Phase I Trial of a Novel Diphtheria Toxin/Granulocyte Macrophage Colony-stimulating Factor Fusion Protein (DT_{388}GMCSF) for Refractory or Relapsed Acute Myeloid Leukemia[1]

Arthur E. Frankel,[2] Bayard L. Powell, Philip D. Hall, L. Douglas Case, and Robert J. Kreitman

Wake Forest University Baptist Medical Center, Winston-Salem, North Carolina 27157 [A. E. F., B. L. P., L. D. C.]; Medical University of South Carolina, Charleston, South Carolina 29425 [P. D. H.]; and Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20892 [R. J. K.]

ABSTRACT

Purpose: **Patients with relapsed or refractory acute myeloid leukemia have a poor prognosis. We tested the safety and efficacy of a diphtheria fusion protein [diphtheria toxin $(DT)_{388}$ granulocyte-macrophage colony-stimulating factor (GMCSF)] directed against the GMCSF receptor that is strongly expressed by leukemic blasts.**

Experimental Design: **DT_{388}GMCSF fusion protein containing the catalytic and translocation domains of DT_{388} fused to human GMCSF was administered in an interpatient dose escalation trial by 15 min i.v. infusion daily for up to 5 days.**

Results: **The maximal tolerated dose was 4 μg/kg/day. The dose-limiting toxicity was liver injury and occurred at the 4.5–5-μg/kg/day dose level. Among nine treated patients at these doses, one patient developed liver failure, and one patient had transient hepatic encephalopathy. There was a positive correlation between peak serum DT_{388}GMCSF levels and serum aspartate aminotransferase ($P = 0.0002$). DT_{388}GMCSF did not damage hepatic cell lines *in vitro*; however, DT_{388}GMCSF binds macrophages and induces cytokine release *in vitro*. Among the treated patients, we observed an early elevation in serum levels of interleukin (IL)-18 and a later rise in IL-8 but no significant changes in IL-1β, IL-6, IFNγ, macrophage inflammatory protein-1α, tumor necrosis factor α or IL-12. The IL-18 elevations occurred before elevations of liver enzymes and correlated with peak aspartate aminotransferase levels ($P = 0.005$). Of the 31 patients who were resistant to chemotherapy, 1 had a complete remission and 2 had partial remissions; all 3 of these patients were treated at or above the maximal tolerated dose, all 3 responding patients had baseline marrow blast percentage of <30%, whereas only 6 of the nonresponding 28 patients had less than 30% marrow blasts. Five of these six patients were treated with subtherapeutic doses. Eight (42%) of 19 patient courses at <4 μg/kg/day and 8 (40%) of 20 patient courses at 4–5 μg/kg/day showed marrow blast reductions at day 12. Patients with higher pretreatment anti-DT_{388}GMCSF levels had significantly lower peak DT_{388}GMCSF levels ($P = 0.0001$).**

Conclusions: **DT_{388}GMCSF can produce complete and partial remissions in patients with chemotherapy-resistant acute myeloid leukemia, but methods to prevent liver injury are needed before more widespread application of this novel agent.**

INTRODUCTION

AML[3] is the most common acute leukemia in adults with 10,000 new cases/year in the United States (1). With combination induction and consolidation chemotherapy using cytarabine and topoisomerase II inhibitors such as daunorubicin and etoposide, complete remission rates of about 70% have been achieved (2). However, most patients ultimately relapse and die from the disease or complications of treatment. The prognosis is dismal for patients with relapsed or refractory AML. Except for the minority of patients who undergo allogeneic bone marrow transplants or have initial complete remissions of >12 months, patients receive salvage therapy similar to that used in their induction/consolidation and have a median survival of weeks to months (3). Two-year survival in this subgroup of relapsed and refractory patients is rare.

Chemotherapy-resistant blasts contribute to treatment failures in AML patients (4). These blasts are resistant to multiple drugs and often show altered expression of one or more resistance proteins that influence drug efflux, drug metabolism, substrate levels, or cell death regulation (5, 6). Because most of the multidrug resistance phenotypes target low-molecular-weight inhibitors of DNA synthesis or cell proliferation, we searched for an agent that could induce leukemic cell death by mechanisms other than damage to DNA or cell division.

Received 9/8/01; revised 1/23/02; accepted 1/30/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by the Leukemia and Lymphoma Society (LSA6114-99), NIH (1R01CA76178, 1R21CA90550, and M01RR07122), and the Wake Forest University School of Medicine.
[2] To whom requests for reprints should be addressed, at Hanes 4046, Wake Forest University School of Medicine, Medical Center Drive, Winston-Salem, NC 27157. Phone: (336) 716-3313; Fax: (336) 716-0255; E-mail: afrankel@wfubmc.edu.

[3] The abbreviations used are: AML, acute myeloid leukemia; GMCSF, granulocyte-macrophage colony-stimulating factor; MTD, maximal tolerated dose; DLT, dose-limiting toxicity; TNF, tumor necrosis factor; AST, aspartate aminotransferase; ALT, alanine aminotransferase; DT, diphtheria toxin; CPK, creatine phosphokinase; VLS, vascular leak syndrome; ANC, absolute neutrophil count; IND, investigational new drug; GGT, gamma-glutamyl aminotransferase; EIA, enzyme immunoassay.

Table 1 Clinical characteristics of DT_{388}GMCSF-treated AML patients

Patient no.	Age (yr)/ Sex	Disease status (yr from diag)[a]	Treatment history	Cytogenetics	Circulating blast count (/μl)	Bone marrow blast %
1	69/F	Ref (2)	Hydrea; Topo	46XiX(p10)	0	5
2	55/M	1st Rel (1)	AlloBMT; DLI; Ara-C	Del7(q22)	1,127	6
3	66/F	1st Rel (1)	Ida/Ara-C × 2	+8,+11,t(1,16)	0	10
4	46/F	2nd Rel (5)	Dauno/Ara-C; Ara-C; AutoBMT	Normal	72	32
5	69/F	Ref (1)	Mito/Ara-C; Ara-C; CTX/VP16	Normal	8,024	10
6	41/F	Ref (1)	Dauno/Ara-C; Ara-C/VP16; AutoBMT	+21,+22	10,004	98
7	84/F	Ref (1)	Cytarabine; 6TG; Mito; Vinc	ND	684	93
8	61/M	1st Rel (1)	Ida/Ara-C; Ara-C	ND	96	30
9	76/M	Ref (0.5)	Ida/Ara-C; Ara-C; CTX/VP16	ND	240	10, LC
10	53/F	Ref (1)	Ida/Ara-C; Ara-C; Topo	+19,+1-2r	276	30
11	60/M	Ref (2)	Dauno/Ara-C; Ara-C; Topo; Mito	+13	4,485	87
12	27/F	Ref (1)	Dauno/Ara-C; Ara-C; VP16/Mito; Topo/Ara-C; Flud/Ara-C	t(1;9)(q21;q21)	15,696	90
13	77/M	Ref (1)	Dauno/Ara-C; Ara-C; VP16/Mito; Topo/Ara-C	Normal	4,324	89
14	24/F	1st Rel (0.5)	Ida/Ara-C; Ida	+8, +11, +13	0	35
15	45/M	2nd Rel (2.5)	VP16/Ara-C; Dauno/Ara-C; Auto BMT	ND	616	80
16	25/M	Ref (1.5)	Dauno/VP16/Ara-C; Ara-C; Auto BMT; Mito/VP16/Ara-C; Topo/Ara-C	Del11(q22), Del5(q12)	0	40
17	52/F	Ref (1)	Ida/Ara-C; Ara-C; Mito/VP16/Ara-C; VP16/Ara-C; Topo/Ara-C	Normal	6,300	95
18	39/F	Ref (2)	Dauno/Ara-C; Ara-C; MTX/Ara-C/Ida; CTX	t(8;21)	0	52
19	52/F	3rd Rel (2)	Mito/Ara-C; Ara-C; Allo BMT × 2; VP16/Ara-C; IT Ara-C; WBI;	Normal	0	75,LC
20	54/M	Ref (2)	Dauno/VP16/Ara-C; VP16/Ara-C; Ida/Ara-C; Auto BMT	−2,−5,+8,+11,+16,+21	14,168	60
21	73/F	1st Rel (1)	Ida/Ara-C; Ara-C	t(1;11),t(2;11),+4,+6	208	88
22	72/F	Ref (4)	Ida/Ara-C;Topo/Ara-C	Normal	0	28
23	46/M	Ref (0.5)	Ida/VP16/Ara-C; Ara-C	+11,Del7	1,588	82
24	32/F	Ref (1)	Ida/Ara-C; Allo BMT; VP16; CTX	ND	424	14
25	77/M	Ref (1)	Ida/Ara-C × 2	Del7(q22)	50	15
26	67/M	Ref (0.5)	Topo; Topo/Ara-C; Mito/Ara-C	ND	0	91
27	61/M	Ref (1)	Dauno/Ara-C; VP16/Ara-C; CTX/VP16; Auto BMT	Normal	0	23
28	57/F	Ref (0.5)	CTX/Ara-C/Topo	t(2;8),Del8,−5,Del7	0	11
29	12/F	Ref (0.5)	Dauno/Ara-C/6TG; Ara-C; Allo BMT	+19	0	87
30	31/M	Ref (1)	Dauno/Ara-C; VP16/Ara-C; Flud/CPT11; Gem/CPT11; Mylotarg; CTX/VP16	Normal	500	92
31	80/F	Ref (1)	Dauno/Ara-C/VP16; Ara-C; Mylotarg	ND	60	47

