Phase I Study of a Bispecific Ligand-Directed Toxin Targeting CD22 and CD19 (DT2219) for Refractory B-cell Malignancies

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Abstract

Purpose: The novel bispecific ligand-directed toxin (BLT) DT2219 consists of a recombinant fusion between the catalytic and translocation enhancing domain of diphtheria toxin (DT) and bispecific single-chain variable fragments (scFV) of antibodies targeting human CD19 and CD22. We conducted a phase I dose-escalation study to assess the safety, maximum tolerated dose, and preliminary efficacy of DT2219 in patients with relapsed/refractory B-cell lymphoma or leukemia.

Experimental Design: DT2219 was administered intravenously over 2 hours every other day for 4 total doses. Dose was escalated from 0.5 μg/kg/day to 80 μg/kg/day in nine dose cohorts until a dose-limiting toxicity (DLT) was observed.

Results: Twenty-five patients with mature or precursor B-cell lymphoid malignancies expressing CD19 and/or CD22 enrolled to the study. Patients received median 3 prior lines of chemotherapy and 8 failed hematopoietic transplantation. All patients received a single course of DT2219; one patient was retreated. The most common adverse events, including weight gain, low albumin, transaminitis, and fever were transient grade 1–2 and occurred in patients in higher dose cohorts (≥40 μg/kg/day). Two subjects experienced DLT at dose levels 40 and 60 μg/kg. Durable objective responses occurred in 2 patients; one was complete remission after 2 cycles. Correlative studies showed a surprisingly low incidence of neutralizing antibody (30%).

Conclusions: We have determined the safety of a novel immunotoxin DT2219 and established its biologically active dose between 40 and 80 μg/kg/day × 4. A phase II study exploring repetitive courses of DT2219 is planned.

Introduction

DT2219, a recombinant fusion protein, contains the catalytic and translocation enhancing domain of diphtheria toxin (DT390) fused with bispecific single-chain variable fragments (scFV) of antibodies targeting human CD19 and CD22 cell surface receptors (1). The protein is engineered so that the native binding region of diphtheria toxin (DT) is replaced by the more avidly bound scFV. After binding, CD19 and CD22 readily internalize (2, 3) to promote toxin entry into the cytosol, inhibition of protein synthesis, and subsequent apoptotic cell death (4). Notably, previous preclinical studies showed that the combination of two different scFVs and a toxin on the same single-chain molecule resulted in greater anticancer activity compared with monomeric anti-CD19 or anti-CD22 connected with truncated DT (5). In addition, xenograft studies demonstrated significant inhibition of CD22+/CD19+ Raji tumor growth and an enhanced therapeutic effect with repetitive dosing in vivo (1).

CD19, a 95-kDa membrane glycoprotein, is ubiquitous present on the surface of all stages of B lymphocyte development and is also expressed on most B-cell mature lymphoma cells and leukemia cells (6). CD22 is a 135-kDa glycoprotein expressed on B-lineage lymphoid precursors, including precursor B acute lymphoblastic leukemia, and often is coexpressed with CD19 on mature B-cell malignancies (7). DT mediates potent cell-cycle-independent cell death and therefore can be particularly effective as an alternative therapy for chemotherapy-refractory malignant (8). We conducted a phase I dose-escalation study to assess safety, maximum tolerated dose (MTD), and preliminary efficacy in patients with chemorefractory B-cell lymphoma or leukemia expressing CD19 and/or CD22.

Patients and Methods

Patients

All patients gave written informed consent to treatment on the Institutional review board (IRB)-approved treatment protocol in accordance with Declaration of Helsinki. This clinical trial was registered at clinicaltrials.gov (NCT 00889408). DT2219 was cGMP manufactured at the University of Minnesota under FDA IND-application (IND number 1000780). Inclusion criteria included: age >12 years, CD19 and/or CD22 expressing B-cell lymphoma or leukemia refractory to conventional therapy, and...
adequate performance and organ function [creatinine ≤1.5 upper
limit of normal (ULN), liver function tests ≤2.5 × ULN; serum
albumin ≥3 g/dL, left ventricular ejection fraction ≥40%]. We
excluded patients with active infections, serious concurrent med-
ical problems, history of penicillin allergy, and more recently
amended the protocol to also exclude patients with history of
central nervous malignancy. Patients were treated at the Scott and
White Medical Center, MD Anderson Cancer Center, and Masonic
Cancer Center, University of Minnesota.

