from the time of initial diagnosis, at relapse, or at the time of consideration for enrollment on study. All patients had to have evaluable disease by physical exam, radiographic studies, or bone marrow exam. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 2. At least 3 weeks had to have elapsed since any prior therapy. At protocol entry, all patients were required to have a total bilirubin ≤2.0 mg/dl, aspartate aminotransferase and alanine aminotransferase ≤3 times the upper limit of normal, serum creatinine ≤2.0 mg/dl, hematocrit ≥25%, platelet count ≥50,000/μl, and absolute neutrophil count ≥500/μl. Patients were excluded for any history of CNS infection, CNS infiltration by tumor, or CNS irradiation ≥3000 cGy. Patients could not be receiving corticosteroids and had to be without evidence of uncontrollable infection, including a positive HIV serology. Patients could not have clinically significant cardiac or pulmonary disease. The clinical protocol was approved by the institutional review boards of the Dana-Farber Cancer Institute and Massachusetts General Hospital (Boston, MA), and all patients signed an informed consent approved by these committees.

Study Design. Eligible patients were treated in the outpatient clinic at either the Dana-Farber Cancer Institute or Massachusetts General Hospital with daily bolus doses of anti-B4-bR, administered over 1 h via a central venous access line, for 5 consecutive days. Patients were dosed according to calculated LBM or actual weight, whichever was lower. LBM calculations were made based upon the following equations:

\[
LBM_{female} = 45.5\ kg + 2.3 \times \text{(height in inches above 5 feet)}
\]

\[
LBM_{male} = 50.0\ kg + 2.3 \times \text{(height in inches above 5 feet)}
\]

Doses and schedules were not modified during the 5-day cycle. The first three patients were treated at 40 μg/kg LBM/day, a dose 10 μg/kg LBM/day below the MTD established by a prior study (9). This lower dose was used because the formulation of anti-B4-bR had changed since the prior study. Subsequent patients were all treated at 50 μg/kg LBM/day. Patients were eligible for retreatment at the same dose on a 28-day cycle if they continued to meet protocol eligibility requirements, had no evidence of disease progression, and failed to develop HAMA or HARA responses.

Documentation of all measurable disease was performed in all patients within 2 weeks before treatment, and again after treatment, using pertinent studies to evaluate sites of previous disease. Blood samples were drawn before, during, and after IT infusion from several patients for pharmacological and HAMA/HARA measurements. Physical exams and blood work were performed on days 0, 4, 14, and 21 of each treatment cycle. Patients were queried for side effects at each of these visits. Incisional or core lymph node biopsies or FNAs of peripheral lymph nodes for immunoperoxidase studies were obtained from some patients on either day 3, 4s or 5 of cycle 1. Bone marrow biopsy for immunoperoxidase studies was performed on some patients on day 2, 3, or 4 of cycle 1 if there was prior evidence of bone marrow involvement by lymphoma.

Pharmacology. Samples were drawn for serum levels of anti-B4-bR immediately before the start of the infusion, 30 min into the infusion, at the completion of the infusion, and then 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 6.0 h after completion of the infusion on days 0 and 3 of cycle 1 for the three patients treated at the 40 μg/kg LBM/day level and the first three patients treated at the 50 μg/kg LBM/day. The remainder of the patients treated at the 50 μg/kg LBM/day dose level had blood drawn for serum levels of anti-B4-bR immediately before the infusion, at the completion of the infusion, and 1 h after the infusion on days 0 and 3 of cycle 1. The serum concentration of anti-B4-bR was determined by using ELISAs based on a methodology described previously (7).

Immunohistochemistry. Bone marrow smears or cyto-sin preparations were prepared, air-dried, and stored at −70°C. Frozen sections were cryostat cut (6–8-μm thickness), collected onto poly-lysine-coated slides, air-dried, and fixed in 2% neutral buffered paraformaldehyde at 4°C.
Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Lymphoma grade (IWF	extsuperscript{a})</th>
<th>No. of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low grade</td>
<td>10</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>5</td>
</tr>
<tr>
<td>High grade</td>
<td>1</td>
</tr>
<tr>
<td>No. of prior regimens</td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>6</td>
</tr>
<tr>
<td>3–4</td>
<td>6</td>
</tr>
<tr>
<td>&gt;4</td>
<td>4</td>
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<tr>
<td>Sites of disease at entry</td>
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</tr>
<tr>
<td>Lymph nodes</td>
<td>16</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>7</td>
</tr>
<tr>
<td>Extranodal</td>
<td>1h</td>
</tr>
<tr>
<td>Spleen</td>
<td>4</td>
</tr>
</tbody>
</table>

	extsuperscript{a} International Working Formulation.

	extsuperscript{h} Bone involvement.

