Safety and efficacy of denileukin difftitox in patients with steroid-refractory acute 
graft-versus-host disease after allogeneic hematopoietic stem cell transplantation

Vincent T. Ho, David Zahrleit, Ephraim Hochberg, Eileen Micale, Jesse Levin, Carol Reynolds, Steve Steckel, Corey Cutler, David C. Fisher, Stephanie J. Lee, Edwin P. Alyea, Jerome Ritz, Robert J. Soiffer, and Joseph H. Antin

Denileukin difftitox (Ontak; Ligand Pharmaceuticals, San Diego, CA) is a recombinant fusion protein with selective cytotoxicity against activated T lymphocytes expressing the high-affinity IL-2 receptor. It is composed of human IL-2 fused to the membrane translocation and catalytic domains of diphtheria toxoid. After cellular entry, the toxoid catalyses adenosine diphosphate (ADP)–riboseylation of elongation factor 2, halts protein synthesis, and triggers apoptosis. Steroid-refractory graft-versus-host disease (GVHD) is common after allogeneic hematopoietic stem cell transplantation (HSCT) and is associated with poor survival primarily because of infectious complications. We hypothesize that selective depletion of activated T cells may be effective in treating GVHD while preserving immune function. We hereby report a phase 1 study investigating denileukin difftitox in steroid-refractory acute GVHD.

Study design

This study was approved by the institutional review board at the Dana-Farber Cancer Institute/Harvard Cancer Center. Informed consent was obtained from patients before they enrolled. Diagnosis of acute GVHD was made clinically and supported with biopsies in most cases. Steroid-refractory GVHD was defined as no improvement in GVHD after 2 weeks on corticosteroids (≥ 1 mg/kg methylprednisolone), progression of GVHD after 1 week on corticosteroids, or GVHD flare after taper that was unresponsive to corticosteroid rechallenge. Mechanical ventilation, serum creatinine level 3 mg/dL or higher, dialysis, vasopressor requirement, Eastern Cooperative Oncology Group (ECOG) performance status more than 3, alanine aminotransaminase (ALT) or aspartate aminotransaminase (AST) level equal to or more than 5× upper limits of normal (ULN), and serum albumin level 1.5 g/dL or less were exclusion criteria. Patients were excluded if they received daclizumab within 7 days or had other immune suppressive agents added within 3 days of enrollment.

Three dose schedules were evaluated: level 1, 9 μg/kg intravenously on days 1 and 15; level 2, 9 μg/kg intravenously on days 1, 3, 5, 15, 17, and 19; and level 3, 9 μg/kg intravenously on days 1 to 5 and 15 to 19. Dose escalation was determined by dose-limiting toxicity (DLT) at each dose level. After the maximum tolerated dose (MTD) was determined, 10 additional patients were enrolled to assess efficacy. The initial dose of denileukin difftitox was administered over 60 minutes and subsequent doses over 30 minutes. Patients were premedicated with diphenhydramine, acetaminophen, and corticosteroid. Whenever possible, the daily steroid dose was used as premedication. Patients were monitored at least weekly during the 4-week study period.

Patients were evaluable for toxicity if they received 1 dose of denileukin difftitox. DLT was defined as (1) renal dysfunction (hemodialysis, serum creatinine level > 3 × baseline/ULN), (2) hepatic dysfunction (ALT or AST > 5 × baseline/ULN, total bilirubin > 3 × baseline/ULN with no GVHD on liver biopsy), (3) respiratory failure requiring mechanical ventilation, (4) hypotension requiring vasopressors for more than 24 hours, (5) cardiac arrhythmia requiring electrical intervention, or (6) death from a cause other than GVHD or disease relapse.

GVHD was assessed at study day 29 or at “unenrollment” for patients who developed DLT after day 14. Patients “unenrolled” at any time for progression or death from GVHD were considered nonresponders. Acute GVHD was graded by consensus criteria.