### Treatment plan

In this phase 1 study, patients received DT2219 in a single
course at doses ranging from 0.5 μg/kg/day (1/500th of the MTD
in rabbits) to 80 μg/kg/day i.v. over 2 hours (4 hours for the first
dose) every other day for 4 total doses (days 1, 3, 5, and 8). The
dose was escalated in 9 cohorts until a dose-limiting toxicity
(DLT) was observed (Table 2). The first 15 patients were treated
by rapid escalation design (dose cohorts 1–3) or by standard 3+3
dose escalation design (cohorts 4–6). We applied continual reassessment method (9) to the last 10 patients (dose cohorts
8, 9) with the goal to identify the dose level that corresponds to a
desired toxicity rate of 33% or less using grade 3 or greater
DT2219-related toxicity except blood pressure changes and fever
as the targeted toxicity [based on NCI Common Terminology
Criteria for Adverse Events version (CTCAE 4)]. Administration of
doses 2 to 4 was permitted if predose creatinine was <1.5 × ULN
and there was an absence of DLT. Supportive care included
allopurinol (300 mg/day orally), intravenous fluids, and preme-
dication with diphénylhydramine (25 mg i.v.), acetaminophen
(325 mg orally), hydrocortisone (100 mg i.v.), and ranitidine (50
mg i.v.) 30 minutes before each DT2219 dose.

### Disease reassessment and correlative studies

Disease assessment included physical examination for lymph
node and spleen weekly; blood and marrow evaluation including
flow cytometry assessment for CD19 and CD22 expression and
assessment for minimal residual disease, and computerized
tomography (CT) scan 21–28 days after treatment using Chesson
criteria for lymphoma and leukemia staging (10, 11). Adverse
event collection focused on targeted and unexpected adverse
events (AE) before and after each dose at the following time
points: 1–4 hours, 24 hours, and days 9, 15, 22, and 29 of the cycle.

### Results

Patients and toxicities

We enrolled 25 patients with a median age of 55 years (range,
34–78 years). Patient and disease characteristics are detailed in
Table 1. All patients were evaluable for safety and efficacy. Ten
patients had pre-B acute lymphoblastic leukemia, 5 had chronic
lymphocytic leukemia (CLL), and 10 had non-Hodgkin lympho-
ma. All patients were chemo-refractory with a median of 3 (range,
2–5) prior therapies. Most patients received prior monoclonal
antibody (rituximab, ofatumumab, inotuzumab), none of the
patients received blinatumomab, and eight failed prior hematopoietic cell transplantation (5 autologous and 3 allogeneic).
All tumors were biopsy-confirmed to express CD19 and/or CD22 in
at least 20% of malignant cells. Most tumors (89%) had over 60%
malignant cells CD19 and/or CD22 and 13 expressed both
CD19 and CD22 targets.

All 25 patients received a single course of therapy. One patient
attained partial response after the first cycle and received an
additional 4 dose course after the protocol was amended with
FDA and IRB approval. Twelve patients treated at doses ranging
from 0.5 μg/kg/day to 20 μg/kg/day exhibited no or minimal adverse
reactions (Table 2). All 13 patients treated at dose levels
≥40 μg/kg/every other day × 4 experienced AE attributed to drug
treatment. No infusion toxicity was observed. The most common
transient grade 1–2 AEs included weight gain (range, 5%–14% of