Immunohistochemical staining was performed using the immunoperoxidase techniques of Cordell et al. (13). Briefly, soluble immune complexes and the complex-antibody conjugate were detected with rabbit anti-mouse IgG antibodies (DAKO Corp.) and alkaline phosphatase and mouse monoclonal-anti-alkaline phosphatase complexes were purchased from DAKO Corp. (Carpinteria, CA) and used at a 1:50 dilution. The immunocomplex was detected by staining for its monoclonal antibody moiety with rabbit anti-mouse IgG antibodies (DAKO Corp.) and alkaline phosphatase and mouse monoclonal anti-alkaline phosphatase complexes. A solution containing naphtol AS-TR phosphate (Sigma Chemical Co.), Fast Red RC (Sigma), and levamisole (5 mM, to quench endogenous alkaline phosphatase activity) was used as the substrate-chromogen solution. Positive controls included substitution of anti-B4 antibody with buffered saline or with purified nonspecific mouse IgG, (Coulter Immunology, Hialeah FL, lot no. 809 M124) on samples of bone marrow obtained prior to treatment. HAMA/HARA Detection. HAMA and HARA measurements were determined with serum samples diluted 30-fold with buffer, using previously described ELISA techniques (7). HAMA and HARA were considered positive if the patient’s serum gave a 20% greater ELISA reading than a negative control. HAMA was determined with a standard sample in which a positive reading implied that the serum contained >468 ng/ml of HAMA.

Response Criteria. Responses were assessed 4 weeks after each course of therapy. Criteria for response, stable disease, and progressive disease have been described previously (7).

RESULTS

Patient Characteristics

Sixteen patients (10 males and 6 females) with relapsed NHL were entered onto this Phase II study. Patient characteristics are listed in Table 1. Patient age ranged from 32 to 74 years, with a median age of 55 years. All patients had disease that had relapsed after prior chemotherapy, with seven patients having failed autologous bone marrow transplant. Patients were heavily pretreated, having received between one and six prior chemotherapy regimens, with 10 patients having received more than three previous regimens. Seven patients had bone marrow involvement, four had splenic involvement, and one patient had extranodal disease involving bone.

Dose Received and Pharmacology

The first four patients were treated at a dose of 40 μg/LBM kg daily. An extra patient was added at this dose level, because one of the first three patients did not complete the full 5 days of treatment due to progressive disease. The subsequent 12 patients were treated at a dose of 50 μg/LBM kg daily for 5 consecutive days. Thirteen of 16 patients received only one course of therapy (one patient received only a partial course). Reasons for ineligibility for further therapy were progressive disease in eight cases, HAMA/HARA formation in three cases, patient refusal in one case, and a severe adverse event in another case. Three patients went on to receive a second course. Further courses were not administered because of HAMA/HARA formation in one case and a decision to change treatment plans in the other two cases.

Serum levels of anti-B4-bR were determined by ELISA in all patients, based upon separate detection of the anti-B4 MoAb and blocked ricin moieties of the IT. The peak serum level on day 0 of therapy ranged between 0.39 and 3.2 nm with a mean of 1.9 ± 0.91 nm. The overall mean peak serum concentration, based upon compiled day 0 and day 3 peak values, was 2.7 ± 1.3 nm. Based upon previous in vitro studies, serum levels >0.5 nm would be predicted to kill up to 3 logs of cells after 24-h exposure (9). Fig. 1 depicts the serum levels of anti-B4-bR over the course of a single infusion for the three patients treated at the 50-μg/LBM kg/day dose level that had complete pharmacological studies performed. The peak serum level was reached by the end of the 1-h infusion and decreased to <0.5 nm as soon as 2 h.
postinfusion for one patient, although there was considerable variation. A similar pattern of serum levels of anti-B4-bR was observed with the four patients treated with 40 μg/LBM kg but with lower peak levels (data not shown).

**Toxicity**

**Overall Toxicity.** The anti-B4-bR IT generally was well tolerated. Table 2 summarizes the documented grades 1 through 4 toxicities. As observed in the previous Phase I trials, the major toxicities included hepatic and hematological toxicity, mainly limited to thrombocytopenia. In addition, one patient experienced line sepsis complicated by a myocardial infarction, one patient developed herpes simplex virus reactivation, and one patient developed severe abdominal pain. No fatal toxicities were observed. A number of common grades 1 and 2 side effects were seen. Including low-grade fevers, anorexia, nausea, and mild myalgias. Finally, except for one case of grade 3 rash, no allergic toxicities were seen. There was no apparent nephrotoxicity, pulmonary toxicity, or neurotoxicity.