[a] Diag, diagnosis; LC, leukemia cutis; Ara-C, cytarabine; CTX, cyclophosphamide; VP16, etoposide; Ida, idarubicin; Mito, mitoxantrone; Dauno, daunorubicin; DLI, donor lymphocyte infusion; BMT, bone marrow transplant; TG, thioguanine; Auto, autologous; Allo, allogeneic; r, ring; Flud, fludarabine; IT, intrathecal; WBI, whole brain irradiation; Topo, topotecan; ND, not determined; Ref, refractory; Rel, relapsed; vinc, vincristine; Mylotary, gemtuzumab ozogamicin; Gem, gemcitabine.

[b] Patient 29 was treated under an IND exemption because of age.

One novel class of AML therapeutics are DT fusion proteins consisting of the catalytic and translocation domains of DT genetically fused to AML blast selective ligands (7). The ligand delivers the protein to blast cell surface receptors. After receptor-mediated endocytosis, the fusion protein reaches the early endosomes, to which it is cleaved by furin and, in the acidic environment, inserts into the vesicle membrane and facilitates the escape of the DT-A fragment to the cytosol. In the cytosol, the A fragment ADP-ribosylates elongation factor 2, which leads to the inactivation of protein synthesis and cell death.

Because GMCSF receptors are expressed on the majority of myeloid leukemias but are poorly expressed on early normal hematopoietic stem cells, we chose to fuse GMCSF to the catalytic and translocation domains of DT (DT_{388}; Ref.8). The DT_{388}GMCSF molecule was cytotoxic *in vitro* to chemotherapy-resistant cell lines and therapy-refractory AML patient progenitors but was nontoxic to normal human myeloid progenitors (9–12). Dramatic antileukemic efficacy was observed *in vivo* administering DT_{388}GMCSF to severe combined immunodeficient mice bearing human leukemia (13). On the basis of these results, we manufactured DT_{388}GMCSF under good manufacturing practice and obtained an IND (BB no. 8153) to perform a Phase I dose-escalation trial in patients with relapsed or refractory AML. This report describes the results of that study.

PATIENTS AND METHODS

Eligibility criteria for patient entry into the study included histological bone marrow evidence for AML and age ≥18 years old. Patients were required to have relapsed or refractory leu-

kemia, a Zubrod performance status of ≤2, to have fully recovered from toxicities of prior chemotherapy or radiation therapy, and to have a life expectancy of ≥3 months. Eligible patients had a bilirubin ≤1.5 mg/dl; transaminases ≤5 × upper limit of normal, creatinine ≤1.5 mg/dl; forced expiratory volume (FEV1) ≥70% normal; cardiac ejection fraction ≥50% normal; had no serious concurrent medical problems, uncontrolled infections, central nervous system leukemia, myocardial infarctions in the last 6 months, or history of severe penicillin allergy; and were not pregnant. Written informed consent was obtained from each patient before entry into the study.

DT_{388}GMCSF was produced under good manufacturing practice and aliquoted in vials containing 1 ml of PBS at 1.5 mg/ml and stored at −80°C (14). Before treatment, vials were thawed at room temperature, and appropriate amounts of drug aliquoted in a laminar flow hood for individual daily doses in sterile Eppendorf tubes and were refrozen at −80°C. Each day of treatment, a single dose was rethawed in the laminar flow hood and diluted aseptically in 1 ml of normal saline or 3% saline. A 1-h incubation at room temperature with 3% saline was used after patient 28 to reduce protein aggregates. After premedications with acetaminophen, diphenhydramine, and corticosteroids, the drug was administered as a 15-min infusion through a rapidly flowing i.v. line. Treatments were repeated daily for up to 5 days. Patients could be retreated with Federal Drug Administration approval if they had no unresolved toxicities.

Patients were monitored in-hospital for 2 weeks for toxicities. The National Cancer Institute Common Toxicity Criteria scale was used. Vital signs were measured posttherapy every 15 min for 1 h, every hour for 8 h, and then every 4 h for 7–10 days. Physical exams were done daily throughout the treatment period and for at least 1 additional week. Blood counts, blood chemistries, and urinalysis were done daily during treatment. Drug pharmacokinetics were measured with blood sampling on each treatment day. A previously described bioassay was used to quantitate circulating DT_{388}GMCSF with an assay limit of 0.3 ng/ml (15). Circulating cytokines (IL-1β, IL-6, IL-8, IL-12, IL-18, macrophage inflammatory protein 1α, IFNγ, and TNFα) were measured on the blood samples obtained for pharmacokinetics using enzyme immunoassays (R&D Systems, Minneapolis, MN; or MBL, Nogoya, Japan) following the directions of the manufacturers. Humoral immune response to DT_{388}GMCSF was measured on serum that was obtained pretreatment, day 14, day 30, and day 60 by both a sandwich enzyme immunoassay and cytotoxicity neutralization assay following the methods we have reported previously (16). Clinical response was assessed by blood counts and differentials, exams, and bone marrow aspirate and biopsies on days 12 and 30 and as indicated. A patient was considered in complete remission if there were no circulating blast, no extramedullary leukemia, the marrow blast percentage was <5%, and there was reconstitution of normal hematopoiesis with normal peripheral hemoglobin, platelet count, and neutrophil count without the need for transfusions. A partial remission occurred with the same conditions except for a lack of recovery of normal hematopoeisis.

Patients were treated at one of six dose levels (1, 2, 3, 4, 4.5, or 5 μg/kg/day for 5 days). Three to six patients were treated at each dose level to establish the MTD. Additional patients were added in some cases to better define the toxicity at that dose level. The MTD was defined as the highest dose level at which zero or one patient, among at least six patients, had DLT. DLT was defined as a drug-related ≥ grade 4 nonhematological toxicity or drug-related grade 4 hematological toxicity of >28 days duration. Transient asymptomatic (<2 weeks) grade 4 elevations of transaminases, CPK, or decreases in serum calcium were not considered DLTs on this study.