GVHD responses were scored as: complete response (CR), resolution in all affected organs; partial response (PR), ≥ 50% reduction in GVHD with or without clinical improvement; stable disease (SD), > 50% reduction but < 50% improvement; or progressive disease (PD), ≥ 25% increase in GVHD or progression of GVHD.

From the Divisions of Medical Oncology and Biostatistics, Dana-Farber Cancer Institute, Department of Medicine, Brigham and Women’s Hospital, Boston, MA; Ligand Pharmaceuticals, Inc, San Diego, CA; and Harvard Medical School, Boston MA.


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Reprints: Vincent T. Ho, Dana-Farber Cancer Institute, 44 Binney St, D1B21, Boston, MA 02115; e-mail: vtho@partners.org.

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Results and discussion

Thirty-two patients were enrolled; 30 were assessable. Their characteristics are presented in Table 1. At dose level 1, one of 5 patients experienced DLT. At dose level 2, 3 of 8 patients developed DLT (one each pulmonary embolism, multiorgan failure, hepatic transaminitis). At dose level 3, all 4 patients evaluable for toxicity developed DLT (1 renal failure, 3 transaminitis). Therefore, dose level 2 was considered the MTD.

Hepatic transaminitis was the most common toxicity (26%) and was observed in 4 of 18 patients (22%) at the MTD. In most cases, hepatic transaminases normalized spontaneously. One patient without pre-existing hepatic GVHD died of liver failure with isolated hyperbilirubinemia. Postmortem examination revealed hepatic centrilobular cholestasis and congestion but no evidence of hepatic GVHD. The hyperbilirubinemia was also deemed unlikely to be GVHD because her skin GVHD was resolving on the study drug, and her bilirubin rise coincided with Escherichia coli sepsis. She had also recently started on antiepileptic and antifungal medications with possible hepatic toxicity. Other severe adverse events potentially attributable to denileukin diftitox included infusional reaction in 2 (6%), acute renal failure in one (4%), cardiac tamponade in one (4%), pulmonary embolism in one (4%), and sepsis in 3 (11%). Eight patients died during the study period. Causes of death were GVHD in 2 patients, sepsis/multiorgan failure in 3 patients, and idiopathic pneumonia syndrome, intracranial hemorrhage, and liver failure in one each.

GVHD responses are presented on Table 2. Among the 24 patients evaluable for response, 8 (33%) achieved CR and 9 (38%) PR. Four partial responders entered CR after day 29 without additional therapy, for an overall CR of 50%. The best responses were observed at the MTD, where 6 (46%) of 13 achieved CR. GVHD responses to denileukin diftitox were substantial in patients with skin and intestine involvement and were not restricted to patients with grade II GVHD.

With extended follow-up, 9 of 30 patients treated are alive (median, 7.2 months). Among those who achieved CR or PR that converted to CR, 7 (58%) are living. Conversely, only one of the 12 evaluable patients who did not achieve CR remains alive (P < .001). There have been 5 late deaths among patients in CR, 3 from infection (2 bacterial, 1 fungal), one from chronic GVHD, and one from lymphoma relapse. No lymphoma associated with Epstein-Barr virus or cytomegalovirus disease has been observed.

Flow cytometry was performed on blood samples taken during the study period. Pretreatment absolute CD3+ lymphocytes was statistically significant in patients who achieved CR (P = .03), but not in nonresponders. Serum or plasma

Table 1. Patient characteristics

| No. treated | 30 |
| Median age, y (range) | 43 (20-63) |
| Sex, M/F | 15/15 |

**Diagnosis, no.**
- Acute leukemia/myelodysplastic syndrome: 15
- CML/chronic myeloproliferative disorder: 4
- Chronic lymphocytic leukemia: 6
- Non-Hodgkin lymphoma: 3
- Hodgkin lymphoma: 1
- Plasma cell leukemia: 1

**Conditioning, no.**
- Flu/Bu (nonmyeloablative): 11
- Cy/TBI: 17
- Bu/Cy: 2

**GVHD prophylaxis, no.**
- FK506/MTX: 10
- FK506/mini-MTX/CD8+ T-cell depletion: 8
- FK506/mini-MTX/sirolimus: 3
- FK506/prednisone: 1
- CyA/prednisone/MMF: 3