**Hepatotoxicity.** The DLT of previous Phase I trials had been hepatotoxicity, manifested as reversible elevations of transaminases. Every course of anti-B4-bR administered in this trial was associated with at least a grade 1 elevation of the aspartate aminotransferase and often the alanine aminotransferase as well. Three courses were associated with grade 3 elevations and one with grade 2 elevation. Other measures of liver function, including alkaline phosphatase, bilirubin, and prothrombin time, were not affected adversely. In general, these elevations resolved within 7–14 days after completion of therapy.

**VLS.** Mild manifestations of the vascular leak syndrome, which can include hypoalbuminemia, weight gain, peripheral edema, pulmonary edema, hypotension, and pericardial effusions, were seen in seven patients. Signs and symptoms of VLS in these patients consisted of peripheral edema, weight gain, and mild shortness of breath.

**Hematological Toxicity.** The predominant hematological toxicity consisted of thrombocytopenia. Five of 19 courses were associated with nadir platelet counts <100,000/μL, with one case each of grades 3 and 4 toxicity. Toxicity duration was short, lasting fewer than 7 days. One case of transient grade 4 neutropenia also was observed.

**HAMA/HARA**

Host humoral responses to either the murine anti-B4 MoAb or ricin moieties developed within a narrow time period in about one-third of patients. Six patients developed HARA, and three patients developed HAMA responses a median of 23 days after therapy, with a range of 21–25 days. Follow-up beyond day 28 was not performed in all patients; therefore, some patients subsequently may have developed HAMA/HARA responses that were not recorded. No patients with measurable HAMA or HARA titers were re-treated. One of the three patients who were re-treated developed a HARA response after the second course. No allergic manifestations were associated with HAMA/HARA formation.

**Clinical Responses**

No sustained clinical responses were observed. Seven patients had clinically stable disease, of which two were minor partial responses (i.e., <50% decrease with no new lesions).

**Immunohistochemistry**

**Lymph Nodes.** Lymph node biopsies or FNAs were obtained from seven patients. In one lymph node core biopsy, obtained from a patient immediately preceding day 3 of treatment, the IT could be detected specifically on tumor cells by immunoalkaline phosphatase staining of its anti-B4 component. Many tumor cells were strongly positive, whereas adjacent skeletal muscle cells were negative (see Fig. 2A). A FNA from another patient similarly demonstrated strong staining for the presence of IT, whereas it could not be demonstrated on a FNA specimen from a third patient. Surgical lymph node biopsies from four patients demonstrated staining only of a few scattered cells. *Ex vivo* treatment of serial sections of these tissues with additional anti-B4 MoAb resulted in positive staining of most tumor cells in the lymph nodes and of most tumor cells obtained by FNA.

**Bone Marrow.** Bone marrow aspirates were obtained from four patients after the initiation of anti-B4-bR and from two of these patients prior to therapy. As expected, the two pretreatment samples were negative for IT, by immunoalkaline phosphatase staining of the anti-B4 component. The conjugate could be detected in posttreatment samples of three patients (Fig. 2B). The fourth patient’s bone marrow demonstrated no staining for IT, and when exogenous anti-B4 was added, again essentially no staining was observed, indicating a lack of tumor cells in the sample. *Ex vivo* treatment of the specimens containing tumor cells, with additional anti-B4 MoAb, increased the intensity of the staining as well as the number of stained cells.

**DISCUSSION**

In the earlier Phase I clinical studies with anti-B4-bR, we demonstrated the IT could be given safely by bolus infusion with tolerable, reversible toxicity. We also showed that a continuous infusion schedule, despite offering a theoretic benefit of longer IT exposure time, did not demonstrate any benefit in

**Table 2**

<table>
<thead>
<tr>
<th>System</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
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<td>1</td>
<td>3</td>
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<td>1</td>
</tr>
<tr>
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<td>0</td>
<td>2</td>
<td>1</td>
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<tr>
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<td>0</td>
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<tr>
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<td>0</td>
<td>0</td>
</tr>
<tr>
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<tr>
<td>Fatigue</td>
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<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Nausea/Vomiting</td>
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<td>3</td>
<td>0</td>
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<tr>
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<td>1</td>
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*a Number of patients experiencing particular toxicity, except where noted otherwise.