Table 2 Summary of clinical characteristics of DT_{388}GMCSF-treated AML patients

Characteristics	*n* (%)
Age (yr)	
<60	17 (55)
60–89	14 (45)
Median (range)	55 (12–84)
Sex	
Male	13 (42)
Female	18 (58)
Disease status	
First relapse	4 (13)
Second relapse	2 (6)
Refractory	25 (81)
Cytogenetics	
Good risk	1 (3)
Intermediate risk	8 (26)
Poor risk	15 (48)
Not performed	7 (23)

The cell cytotoxicity assay used the HepG2 hepatocellular carcinoma cell line and was obtained from the American Type Culture Collection (Manassas, VA) in MEM containing 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, Earle's balanced salt solution with 1.5 g/liter sodium bicarbonate and 10% fetal bovine serum (Life Technologies, Inc., Grand Island, NY). Cells were trypsinized and aliquoted at 10^4 cells/well in Costar 96-well flat-bottomed tissue culture plates in 150 μl of medium containing 12 different concentrations of DT_{388}GMCSF or DAB_{389}EGF (a gift from Ligand Pharmaceuticals, Inc, San Diego, CA; Ref. 17), and were incubated at 37°C 5% CO_2 for 48 h. Fifty μl of medium with 1 μCi [^{3}H]thymidine (NEN DuPont, Wilmington, DE) was then added, and, after 18 h, media was removed and cells washed three times with serum-free media, solubilized with 50 μl of 2 M NaOH, neutralized with 50 μl of 2 M HCL, harvested onto glass fiber mats with a Skatron Cell Harvestor (Skatron Instruments, Lier, Norway), and counted on a Betaplate reader gated for ^{3}H. The calculated IC_{50}s were the concentrations of toxin that inhibited thymidine incorporation by 50% compared with control wells.

RESULTS

Patients. Thirty-one patients were treated for a total of 39 courses (8 patients received a second course). The mean age was 54 years (range, 12–84), and there were 13 males and 18 females (Tables 1 and 2). Although the eligibility criteria stipulated age ≥18 years, one child was allowed under a Federal Drug Administration-approved exemption. Four patients were in first relapse, 2 patients were in second relapse, and 25 patients had refractory AML. Six patients had undergone autologous

Table 3 Dose level and drug-related toxic effects of DT_{388}GMCSF in AML patients[a]

Patient no.	Dose μg/kg/day	Drug-related side effects (CTC toxicity grade)	Cause of death (day posttherapy)
1	1	Gr[b] 1 myalgias	Progressive disease (21)
2	1	Gr 2 AST; Gr 1 Alk Phos	Progressive disease (213)
3	1	Gr 1 myalgias; Gr 2 AST; Gr 1 Alk Phos	Progressive disease (230)
4	2	Gr 2 fever; Gr 2 hypocalcemia; Gr 2 hypoalbuminemia; Gr 3 AST	Progressive disease (357)
5	2	Gr 1 Alk Phos	Progressive disease (135)
6	2	Gr 1 Alk Phos	Progressive disease (130)
7	2	Gr 2 fever; Gr 2 hypocalcemia; Gr 3 AST	Progressive disease (60)
8	2	Gr 1 fever; Gr 1 AST; Gr 2 hypocalcemia	Progressive disease (94)
9	2	Gr 1 elevated Cr; Gr 1 Bili; Gr 1 AST; Gr 1 Alk Phos; Gr 3 VLS	Progressive disease (45)
10	3	Gr 1 fever; Gr 2 AST	Progressive disease (130)
11	3	Gr 1 weight gain; Gr 2 AST, Gr 2 VLS; Gr 2 hypoalbuminemia; Gr 2 hypocalcemia; Gr 2 Bili; Gr 2 hypotension; Gr 1 Alk Phos	Progressive disease (105)
12	3	Gr 2 AST; Gr 2 hypoalbuminemia; Gr 1 bradycardia	Post-BMT GVHD (72)
13	4	Gr 1 edema; Gr 1 hypoalbuminemia; Gr 2 hypotension; Gr 2 hypocalcemia; Gr 2 AST	Sepsis (13)
14	4	Gr 1 Alk Phos; Gr 2 AST	Post-BMT GVHD (90)
15	4	Gr 1 fever; Gr 2 hypoalbuminemia; Gr 2 GGT; Gr 2 hypocalcemia; Gr 3 CPK; Gr 3 ALT; Gr 4 AST	Alive (600+)
16	4	Gr 1 AST; Gr 2 hypocalcemia; Gr 2 hypoalbuminemia	Progressive disease (90)
17	4	Gr 1 bradycardia; Gr 1 AST; Gr 1 Alk Phos; Gr 1 hypoalbuminemia; Gr 2 hypocalcemia	Progressive disease (90)
18	4	Gr 1 AST; Gr 1 bradycardia	Progressive disease (142)
19[c]	4	Inevaluable for toxicities	Heart and renal failure (10)
20	4	Gr 1 AST	Progressive disease (34)
21	5	Gr 1 Alk Phos; Gr 1 fever; Gr 2 AST, Gr 2 CPK; Gr 3 hypocalcemia	Progressive disease (37)
22	5	Gr 1 GGT; Gr 3 ALT; Gr 3 CPK; Gr 3 hypocalcemia; Gr 4 AST	Pneumonia (380)
23	5	Gr 1 ALT; Gr 1 CPK; Gr 2 AST; Gr 2 hypocalcemia	CNS hemorrhage (17)
24	5	Gr 1 Alk Phos; Gr 1 CPK; Gr 2 AST; Gr 2 Bili; Gr 3 GGT; Gr 3 ALT; Gr 3 hypocalcemia	Progressive disease (350)
25	3	Gr 1 Alk Phos, Gr 2 Bili; Gr 2 AST; Gr 2 Cr; Gr 2 CPK; Gr 3 ALT; Gr 3 hypocalcemia	Progressive disease (40)
26	4	Gr 1 bradycardia; Gr 1 CPK; Gr 1 Alk Phos; Gr 1 GGT; Gr 2 hypocalcemia; Gr 2 AST	CNS hemorrhage (9)
27	4.5	Gr 1 Alk Phos; Gr 1 hypoalbuminemia; Gr 2 GGT; Gr 2 AST; Gr 2 CPK; Gr 3 ALT, Gr 3 hypocalcemia	Mylotarg liver failure (60)
28	4.5	Gr 1 hyperkalemia; Gr 2 Cr; Gr 2 PT; Gr 2 fever; Gr 2 Alk Phos; Gr 2 GGT; Gr 2 hypoalbuminemia; Gr 3 CPK; Gr 3 PTT; Gr 4 GGT; Gr 4 ALT; Gr 4 AST; Gr 4 hypocalcemia; Gr 5 hepatic failure	Liver failure (19)
29	4.5	Gr 1 hypofibrinogenemia; Gr 1 PT; Gr 2 Bili; Gr 2 hypoalbuminemia; Gr 3 AST; Gr 3 ALT	Progressive disease (38)
30	4.5	Gr 1, Bili; Gr 1 PT; Gr 1 hyperkalemia; Gr 2 hypocalcemia; Gr 2 hypofibrinogenemia	Progressive disease (31)
31[d]	4.5	Gr 1 PT; Gr 2 hypoalbuminemia; Gr 2 Bili; Gr 3 hypocalcemia; Gr 3 ALT; Gr 4 AST; Gr 4 hepatic encephalopathy; Gr 4 renal failure	Renal failure (18)

[a] Patients nos. 2, 3, 4, 8, 11, 12, 22, and 24 had two courses, and all toxicities from all courses are listed with highest grade observed.

[b] Gr, grade; Bili, bilirubin; Alk Phos, alkaline phosphatase; BMT, bone marrow transplant; GVHD, graft-versus-host disease; CNS, central nervous system; PT, protime; PTT, partial thromboplastin time; Cr, creatinine; GGT, gamma glutomylaminotransferase; CTC, common toxicity criteria.