Correlative studies included assessment of pharmacokinetics,
normalizing antibody, and immunophenotype of peripheral
blood and marrow for CD22, CD19, and CD20. Cell suspensions
were stained with the following monoclonal antibodies: PerCP-Cy5.5-anti-CD3 (OKT3, Tonbo Biosciences 65-0037); APC-
anti-CD45 (HI30, Tonbo Biosciences 20-0459); FITC-anti CD19
(BU-12), FITC-anti CD20 (clone 2H7, ebioscience, 11-0209-42);
FITC-anti CD22 (Invitrogen MHCD2201). Phenotypic acquisi-
tion of cells was carried out on the BD Accuri C6 and analyzed
with BD Accuri C6 software. The presence of DT2219 in serum
was measured by the ability of diluted serum to inhibit proliferation
of CD22+CD19+ Raji indicator cells and then extrapolating DT2219
concentration using standard curve comparison, as described
previously (12). The presence of CD19 and CD22 on lymphoid
tumor samples obtained from patients before the therapy has
been evaluated using standard immunohistochemistry on for-
malin-fixed, paraffin-embedded tissues and, where possible, by
flow cytometry. We also measured CD19-, CD22-, CD20-, and
CD3-expressing peripheral blood cells at weekly intervals. Finally,
the presence of neutralizing antibodies was measured with an
assay where patient serum was used to block the activity of
DT2219 in vitro (5). Peripheral mononuclear cells and serum
samples were collected pretreatment and posttreatment at days 1,
8, 15, 21, and 28 and stored at −80°C.

Statistical analysis

Patients and disease characteristics were summarized using
descriptive statistics. For binary endpoints such as toxicity and
clinical response, frequencies and proportions were calculated.
For continuous endpoints such as area under the curve (AUC),
summary statistic including median and range (minimum and
maximum) were used. All statistical analyses were performed with
Statistical Analysis System software version 9.3 (SAS Institute,
Inc.).

### Translational Relevance

In a phase 1 clinical trial, we report the safety, dosing
feasibility, biologic activity, and clinical efficacy of DT2219;
A novel recombinant protein engineered by fusing the truncated
diphtheria toxin (DT390) with bispecific single-chain variable
fragments of antibodies targeting human CD19 and
CD22. Bispecific immunotoxins represent a novel therapeutic
strategy targeting tumor-specific antigens while limiting sys-
temic toxicity. DT2219 will be further developed for therapy of
mature or precursor B-cell lymphoid malignancies. In the future,
DT2219 can be used in combination with other tar-
gated agents providing a safer and nongenotoxic alternative to
chemotherapy.
baseline), peripheral edema, and hypoalbuminemia consistent with capillary leak syndrome, grade 1–2 fever, and fatigue (Table 2). Seven patients experienced isolated mild elevation of liver function tests (1.1–2.1 × ULN) without hyperbilirubinemia, which resolved within 3 to 7 days. Thrombocytopenia and anemia occurred in 5 patients; however, marrow involvement by underlying lymphoma or leukemia often contributed to cytopenias. Whereas lactate dehydrogenase (2–2.3-fold) transiently increased in 4 patients after the first dose, clinical tumor lysis or acute cytokine release syndrome did not occur. Most AEs were recognized during routine monitoring before the second or third dose of DT2219. All AEs were brief and resolved completely within one week. Two patients experienced DLTs: the first DLT occurred at the 40 μg/kg dose level in a 71-year-old patient with ALL who developed back pain along with acute lower extremity weakness after the third dose of study drug. While the patient had a recent history of CNS leukemia before enrollment, brain MRI and cerebrospinal fluid studies at the time of AE were negative for leukemic CNS involvement. This patient died of rapidly progressive disease. No neurologic adverse effects of any grade occurred in the next 10 patients treated at this or higher doses (40–80 μg/kg). The second DLT event occurred at the 60 μg/kg dose level in a 55-year-old patient who developed grade 3 capillary leak and manifested as hypoxemia, hypotension, pulmonary edema, and hypoalbuminemia in combination with febrile neutropenia. The patient was hospitalized and treated with oxygen, intravenous antibiotics, hydration, and diuresis. Her symptoms improved with supportive care to grade 2 after 2–3 days and completely resolved in 10 days.