**Stem cell source, no.**
- Marrow: 6
- PBSCs: 24

**Donor, no.**
- Matched related: 2
- Matched unrelated: 26
- Antigen mismatched related: 1
- Antigen mismatched unrelated: 1

**GVHD grade at enrollment, no.**
- Grade I: 11
- Grade II: 13
- Grade III: 6

**Denileukin diftitox dose level**
- Level 1: 9 μg/kg d 1, 15
- Level 2: 9 μg/kg d 1, 3, 5, 15, 19
- Level 3: 9 μg/kg d 1-5, 16-19

*CML* indicates chronic myelogenous leukemia; PBSCs, peripheral blood stem cells; Cy/TBI, cyclophosphamide/total body irradiation; Bu/Cy, busulfan/cyclophosphamide; Flu/Bu, fludarabine/busulfex; FK506/MTX, tacrolimus/methotrexate; pred, prednisolone; CyA, cyclosporine A; MMF, mycophenolate mofetil.

*Eight patients treated in dose-escalation phase, 10 on MTD expansion phase of trial.

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**Table 2. GVHD response to denileukin diftitox by dose level, organ involvement, and severity.**

<table>
<thead>
<tr>
<th>Drug dose</th>
<th>CR, no. (%)</th>
<th>PR, no. (%)</th>
<th>OR, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Level 1</strong></td>
<td>1/7 (14)</td>
<td>4/7 (57)</td>
<td>5/7 (71)</td>
</tr>
<tr>
<td><strong>Level 2</strong></td>
<td>6/13 (46)</td>
<td>3/13 (23)</td>
<td>9/13 (69)</td>
</tr>
<tr>
<td><strong>Level 3</strong></td>
<td>1/4 (25)</td>
<td>2/4 (50)</td>
<td>3/4 (75)</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>8/24 (33)</td>
<td>9/24 (38)</td>
<td>17/24 (71)</td>
</tr>
</tbody>
</table>

**Organs involved**
- Skin: 7/16 (44)
- Intestine: 9/16 (56)
- Liver: 1/4 (25)

**GVHD grade at enrollment**
- II: 1/8 (13)
- III: 5/13 (38)
- IV: 2/3 (67)

*Only 1 patient at this level completed the intended 10 doses due to toxicity.
†Four of 9 patients with a PR converted to CR after day 29 without additional therapy.
soluble IL-2 receptor levels remained stable over the treatment period. There was no correlation between soluble IL-2 receptor level and clinical response.

We have determined that denileukin diftitox can be safely administered to patients after allogeneic HSCT. Reversible hepatic transaminitis is the DLT and 9 μg/kg intravenously on days 1, 3, 5, 15, 17, and 19 is the MTD. The incidence of hepatic toxicity at the MTD (22%) is comparable to that previously reported in patients with lymphoma. Serum measurements confirm that denileukin diftitox retains a short half-life of about 1 hour (data not shown). This may have important implications in terms of prolonged immunosuppression.

Denileukin diftitox has significant activity against steroid-refractory acute GVHD. At the MTD, the drug resulted in a 46% CR rate and a 69% overall response (OR) rate. Although CD3⁺CD25⁺ numbers were not affected, peripheral CD3⁺ T lymphocytes were transiently depleted after treatment with denileukin diftitox especially in patients who achieved CR. This observation suggests that cells expressing other components of the IL-2 receptor may internalize denileukin diftitox sufficiently to effect cell lysis in vivo. Alternatively, flow cytometry may not be able to detect low-level expression of high-affinity receptors that may still be sufficient for binding and internalization.

Although survival was improved among the patients who achieved CR, infections remained a common cause of late mortality. Attribution of late infections is difficult because these patients had failed multiple immunosuppressive therapies prior to enrollment. For these reasons, demonstration of overall survival benefit from denileukin diftitox will likely require studies as primary therapy or in a more viable population of steroid-refractory patients.

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References

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