[c] Patient no. 19 was inevaluable for toxicities because of heart and kidney dysfunction which made the patient ineligible for study.

[d] Patient no. 31 had renal failure possibly related to study drug, but patient also had aminoglycoside and amphotericin exposure, and renal insufficiency developed 1 week after therapy.

bone marrow transplants, 4 had previously received allogeneic bone marrow transplants, and 21 had not received transplants. The cytogenetics were poor risk in 15 patients, intermediate risk in 8 patients, and good risk in 1 patient. In seven patients, cytogenetics had not been performed. Prior myelodysplasia had been present in 5 of the 31 patients.

Toxicities. The DLT for this dose/schedule of DT_{388}GMCSF was liver injury. Eight patients were treated at the 4.5–5-μg/kg/day dose level, and two of these patients had severe hepatic toxicity. The dose was reduced after patient 24, although there were no DLTs, to more safely assess the effects of modified prophylaxis (rofecoxib and anti-TNF-α antibody) on the incidence and severity of toxicities. Patient 28 developed liver failure and died 1 week after treatment. This was coincident with florid leukemic progression. Necropsy showed centrilobular hepatic necrosis. Patient 31 developed transient hepatic encephalopathy with elevated ammonia levels 4 days after therapy. The liver dysfunction resolved, but the patient developed renal failure contributed to by aminoglycosides and amphotericin. Without dialysis, the patient died 1 week later. Transient elevations in AST were seen in most patients (Tables 3 and 4). Furthermore, the peak AST level correlated with the dose ($P = 0.002$; Fig. 1*A*) and peak DT_{388}GMCSF level ($P = 0.0002$; Fig. 1*B*). The AST rose disproportionately relative to ALT and GGT. Steroids, calcium channel blocker, and antibody to TNFα did not influence this toxicity. The mechanism for the liver injury is unknown. As shown in Fig. 2, DT_{388}GMCSF did not damage a hepatic cell line. DT_{388}GMCSF may indirectly

Table 4 Relationship of grade 3–5 DT_{388}GMCSF-related toxicities and dose[a]

Dose (μg/kg/day)	No. of patients at each dose	Grade 3–5 drug-related toxicities *n* (%)						
		Transaminasemia	VLS	Elevated CPK	Hypocalcemia	Elevated PTT[b]	Renal failure	Hepatic failure
1	3	0	0	0	0	0	0	0
2	6	2 (33)	1 (16)	0	0	0	0	0
3	4	0	0	0	0	0	0	0
4	8	1 (12)	0	1 (12)	0	0	0	0
4.5	5	4 (80)	0	0	3 (60)	1 (20)	1 (20)	1 (20)
5	4	2 (50)	0	1 (25)	3 (75)	0	0	1 (25)

[a] Patient no. 19 was not evaluable for toxicities because pretreatment ineligibilities (cardiac and renal failure). The only irreversible toxicities were hepatic failure in patient no. 28 and renal failure in patient no. 31. The latter patient also had aminoglycoside and amphotericin exposure during therapy.

[b] PTT, partial thromboplastin time.

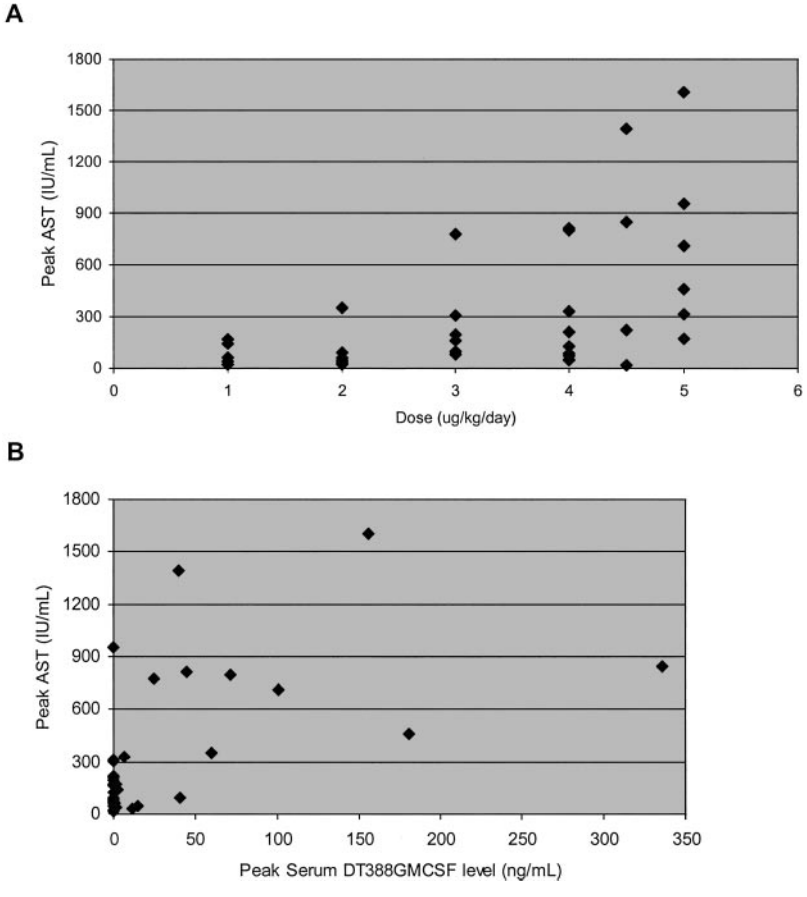

Fig. 1 Plot of peak AST *versus* dose (*A*) and *versus* peak DT_{388}GMCSF concentration (*B*). Patient 28 values are not displayed.

injure hepatocytes by causing inflammatory cytokine release from liver Kupffer cells, which are known to have GM-CSF receptors. No liver biopsies have been performed in DT_{388}GMCSF-treated patients, but elevated circulating levels of two inflammatory cytokines, IL-8 and IL-18, have been observed (Table 5). There was a significant positive association of IL-18 levels with AST levels ($P = 0.05$; Table 6). The elevations of IL-18 occurred earlier (by day 2) than the elevations of IL-8 (day 4–8; data not shown).

Other drug-related side effects include fever, chills, hypoxemia, and transient hypotension postinfusion. These were rare with corticosteroid prophylaxis beginning with patient 10. A transient asymptomatic sinus bradycardia occurred in some patients that was responsive to oral theophyllines. Echocardiograms during and after treatment did not show reduced cardiac function. There were no cardiac enzyme elevations (CPK-MB fractions) and no evidence of heart block by electrocardiography. Asymptomatic, transient hypocalcemia occurred between days 4 and 10. The incidence of hypocalcemia was dose dependent. Neither neurological nor muscular abnormalities were observed. When measured, the vitamin D levels were normal and parathyroid hormone was slightly increased. Serum

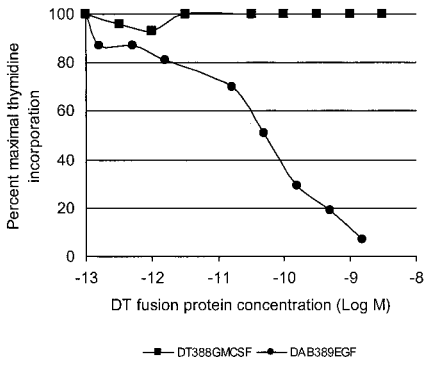

Fig. 2 DT fusion protein inhibition of thymidine incorporation by the HepG2 hepatocellular carcinoma cell line. Experiments were performed as described in "Patients and Methods." ■, DT_{388}GMCSF; ●, DAB_{389}EGF. IC_{50} was >3000 pM for DT_{388}GMCSF and >59 pM for DAB_{389}EGF.

calcium values of <7 mg/dl were treated with i.v. calcium gluconate. Serum calciums were not corrected for the serum albumins. Transient CPK and lactate dehydrogenase elevations were observed in most patients and paralleled the changes in AST. However, there were no signs of muscle injury or RBC injury measured by serum aldolase and haptoglobin or peripheral smear. VLS with the combination of edema, weight gain, hypoxia, hypotension, and hypoalbuminemia was noted only in patients 9 and 11. Patient 9 had not been receiving thyroid medications for 1 week, and the symptoms resolved with diuresis and reinstitution of thyroxine. Patient 11 had fluid overload combined with corticosteroids that also resolved with diuresis. The other patients had signs of the components of VLS with weight gain and/or hypoalbuminemia, but there was no associated edema, hypoxemia, or hypotension. The incidence and frequency of toxicities to DT_{388}GMCSF are shown in Tables 3 and 4.