Pharmacologic and immunologic studies

At the time of enrollment, most patients exhibited low peripheral blood B-cell counts [median B-cell count 3.5% (<0.1 × 10⁹ cells/μL); range, 0%–52%; n = 10] often associated with prior rituximab, corticosteroids, and chemotherapy. The effect of DT2219 on B lymphocytes in a patient with an extramedullary ALL relapse shortly after allogeneic HCT was observed with gradual decline in number of peripheral blood CD19- and CD22-expressing cells after 4 doses of DT2219 (Fig. 1A). The possibility that DT2219 may interfere with fluorochrome-labeled anti-CD19 and anti-CD22 was excluded by examining CD20⁺

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Table 1. Patients and disease characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of subjects (N = 25)</th>
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<tr>
<td>Age median (range)</td>
<td>55 (34–78)</td>
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<td>Gender (male/female)</td>
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<td>Race</td>
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<tr>
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<tr>
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<tr>
<td>Non-Hodgkin lymphoma</td>
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<td>Disease status</td>
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<tr>
<td>Relapsed refractory</td>
<td>14</td>
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<td>Site of disease</td>
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<td>Lymph nodes</td>
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<tr>
<td>Extra lymphatic sites</td>
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</tr>
<tr>
<td>CD19 and CD22 expression on tumor</td>
<td></td>
</tr>
<tr>
<td>CD19 only</td>
<td>11</td>
</tr>
<tr>
<td>CD22 only</td>
<td>1</td>
</tr>
<tr>
<td>CD19 and 22 both</td>
<td>13</td>
</tr>
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<td>Prior therapy</td>
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<td>Lines median (range)</td>
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<tr>
<td>Inotuzumab</td>
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<tr>
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<tr>
<td>Allogeneic hematopoietic cell transplantation</td>
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Table 2. Treatment detail and AEs

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<tr>
<th>Cohort</th>
<th>Escalation detail</th>
<th>DT2219 dose μg/kg/day</th>
<th>Doses received</th>
<th>Total dose per cycle in μg</th>
<th>N</th>
<th>Drug-related AEs (CTCAE v4.03 toxicity grade)</th>
<th>DLT</th>
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<td>1</td>
<td>Rapid escalation</td>
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<td>2</td>
<td>1.25</td>
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<td>3</td>
<td>2.5</td>
<td>4</td>
<td>10</td>
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<td>1</td>
<td>Grade 1 fever (n = 1)</td>
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<tr>
<td>4</td>
<td>Standard escalation</td>
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<td>4</td>
<td>20</td>
<td>3</td>
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<td>No</td>
</tr>
<tr>
<td>5</td>
<td>10.0</td>
<td>4</td>
<td>40</td>
<td>4*</td>
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<td>6</td>
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<td>4</td>
<td>80</td>
<td>3</td>
<td>3</td>
<td>Grade 1 ALT elevation (n = 1) Grade 2 ALT, AST elevation (n = 1)</td>
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</tr>
<tr>
<td>7</td>
<td>40.0</td>
<td>4⁹</td>
<td>160</td>
<td>5</td>
<td>5</td>
<td>Grade 1 AST, Grade 2 hypoalbuminemia (n = 1) Grade 2 capillary leak syndrome (n = 2)</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Continual Reassessment</td>
<td>60.0</td>
<td>4⁵,⁴</td>
<td>240</td>
<td>5</td>
<td>Grade 1-2 capillary leak syndrome (n = 3) Grade 2 anemia (n = 1) Grade 3 thrombocytopenia (n = 2) Grade 2 fever (n = 2) Grade 4 neutropenia (n = 1) Grade 3 capillary leak syndrome (n = 2) Grade 3 neutropenic fever (n = 1) Grade 2 hearing loss (n = 1) Grade 1 hypocalcemia (n = 1)</td>
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<tr>
<td>9</td>
<td>80.0</td>
<td>4</td>
<td>320</td>
<td>3</td>
<td>3</td>
<td>Grade 1 hypocalcemia (n = 1) Grade 1-2 capillary leak syndrome (n = 2) Grade 1 vomiting (n = 1) Grade 3 hypocalcemia (n = 1) Grade 1 ALT ALT elevation (n = 2) Grade 2 fatique (n = 2)</td>
<td>No</td>
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</tbody>
</table>

⁹One patient at the 10 μg/kg/day was less than 12 years old and enrolled after receiving permission from the local IRB.
⁸Patient with DLT received 3 doses of DT2219.
⁴One patient at the 60 μg/kg/day was retreated 8 weeks later with second cycle at dose 40 μg/kg/day.
⁵One patient was dose reduced for fourth injection to 40 μg/kg/day due to capillary leak syndrome.