*b Number of courses during which this toxicity was observed.
terms of response or toxicity over a bolus infusion regimen. The present study was conducted to establish the efficacy of bolus-dose anti-B4-bR in a more uniform population of patients than those treated on the Phase I trials. The IT was administered at the previously established MTD on an identical schedule, with the exception that doses in this study were based upon ideal body weight, in an effort to minimize toxicity, because anti-B4-bR is a hydrophilic molecule. Furthermore, because lower than expected efficacy has been attributed by many investigators to poor IT penetration into tumor masses, we performed biopsies of bone marrow and accessible lymph nodes to document whether anti-B4-bR could be identified within sites of disease. Finally, serum measurements of anti-B4-bR were performed to confirm that the pharmacology of the reformulated IT was identical to that seen in the initial Phase I study.

Sixteen patients with multiply relapsed NHL were enrolled and received a total of 18 full courses of the anti-B4-bR IT (one patient discontinued therapy on day 2 because of progressive disease). Pharmacokinetic studies demonstrated that although there was considerable variation in the absolute levels achieved in a given patient, the overall behavior was similar to that observed in the Phase I trial of bolus dose anti-B4-bR. The peak level was achieved within the first 1–2 h and routinely exceeded the presumed therapeutic threshold of 0.5 nM. Except for one patient, who received the 40-μg/LBM kg dose, all patients maintained serum levels above this threshold for at least several hours. There did not appear to be any significant accumulation of anti-B4-bR over successive days of therapy.

The toxicity profile also was similar to that observed previously. The most common side effects included near universal elevations of the hepatic transaminases, which in all cases was reversible. Also, many patients experienced mild to moderate systemic side effects, such as fatigue, nausea, and anorexia. Although a number of grade 3 and 4 toxicities were observed, again most were reversible, including thrombocytopenia and neutropenia. Several infectious complications were also observed, including one case of herpes simplex virus reactivation, one Hickmann catheter infection leading to cellulitis, and one case of sepsis. In general, life-threatening toxicities were rare. These results combined with the pharmacology data confirm that anti-B4-bR can be delivered reliably and with tolerable toxicity.

The immunohistochemistry studies of lymph nodes and bone marrow demonstrate that anti-B4-bR does not consistently
penetrate into all sites of disease. The bone marrow aspirate studies demonstrate that in the three cases in which there was tumor involvement of the bone marrow, anti-B4-bR could be detected associated with tumor cells. The fourth bone marrow specimen, which lacked tumor cells, confirmed the specificity of anti-B4-bR, because no IT could be identified. Penetration into lymph nodes appeared to be more problematic. Two samples showed abundant staining for IT, whereas the remaining five samples showed scattered to no staining, despite containing B4-positive tumor cells. Thus, anti-B4-bR may indeed suffer from an inability to gain access to all sites of tumor involvement, limiting its efficacy in solid hematological malignancies such as B-cell NHL. A similar differential pattern of tissue penetration was observed with the unconjugated humanized MoAb, CAMPATH-1H (14, 15). In this instance, patients with chronic lymphocytic leukemia experienced significant responses in peripheral blood and bone marrow but not involved lymph nodes.

The clinical correlate of inconsistent tumor penetration was the poor response rate observed in this study. Although seven patients had stable disease (of which two could be considered minor responses), there were no significant clinical responses observed. The patients in this study all were treated previously and may have had varying degrees of resistant disease; nevertheless, these results are disappointing. Impaired IT delivery to bulky sites of disease may be one explanation for lack of response observed in this study. The use of a bolus dosing regimen, with high peak levels and therefore presumably a large serum-to-tumor gradient driving IT diffusion into tissues, did not appear to be sufficient to overcome this obstacle to IT therapy.

Experiments with radiolabeled MoAbs offer another explanation for poor IT penetration into lymph nodes. Localization studies using tumor-specific radiolabeled MoAbs demonstrate up to 50-fold greater penetration into solid tumors such as colon or ovarian carcinoma compared with lymph nodes involved with NHL (16). These findings argue that bulk may not be the sole obstacle limiting penetration of MoAbs; rather, such factors as poor capillary permeability and the presence of tight junctions in the vasculature of lymph nodes may present another formidable barrier to adequate IT penetration. Finally, the resistance of tumor cells to blocked ricin may be significant.

In conclusion, the anti-B4-bR IT can be delivered safely to patients with relapsed NHL with a predictable serum pharmacokinetic profile. Nevertheless, this agent, and perhaps other similarly constructed ITs, suffer from poor penetration into tissue sites of tumor, limiting efficacy. As a single agent, anti-B4-bR will have little role in the treatment of NHL. Perhaps efficacy may be enhanced in synergy with cytoreductive conventional chemotherapy or in the setting of minimal residual disease.

ACKNOWLEDGMENTS

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REFERENCES

Clinical Cancer Research

Phase II clinical trial of bolus infusion anti-B4 blocked ricin immunoconjugate in patients with relapsed B-cell non-Hodgkin’s lymphoma.


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