On the basis of the occurrence of grade 4 and 5 liver toxicities in patients 31 and 28, respectively, we determined the MTD to be 4 μg/kg/day, and nine patients have been treated at that dose to date without DLT.

Pharmacokinetics. Pharmacokinetic data were obtained for the first infusion of each course on all 31 patients and for the first and last infusion on 5 patients. The peak DT_{388}GMCSF serum level occurred at 2 min postinfusion, and the concentration decreased over time exponentially with a $t_{1/2}$ of ~30 min (Fig. 3). The peak fusion protein concentration was not significantly correlated with the dose ($P = 0.53$; Fig. 4*B*) but was correlated with the pretreatment antibody titer ($P = 0.0001$; Table 7; Fig. 4*A*; and see "Immune Response"). Interestingly, in the five patients for whom data were available, the peak DT_{388}GMCSF concentrations were higher on day 5 than on day 1.

Immune Response. We do not have a vaccination history for the 31 patients in the trial. However, most patients likely received their full immunization against DT in childhood. The EIA showed that 28 (90%) of 31 were positive (*i.e.*, had circulating anti-DT_{388}GMCSF antibodies) in concentrations ranging from 0.2 to 9.4 μg/ml with a median of 1.85 μg/ml (Table 7). Patients with low pretreatment antibody titers were more likely to have measurable peak circulating DT_{388}GMCSF. The median and range of antibody for those with undetectable peak fusion protein during the first course were 2.5 μg/ml (1.1–9.4 μg/ml; $n = 15$), and the same antibody levels for those with measurable peak fusion protein were 0.6 μg/ml (0–3.7 μg/ml; $n = 16$). The difference was highly significant ($P < 0.001$). Only 2 of 11 patients with EIA antibody concentration above 2.2 μg/ml had detectable peak DT_{388}GMCSF compared with 14 of 20 with EIA antibody concentrations of <2.2 μg/ml.

After 15–60 days, 21 of 25 evaluable patients had increased antibody titers ranging from 0.2 to 6613 μg/ml (Table 7). One patient had no change in antibody titer and three patients had decreased antibody titers. Of the three patients with no humoral immune response to DT_{388}GMCSF, one patient had received two prior allogeneic bone marrow grafts, one patient had undergone a prior autologous bone marrow transplant, and the last patient was heavily pretreated with fludarabine.

Clinical Response. Three clinical remissions were observed (Table 8). Six of the 31 patients had relapsed disease. The three responses were seen in this group. No responses were seen in the refractory patients. Patient 22 was a 72-year-old female who developed AML in April 1996. She had normal cytogenetics and received idarubicin plus cytarabine (3 + 7), achieving a complete remission. She had no consolidation therapy, relapsed in January 2000 and received salvage therapy consisting of topotecan and high-dose cytarabine. However, she was refractory, and bone marrow biopsy on March 8, 2000, showed 28% blasts confirmed by flow cytometry (Fig. 5*A*). She received a 5-day course of DT_{388}GMCSF at 5 μg/kg/day complicated only by asymptomatic and transient transaminasemia, elevated CPK, and hypocalcemia from April 10 to April 14, 2000. Before therapy, she had an ANC of 280/μl, a platelet count of 64,000/μl, and no circulating blasts. One and 2 months posttherapy, her bone marrow showed 1–3% blasts by morphology and flow cytometry (Fig. 5*B*). She had recovery of platelets by day 60 to 158,000/μl but continued to be neutropenic (ANC of 279/μl). She was active spending most days out of the home and did not require antibiotics. By August 17, 2000, she had normalization of counts with an ANC of 1,320/μl, a platelet count of 238,000/μl, and a hemoglobin of 12.0 g/dl, and did not require transfusions or antibiotics. Repeat bone marrow exam on November 15, 2000, showed no morphological evidence of increased blasts, but there were 8% blasts by flow cytometry. Her blood counts remained normal with an ANC of 1,600/μl, a hemoglobin of 14.2 g/dl, and a platelet count of 180,000/μl. By March 29, 2001, she had a recurrence of pancytopenia (ANC of 420/μl, platelet count of 76,000/μl, and hemoglobin of 12.8 g/dl). There were no circulating blasts, but the bone marrow examination showed 8% blasts by morphology and 12% blasts by flow cytometry. She received a second course of DT_{388}GMCSF at 5 μg/kg/day for 5 days, and the day-12 bone marrow showed disappearance of the blasts by morphology and flow cytometry. By day 17 after the second course, her ANC was 1,000/μl However, she remained thrombopenic (platelet count of 7/μl)and anemic (hemoglobin of 7.1 g/dl). On day 21, she developed a pneumonia, confirmed by chest X-ray, but de-

Table 5 Serum cytokine levels in DT_{388}GMCSF-treated AML patients[a]

Patient no.	Peak serum level (pg/ml)								Peak level (IU/ml)
	IL-18	IL-1β	IL-6	IL-8	MIP-1α[b]	IFNγ	TNFα	IL-12	AST
25	9996	10	8	73	64	9	10	5	778
22A[c]	7620	3	4	507	40	51	3	10	1607
27	5590	10	6	99	23	26	23	1	848
28	4450	8	5	279	40	9	84	1	8900
31	3661	4	397	3936	82	11	7	7	1394
26	2986	2	5	155	63	10	29	4	331
24B[c]	2600	4	3	61	253	139	36	3	173
23	1855	4	8	179	79	118	4	50	315
22B	1145	11	198	2003	37	1	6	1	710
24A	935	4	2	105	270	41	15	12	956
29	565	10	4	160	47	14	2	4	199
30	225	4	26	138	37	47	2	10	17

[a] Serum levels were determined at time points described in text and assayed by EIA as described in text. Patients are listed in descending order of maximal peak IL-18 levels.
[b] MIP, macrophage inflammatory protein.
[c] A and B refer to the same patient but different courses. (See "Patients and Methods.").

Table 6 Spearman rank correlations between selected parameters: *n*, correlation (*r*), *P*[a]

	Age			Pre-Ab[b]			Peak DT_{388}GMCSF			Peak AST			Peak IL-18		
	n	*r*	(*P*)	*n*	*r*	(*P*)	*n*	*r*	(*P*)	*n*	*r*	(*P*)	*n*	*r*	(*P*)
Dose	31	−0.20	(0.281)	31	0.06	(0.742)	31	0.12	(0.534)	31	0.53	(0.002)	10	−0.24	(0.500)
	8	−0.15	(0.729)	8	−0.61	(0.106)	8	0.10	(0.821)	8	0.73	(0.039)			
Age				31	−0.40	(0.027)	31	0.46	(0.010)	31	0.21	(0.266)	10	0.81	(0.005)
				8	0.56	(0.146)	8	0.44	(0.280)	8	0.02	(0.955)			
Pre-Ab							31	−0.64	(0.001)	31	−0.45	(0.011)	10	−0.88	(0.001)
							8	0.02	(0.971)	8	−0.73	(0.040)			
Peak DT_{388}GMCSF										31	0.62	(0.001)	10	0.81	(0.005)
										8	0.55	(0.162)			
Peak AST													10	0.64	(0.048)

[a] First row gives correlations for the first dose; second row gives correlations for the second dose. Correlations between selected parameters and response were not done because only seven patients had day-30 marrow blast reductions.
[b] Pre-Ab, antibody level pretreatment.