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Figure 1.

Immunologic and pharmacokinetic studies. A, peripheral blood mononuclear cell (PBMC) analysis of a representative patient is shown. PBMCs were enriched from patient blood and collected at various times post-treatment. Flow cytometry was used to count cells expressing CD22, CD19, CD20, or CD3. B, a bioassay was used to determine the area under the curve (AUC) of serum DT2219 levels in serum by measuring the ability of diluted serum to inhibit proliferation of CD22+CD19+ Raji indicator cells. Drug serum levels at various times were analyzed using prism 5.0 software to calculate AUC. A concentration-time curve is shown for our second patient at 60 minutes; T1/2 was 59 minutes. C-F, DT2219 serum levels and neutralizing antibodies. C, a nonresponding patient treated at the 80 mg/kg dose level showing no evidence of DT2219 in serum. D, high levels of neutralizing antibodies in this same patient at day 8 through 22. In contrast, E shows the patient that completely responded to 60 mg/kg/day DT2219 had a serum drug concentration and F shows this same patient had no detectable neutralizing antibodies. DT2219 serum levels were calculated from assays in which various serum dilutions were tested for their ability to inhibit Raji cell proliferation. Serum collected prior to drug administration served as a negative control. Neutralization assays were performed based on the ability of undiluted patient serum samples to block the killing of a 99% inhibitory dose of DT2219. The percentage of neutralization was calculated.

cells, which also declined over time. The B-cell depletion was specific as CD3+ T-cell levels remained constant during the testing interval.

We also measured the circulating concentration of DT2219 in a functional pharmacokinetic bioassay (Fig. 1B). Patients treated at dose levels 0.5–20 µg/kg/day had no detectable drug in serum when sampled on day 1 and 8 at 15, 30, 45, 60, and 120 minutes postinfusion. All evaluable patients at the University of Minnesota treated with ≥40 µg/kg/dose (n = 10) demonstrated detectable levels of DT2219 with the exception of one (Fig. 1C) with preexisting antibodies to DT (Fig. 1D). The AUC after the first dose (4-hour infusion) was lower at a median of 285 µg/mL × minutes (range, 0–2,020; n = 8) compared with drug levels after the fourth dose (2 hours infusion; AUC median 1,249 µg/mL × minutes; range, 0–1,692; n = 7). A representative AUC is shown in Fig. 1B. The drug half-life ranged from 59 to 110 minutes (n = 4).

Because the recombinant immunotoxin contains a bacterial toxin, immunogenicity is expected and can be a major barrier to
the potential activity of bacterial toxin-based drugs. We measured serum neutralizing antibodies in all patients treated with ≥40 μg/kg/dose at days 1, 8, 15, 29, 35, and 42 (n = 9). Neutralizing antibodies developed in 3 evaluable patients (30%) at dose levels between 40 and 80 μg/kg at median of one week (range, 1–2 weeks) after the first dose of DT2219. One patient had pre-formed anti-DT antibody that we detected at screening and attributed to prior DT immunization. In some patients, the presence of neutralizing antibodies inversely correlated with the serum concentration of DT2219 (Fig. 1C); however, no consistent pattern was recognized.

Clinical responses

Twenty-five patients were evaluable for response, recognizing that only 9 patients in the highest dose cohorts had measurable drug levels. Three patients had biopsy performed at the time of progression and all 3 demonstrated persistence of one or both CD19/CD22 antigens. Treatment produced an objective tumor response in two of these patients. After a single course of DT2219 at dose level 40 μg/kg/day × 4, a 77-year-old patient with chemotherapy-refractory CD19+/CD22- CLL experienced a 40% reduction in cervical and axillary adenopathy with decrease of an abdominal tumor mass at day 28 after treatment, which was sustained for 2 months. (Fig. 2A) Patient was in continuous partial remission when she received a salvage ibrutinib therapy. A second response occurred in a 53-year-old patient with relapsed CD19+/CD22+ diffuse large B cell lymphoma (dose level 60 μg/kg) who experienced a 75% reduction in size of lymphoma lesion after a single course complicated by a grade 3 capillary leak syndrome. Eight weeks later, after FDA approval, this patient received a second DT2219 course at a reduced dose of 40 μg/kg/dose × 4, which resulted in a complete resolution of a subcutaneous mass and pelvic lymphadenopathy (Fig. 2B). Second patient is alive and in complete remission with no neutralizing antibodies (Fig. 1F), currently at 8 months after therapy. We observed no correlation between CD19 and CD22 target expression and clinical activity in this small cohort.