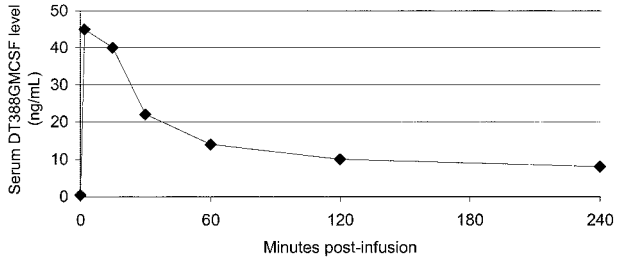

Fig. 3 Serum levels in patient 15 after first infusion. Assay was performed as described in "Patients and Methods."

clined aggressive management and died on day 24 post-therapy.

Patient 24 was a 33-year-old female diagnosed with AML in November 1998 and induced with idarubicin plus cytarabine (3 + 7) followed by an allogeneic bone marrow transplant in June 1999. She developed bronchiolitis obliterans, requiring chronic corticosteroids, and had relapse of her AML in April 2000. Bone marrow exam showed 14% blasts. She had 300/μl circulating blasts and thrombopenia (platelet count of 23,000/μl). She received DT_{388}GMCSF at 5 μg/kg/day for 5 days, and, again, the only side effects were transient, asymptomatic transaminasemia and hypocalcemia. Her day-30 bone marrow showed 2% blasts, but she remained pancytopenic with an ANC of 75/μl, a platelet count of 25,000/μl and a hemoglobin of 10.8 g/dl. She was asymptomatic. She received a course of granulocyte colony-stimulating factor (G-CSF) to stimulate recovery of normal myelopoiesis, however, by day 60, a repeat bone marrow showed an increase in blast percentage to 15%. She was again treated with DT_{388}GMCSF at 5 μg/kg/day complicated only by asymptomatic and transient transaminasemia. Her marrow blasts at day 30 were 5–10%. She continued to have neutropenia and thrombopenia. She died 1 year after initiating fusion protein therapy in April 2001.

Patient 25 was a 70-year-old male diagnosed with AML in March 2000 and treated with idarubicin plus cytarabine for induction and consolidation. He relapsed in April 2000 with a bone marrow showing 15% blasts. He had circulating blasts

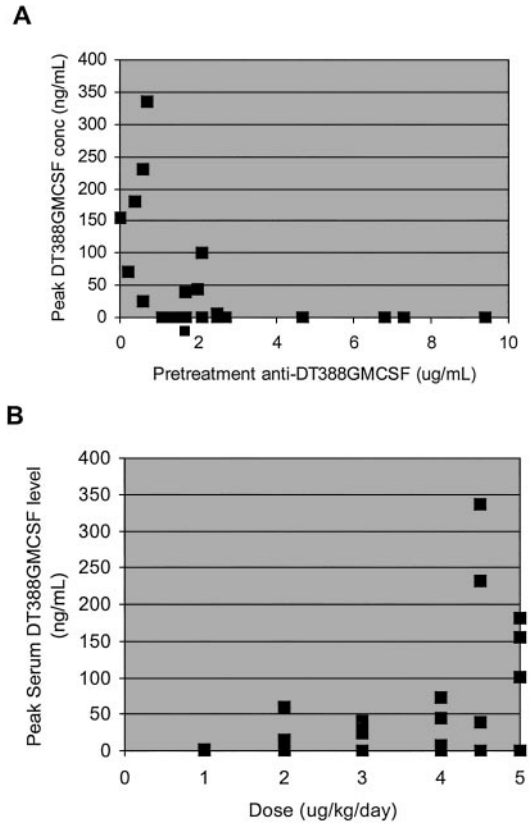

Fig. 4 Plot of pretreatment anti-DT_{388}GMCSF serum levels *versus* peak serum DT_{388}GMCSF level (*A*) and dose *versus* peak serum DT_{388}GMCSF level (*B*). Fusion protein and antibody concentrations were determined as described in "Patients and Methods."

(50/μl), thrombopenia (platelet count of 34,000/μl), and anemia (hemoglobin 8.3 g/dl). He was treated from 6/22/00 to 6/26/00 with DT_{388}GMCSF at 3 μg/kg/day. His course was complicated by transient renal insufficiency attributed to rofecoxib and transient, asymptomatic transaminasemia, hypocalcemia, and elevated CPK. The day-12 marrow showed 1% blasts, and the day-30 marrow showed 4% blasts. However, he remained anemic and thrombopenic. He declined further fusion protein or other therapy and died from progressive disease 40 days post-therapy.

DISCUSSION

The diphtheria fusion protein DT_{388}GMCSF unexpectedly produced liver injury characterized by transient transaminasemia and, rarely, more severe liver dysfunction. The MTD of 4 μg/kg/day for 5 days or 20 μg/kg total per course is much lower than that observed for the other systemically administered clinically active fusion proteins ONTAK, LMB–2, and BL22 (120, 120, and 135 μg/kg/total, respectively; Refs. 19, 21, 22) and resembles the low MTDs of 18 and 3 μg/kg/total DAB_{389}EGF and erb-38, respectively (23). Both DAB_{389}EGF and erb-38 bound hepatocytes and produced direct liver injury (see Fig. 3). In contrast, DT_{388}GMCSF does not directly bind or damage hepatocytes.

Table 7 Summary of antibody titers and peak serum DT_{388}GMCSF levels[a]

Dose (μg/kg/day)	Pretreatment antibody titer median (range)	Peak serum DT_{388}GMCSF median (range)	Day 30 antibody titer median (range)
First course			
1	0 (0–3.7)	1.7 (0.5–2)	8.5 (2–6617)
2	1.3 (0.3–4.1)	0 (0–60)	4.7 (0.7–150)
3	1.4 (0.3–2.7)	0	1.7 (1.6–34)
4	2.5 (0.2–7.3)	0 (0–72)	11 (1.4–599)
4.5	1.7 (0.6–9.4)	40 (0–336)	ND[b]
5	0.4 (0–2.1)	0 (0–181)	2.1 (1–177)
Second course			
1	2 (2–257)	0 (0–2.2)	5400 (5400–6617)
2	22 (22–37)	0	797
3	1.6 (1.6–1.7)	0	3.1
5	1.6 (1.6–2.1)	0 (0–101)	ND

[a] The circulating fusion protein assay limit was 0.3 ng/ml, and undetectable drug levels were listed as 0. Inter- and intra-assay variability was ≤50%. The antibody assay limit was 0.1 μg/ml with 30% intra- and interassay variability.[16] Patients 1 and 19 were assayed on day 15, and patients 3B, 5, and 10 were assayed on day 60. Methods described in the "Patients and Methods."

[b] ND, not determined.

Table 8 Summary of effects of DT_{388}GMCSF on bone marrow blasts

Dose (μg/kg/day)	Change in % marrow blasts pre-day 12 median (range)	Change in % marrow blasts pre-day 30 median (range)
First course		
1	4 (−3 to 20)	5 (0 to 95)
2	−7 (−10 to 57)	0 (−3 to 62)
3	0 (−71 to 3)	−61 (−70 to 0)
4	−15 (−42 to 5)	5 (−25 to 20)
4.5	7 (−23 to 45)	0 (−5 to 19)
5	−18 (−28 to 56)	−12 (−26 to −12)
Second course		
1	0 (0 to 10)	5 (5 to 17)
2	4 (4 to 10)	10
3	0 (0 to 63)	35
5	−12 (−15 to −12)	55

We hypothesize that the fusion protein bound the GMCSF receptor-positive macrophages in the liver (Kupffer cells) and triggered cytokine release. We have observed extremely elevated circulating levels of IL-18 in treated patients. IL-18 is a known pro-inflammatory cytokine that can be released by stimulated Kupffer cells and can damage hepatocytes by inducing their expression of Fas, leading to Fas ligand-mediated cell death (24, 25). Other laboratories have shown that cyclosporin, FK506, and monoclonal anti-IL-18 antibodies can provide effective prophylaxis *in vivo* for IL-18-inducing liver toxins (26, 27). Whereas our data are consistent with the hypothesis of Kupffer cell cytokine-induced liver injury, the cause of the DT_{388}GMCSF hepatotoxicity is not identified definitively.

The humoral immune response to DT fusion proteins clearly affected circulating levels of DT_{388}GMCSF and likely influenced clinical benefit at the low doses used in this study. Approaches to circumvent the immune response are to carefully

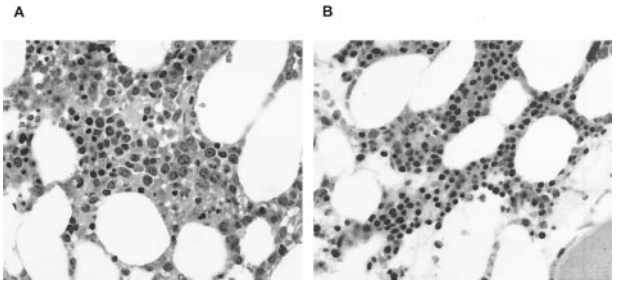

Fig. 5 Photomicrographs of H&E-stained sections of bone marrow biopsies obtained pretreatment (*A*) and 1-month posttreatment with DT_{388}GMCSF (*B*).

select patients with low pretreatment antibody titers or to alter the schedule and use fewer but higher doses. Another approach is to engineer and develop other targeted toxins that can intoxicate malignant myeloid cells. An antibody to CD33 has been humanized and conjugated to recombinant gelonin, a plant ribosome-inactivating protein (28). Human proteins such as eosinophil-derived neurotoxin, a member of the RNase family, may be engineered to target myeloblasts (29). This toxin, combined with a human-derived ligand peptide, should be less immunogenic in patients.

We observed clinical activity with DT_{388}GMCSF in highly chemoresistant AML patients. This observation confirms the antileukemic activity observed with this drug in tissue culture and animal models (10–13). Similar fusion proteins (DAB_{389}IL-2, Tf-CRM107, LMB-2, and LMB-7) directed to other receptors have yielded 30–80% response rates in cutaneous T-cell lymphoma, high-grade gliomas, and hairy cell leukemia (19–22). The observed response rate of 10% is significantly lower than with these other fusion proteins. There are several possible explanations for this difference. First, DT_{388}GMCSF may be less active in patients than are the other proteins. The preclinical data does not support this hypothesis. In tissue culture, leukemic progenitors were killed with picomolar concentrations of drug (9–12). *In vivo,* long-term remissions were produced with DT_{388}GMCSF (13). These results compare favorably with the other clinically efficacious fusion proteins. A second hypothesis is that most patients were treated at suboptimal doses, and very few patients received therapeutic amounts of DT_{388}GMCSF. Only 18 of 31 patients received doses at or above the MTD (4 to 5 μg/kg/day). A slow interpatient dose escalation was done because of the novel nature of this drug and the comorbidities and non-drug-related toxicities that are common in these highly pretreated leukemic patients. A third hypothesis is that the early occurrence of DLT limited the dose escalation and prevented administration of adequate dose levels to achieve a higher response rate. The liver toxicity precluded dose escalation, and reducing the liver toxicity should permit dose escalation. A fourth factor that likely contributed to the low response rate was the presence of pretreatment anti-DT antibodies in many of the patients. Whereas the influence of anti-DT antibodies is less pronounced when higher doses of DT fusion proteins are given (19), when only 4–5 μg/kg fusion protein can be administered, the antibodies likely reduce blast exposure to drug. We failed to observe circulating drug in most patients with high anti-DT antibody titers. Fifth, there may be significant patient-to-patient variations in sensitivity to DT_{388}GMCSF that were not detected with the previously used colony-forming assay (AML-CFC; Refs. 9–12). In support of this hypothesis, we have found dramatic differences in patient leukemic blast sensitivity to DT_{388}GMCSF using a 3-day proliferation assay.[4] However, patient blast GM-CSF receptor content and DT_{388}GMCSF sensitivity was not measured prospectively in our study patients. Finally, the patients treated on this study, in most instances, had very advanced disease. In only 12 of 39 courses were patients treated who had <30% marrow blasts. Patients with extensive tumor burden may be less sensitive to biologicals such as DT_{388}GMCSF, particularly at the low dose levels used in this study. All of the responses observed occurred among the 12 patients with low marrow-blast percentage.

We are committed to discovering approaches to improve the preliminary response rate observed in the Phase I study. First, we are investigating the molecular mechanism for the liver toxicity and looking for methods of prophylaxis that will protect the liver without modifying the antileukemic efficacy. We are testing oral glycine prophylaxis to block Kupffer cell activation and liver injury from DT_{388}GMCSF (30). The selective glycine inhibition of liver macrophage should not impair the antileukemic activity of DT_{388}GMCSF. Second, we are evaluating prognostic factors that may identify a patient category more likely to respond, including low pretreatment anti-DT antibody titer, high sensitivity of pretreatment blasts to DT_{388}GMCSF, and low pretreatment leukemic burden. Third, we will give the fusion protein on a twice-weekly-for-2-weeks schedule as has been used successfully for the *Pseudomonas* exotoxin fusion proteins (*i.e.,* LMB-2 and BL22). Such a schedule may permit higher individual doses to be given and yield better blast saturation both in the blood and marrow and other extravascular sites.

Although multiple issues need to be addressed to improve the therapeutic index of the DT_{388}GMCSF fusion protein for therapy of myeloid malignancies, the preliminary results are encouraging and suggest that additional preclinical and clinical development is warranted.

ACKNOWLEDGMENTS

We thank Drs. Samer Renno, Itzvan Molnar, and Andrew Mone for their assistance in developing some of the *in vitro* cytokine assays, patient serum DT_{388}GMCSF assays, and patient serum cytokine assays used in this study. We also thank Jason Ramage and Jennifer Black for excellent technical assistance, Cynthia Patrick for clinical protocol nursing support, Dr. Mark Willingham for assistance with photomicroscopy, and Dr. Charles McCall for advice and guidance during this study. We also thank Dr. Dianna Howard at the University of Kentucky Medical Center for the care of two of the patients on protocol.

REFERENCES

1. Greenlee, R. T., Hill-Harmon, M. B., Murray, T., and Thun, M. Cancer statistics, 2001. CA Cancer J. Clin., *51:* 15–36, 2001.

[4] A. E. Frankel, unpublished observations.

2. Bennett, J. M., Young, M. L., Andersen, J. W., Casssileth, P. A., Tallman, M. S., Paietta, E., Wiernik, P. H., and Rowe, J. M. Long-term survival in acute myeloid leukemia: the Eastern Cooperative Oncology Group Experience. Cancer (Phila.), *80* (Suppl. 11)*:* 2205–2209, 1997.

3. Estey, E. H. Treatment of relapsed and refractory acute myelogenous leukemia. Leukemia (Baltimore), *14:* 476–479, 2000.

4. Lowenberg, B., and Sonneveld, P. Resistance to chemotherapy in acute leukemia. Curr. Opin. Oncol., *10:* 31–35, 1998.

5. Sauerbrey, A., Zintl, F., Hermann, J., and Volm, M. Multiple resistance mechanisms in acute nonlymphoblastic leukemia (ANLL). Anticancer Res., *18:* 1231–1236, 1998.