Discussion

We have established the safety and dosing feasibility of a novel CD19/CD22 bispecific ligand-directed toxin DT2219. We also demonstrated that the current dosing schedule and route of administration achieves drug levels capable of biologic and clinical response against CD19/22-expressing lymphoid malignancies refractory to standard therapies with a surprisingly low incidence of neutralizing antibody responses. The current phase I study shows that although MTD was not reached, the drug can be administered safely up to 80 μg/kg/day at days 1, 3, 5, and 8 and for a total of 4 doses. The first dose infused over 4 hours as a safety precaution was always well tolerated. All other doses were administered over 2 hours. Interestingly, the AUC measured for the first dose was almost always lower than the AUC measured for the fourth dose suggesting the importance of shorter infusion time for immunotoxins with brief half-life. Early on-target saturation may also play a role in low AUC at the onset of therapy; yet the DT2219 dosing in 4 infusions 1 to 2 days apart resulted to adequate drug levels, biologic effectiveness, and tolerable toxicity. Although clinical responses to DT2219 were observed at doses 40 and 60 μg/kg/day, the 4 doses as administered in this trial maybe inadequate to induce deeper remissions. In one patient who achieved partial remission after 1 cycle, an additional cycle led to complete tumor elimination. The rationale for improved efficacy with repetitive dosing is supported by others who are developing immunotoxin conjugates using bacterial toxins, such as the anti-CD22 moxerumomab pasudotox for hairy cell leukemia or SI-401, an IL3 receptor-DT fusion protein for myeloid malignancies (12–14).

In our experience, increasing the number of consecutive doses per cycle is unlikely to be tolerated; however, the treatment schedule with repetitive cycles of four every other day doses at least a week apart should be explored in future studies.

An important observation in this study is the lack of neutralizing antibodies formation in 7 of 10 of the evaluable patients treated at the 3 highest dose cohorts. In other trials involving DT-related immunotoxins in non-B-cell malignancies, neutralizing antibody responses have been frequent. One potential explanation is that prior rituximab therapy and B-cell lymphopenia contributed to a blunted humoral response that can last up to 1 year (4). As is typical for most immunotoxins, the potential toxicity of greatest concern at higher doses was capillary leak syndrome. The underlying mechanism at least in part involves pinocytosis of the immunotoxin by endothelial cells, which is dose-dependent and thus of a particular concern at higher drug concentrations (15).

Drug development strategies to engineer toxins that do not induce
capillary leak syndrome are underway (16, 17). However, despite capillary leak in many patients at the higher dose levels (40–80 µg/kg/day), this side effect was manageable and fully reversible. In contrast to recently approved anti-CD19 targeting bispecific anti-body blinatumomab, which produced neurotoxicity in 11% of patients, DT2219 therapy caused no grade 1–2 neurotoxicity and only a single grade 3 paraparesis of an uncertain drug causality. (18) Importantly, other complications inherent in the use of many experimental immunotherapeutic agents such as infusion-related reactions, pyrexia, tumor lysis, or cytokine release syndrome were not observed in this study (19).

In conclusion, we have demonstrated safety, dosing feasibility, and preliminary clinical activity of a bispecific ligand-directed toxin in chemotherapy refractory B-cell lymphoid malignancies. In contrast to cytostatic chemotherapy, DT2219-mediated tumor cell killing is cell cycle and p53 independent (8), making it a particularly attractive therapy for overcoming resistance to standard chemotherapeutics in lymphoma.

A phase I/II clinical study designed to administer sequential cycles of this unique heterodimeric bispecific antibody toxin conjugate is underway.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Authors’ Contributions
Conception and design: M.R. Verneris, C. Ustun, V. Bachanova, D.A. Vallera
Development of methodology: H. Kantarjian, D.A. Vallera

References
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