6. List, A., Spier, C., Grogan, T., Johnson, C., Roe, D., Greer, J., Wolff, S., Broxterman, H., Scheffer, G., Scheper, R., and Dalton, W. Overexpression of the major vault transporter protein lung-resistance protein predicts treatment outcome in acute myeloid leukemia. Blood, *87:* 2464–2469, 1996.

7. Frankel, A. E., Powell, B. L., Vallera, D. A., and Neville, D. A. Chimeric fusion proteins (diphtheria toxin-based). Curr. Opin. Investig. Drugs, *2:* 1294–1301, 2001.

8. Kreitman, R. J., and Pastan, I. Recombinant toxins containing human granulocyte-macrophage colony-stimulating factor and either *Pseudomonas* exotoxin or diphtheria toxin kill gastrointestinal cancer and leukemia cells. Blood, *90:* 252–259, 1997.

9. Frankel, A. E., Hall, P., Burbage, C., Vesely, J., Willingham, M. C., Bhalla, K., and Kreitman, R. J. Modulation of the apoptotic response of human myeloid leukemia cells to a diphtheria toxin-granulocyte-macrophage colony stimulating factor (GMCSF) fusion protein. Blood, *90:* 3654–3661, 1997.

10. Hogge, D. E., Willman, C. L., Kreitman, R. J., Berger, M., McLain, C., Hall, P. D., Kopecky, K. J., Tagge, E. P., and Frankel, A. E. Malignant progenitors from patients with acute myelogenous leukemia are sensitive to a diphtheria toxin-granulocyte-macrophage colony stimulating factor (GMCSF) fusion protein. Blood, *92:* 589–595, 1998.

11. Feuring-Buske, M., Frankel, A. E., Gerhard, B., and Hogge, D. Cytotoxicity of diphtheria toxin 388-granulocyte-macrophage colony stimulating factor (DT_{388}-GMCSF) fusion protein for acute myelogenous leukemia (AML) stem cells. Exp. Hematol., *28:* 1390–1400, 2000.

12. Frankel, A. E., Lilly, M., Kreitman, R. J., Hogge, D., Beran, M., Freedman, M. H., Emanuel, P. D., McLain, C., Hall, P., Tagge, E. P., Berger, M., and Eaves, C. Diphtheria toxin fused to granulocyte-macrophage colony-stimulating factor is toxic to blasts from patients with juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. Blood, *92:* 4279–4286, 1998.

13. Hall, P. D., Kreitman, R. J., Willingham, M. C., Tagge, E. P., and Frankel, A. E. Diphtheria toxin fused to granulocyte-macrophage colony-stimulating factor prolongs the survival of scid mice bearing human acute myelogenous leukemia. Leukemia (Baltimore), *13:* 629–633, 1999.

14. Frankel, A. E., Ramage, J., Latimer, A., Feely, T., Delatte, S., Hall, P., Tagge, E., Kreitman, R. J., and Willingham, M. C. High level synthesis and maximal purification of the recombinant diphtheria fusion toxin DTGM for Phase I clinical trials. Protein Expr. Purif., *16:* 190–201, 1999.

15. Hotchkiss, C. E., Hall, P. D., Cline, J. M., Willingham, M. C., Kreitman, R. J., Gardin, J., Latimer, A., Ramage, J., Feely, T., and Frankel, A. E. Toxicology and pharmacokinetics of DTGM, a fusion toxin consisting of a truncated diphtheria toxin (DT_{388}) linked to human granulocyte-macrophage colony stimulating factor (GM-CSF), in cynomolgus monkeys. Toxicol. Appl. Pharmacol., *158:* 152–160, 1999.

16. Hall, P. D., Virella, G., Beckham, W., and Frankel, A. E. Adult AML patients treated with DTGM diphtheria fusion toxin do not have neutralizing antibodies before or after the first treatment course. Clin. Immunol., *100:* 191–197, 2001.

17. Shaw, J. P., Akiyoshi, D. E., Arrigo, D. A., Rhoad, A. E., Sullivan, B., Thomas, J., Genbauffe, F. S., Bacha, P., and Nichols, J. C. Cytotoxic properties of DAB_{486}EGF and DAB_{389}EGF, epidermal growth factor (EGF) receptor-targeted fusion toxins. J. Biol. Chem., *266:* 13449–13455, 1991.

18. Olsen, E., Duvic, M., Frankel, A., Kim, Y., Martin, A., Vonderheid, E., Jegasothy, B., Wood, G., Gordon, M., Heald, P., Oseroff, A., Pinter-Brown, L., Bowen, G., Kuzel, T., Fivenson, D., Foss, F., Glode, M., Molina, A., Knobler, E., Stewart, S., Cooper, K., Stevens, S., Craig, F., Reuben, J., Bacha, P., and Nichols, J. Pivotal Phase III trial of two dose levels of denileukin diftitox for the treatment of cutaneous T-cell lymphoma. J. Clin. Oncol., *19:* 376–388, 2001.

19. Laske, D. W., Youle, R. J., and Oldfield, E. H. Tumor regression with regional distribution of the targeted toxin Tf-CRM107 in patients with malignant brain tumors. Nat. Med., *3:* 1362–1368, 1997.

20. Kreitman, R. J., Wilson, W. H., White, J. D., Stetler-Stevenson, M., Jaffe, E. S., Giardina, S., Waldman, T. A., and Pastan, I. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. J. Clin. Oncol., *18:* 1622–1636, 2000.

21. Kreitman, R. J., Wilson, W. H., Bergeron, K., Raggio, M., Stetler-Stevenson, M., FitzGerald, D. J., and Pastan, I. Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. N. Engl. J. Med., *345:* 241–247, 2001.

22. Pai-Scherf, L. H., Villa, J., Pearson, D., Watson, T., Liu, E., Willingham, M. C., and Pastan, I. Hepatotoxicity in cancer patients receiving erb-38, a recombinant immunotoxin that targets the erbB2 receptor. Clin. Cancer Res., *5:* 2311–2315, 1999.

23. Nakanishi, K., Yoshimoto, T., Tsutsui, H., and Okamura, H. Interleukin-18 regulates both Th1 and Th2 responses. Annu. Rev. Immunol., *19:* 423–474, 2001.

24. Tsutsui, H., Matsui, K., Okamura, H., and Nakanishi, K. Pathophysiological roles of interleukin-18 in inflammatory liver diseases. Immunol. Rev., *174:* 192–209, 2000.

25. Dinarello, C. A. Interleukin-18, a proinflammatory cytokine. Eur. Cytokine Netw., *11:* 483–486, 2000.

26. Kuinose, M., Iwagaki, H., Morimoto, Y., Kohka, H., Kobashi, K., Sadamori, H., Inagaki, M., Urushihara, N., Yagi, T., and Tanaka, N. Calcineurin antagonists inhibit interferon-γ production by downregulation of interleukin-18 in human mixed lymphocyte reactions. Acta Med. Okayama, *54:* 201–209, 2000.

27. Pagliaro, L. C., Liu, B., Munker, R., Andreef, M., Freireich, E. J., Scheinberg, D. A., and Rosenblum, M. G. Humanized M195 monoclonal antibody conjugated to recombinant gelonin: an anti-CD33 immunotoxin with antileukemic activity. Clin. Cancer Res., *4:* 1971–1976, 1998.

28. Newton, D. L., Nicholls, P. J., Rybak, S. M., and Youle, R. J. Expression and characterization of recombinant human eosinophil-derived neurotoxin and eosinophil-derived neurotoxin-anti-transferrin receptor sFv. J. Biol. Chem., *269:* 26739–26745, 1994.

29. Schemmer, P., Golling, M., Kraus, T., Mayatepek, E., Herfarth, C., and Klar, E. Glycine reduces reperfusion injury in human liver transplantation: our first patients. Transplant. Proc., *33:* 3750–3752, 2001.