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A phase-1 trial of bexarotene and denileukin diftitox in patients with relapsed or refractory cutaneous T-cell lymphoma

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Denileukin diftitox, a genetically engineered fusion protein combining the enzymatically active domains of diphtheria toxin and the full-length sequence for interleukin-2 (IL-2), efficiently targets lymphoma cells expressing the high-affinity IL-2 receptor (IL-2R) consisting of the α/p55/CD25, β/p75/CD122, and γ/p64/CD132 chains. In vitro studies demonstrated that the retinoid X receptor (RXR) retinoid, bexarotene, at biologically relevant concentrations of 10^{-6}M to 10^{-8}M, upregulated both the p55 and p75 subunits of the IL-2R and enhanced 5- to 10-fold the susceptibility of T-cell leukemia cells to denileukin diftitox. To determine whether this biomodulatory effect could be recapitulated in vivo, we treated 14 patients with relapsed or refractory cutaneous T-cell lymphoma with escalating doses of bexarotene (75 mg/day-300 mg/day) and denileukin diftitox (18 mcg/kg per day × 3 days every 21 days) in a phase 1 trial. Overall response was 67% (4 complete responses, 4 partial responses). Modulation of IL-2R expression was observed at or above a bexarotene dose of 150 mg/day. Four patients experienced grade 2 or 3 leukopenia, and 2 had grade 4 lymphopenia. Our results demonstrate that the combination of denileukin diftitox and bexarotene is well tolerated and that even low doses (150 mg/day) of bexarotene are capable of in vivo upregulation of CD25 expression on circulating leukemia cells. (Blood. 2005; 106:454-457)

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Introduction

Denileukin diftitox (ONTAK; Ligand Pharmaceuticals, San Diego, CA) is a fusion protein that consists of the full-length sequence of the interleukin-2 (IL-2) protein genetically fused to the enzymatically active and translocating domains of diphtheria toxin. The IL-2 moiety of the molecule binds to the interleukin-2 receptor (IL-2R) and is internalized via receptor-mediated endocytosis in an acidified vesicle. Upon acidification, the enzymatically active moiety of diphtheria toxin passes into the cytosol where, over several hours, it inhibits protein synthesis via adenosine diphosphate (ADP)–ribosylation of elongation factor-2, ultimately resulting in cell death.[1-4] Binding affinity of denileukin diftitox is highest for the heterotrimeric form of the IL-2R (IL2R α, β, and γ), but the fusion protein is capable of binding with lower affinity and intoxicating leukemia cells expressing the intermediate-affinity receptor (IL-2R β and γ).[5] Expression of IL-2R subunits by leukemia cells is tightly regulated by a number of cellular factors, including cytokine stimulation and expression of activation-associated genes. Denileukin diftitox is effective in the treatment of cutaneous T-cell lymphoma (CTCL) and other types of lymphoid malignancies.[6-9]

Bexarotene (Targretin; Ligand Pharmaceuticals) is a retinoid X receptor (RXR) selective retinoid that has demonstrated clinical efficacy in patients with early- and advanced-stage CTCL.[10,11] In previous studies, we demonstrated that the retinoid acid receptor (RAR) retinoid, all-*trans* retinoic acid, and the RXR retinoid, bexarotene, at biologically relevant concentrations of 10^{-6}M to 10^{-8}M, upregulated both the p55 and p75 subunits of the IL-2R in vitro and enhanced the susceptibility of leukemia cells 5- to 10-fold to denileukin diftitox.[12] The ability of the RXR modulators to form heterodimers with various receptor partners that are important in cellular function and physiology indicates that the biologic activities of bexarotene are more diverse than those of compounds that activate the RARs. Once activated, these RXR receptors function as transcription factors that regulate various cellular processes. To further explore the effects of bexarotene on IL-2R expression and potentially enhance the susceptibility of leukemia cells to denileukin diftitox, we conducted a phase 1 clinical trial of bexarotene administration prior to and during denileukin diftitox in patients with CTCL to determine the safety and tolerability of the combination.

Patients and methods

Patient population

Patients aged 18 years and older with histopathologically confirmed CTCL were eligible for enrollment. All patients had to have progressive disease after at least one systemic therapy other than steroids. Patients previously treated with bexarotene were not eligible. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2. Consent forms approved by the Tufts New England Medical Center institutional review board were signed by all patients prior to therapy.

Treatment plan

Bexarotene was administered by cohort dose escalation at daily doses of 75, 150, 225, or 300 mg/day beginning 7 days before the first dose of denileukin diftitox. Once begun, bexarotene was taken by mouth daily throughout the course of treatment. The initial goal was accrual of 3 patients into each

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dose-cohort with the number of patients per cohort adjusted on the basis of treatment tolerability and effect of bexarotene pretreatment on CD25 expression. No prophylactic antilipid or thyroid therapy was administered in this study. Plasma triglycerides, cholesterol, and serum thyroxin levels were measured at every study visit. Atorvastatin (Pfizer, New York, NY) and levothyroxine (Abbott, Abbott Park, IL) were administered for evidence of hyperlipidemia or hypothyroidism, respectively. Denileukin diftitox was initiated on day 8 at a dose of 18 mcg/kg per day for 3 doses every 21 days. Denileukin diftitox was infused over 40 to 60 minutes on day 1 of the first cycle, and if well tolerated, was given over an infusion time of at least 15 minutes for subsequent doses. Daily premedication included 8 mg intravenous dexamethasone, 25 mg to 50 mg diphenhydramine given orally, and 650 mg acetaminophen given orally. Patients were treated with at least 4 cycles of therapy or until best response or response plateau. Response was determined based on skin scoring and on overall disease burden, including measurement of adenopathy and blood involvement. Photographs were taken at baseline and at response or progression.

CD25 expression assessment

Only the CD25 component of the IL-2R was evaluated. CD25 expression was assessed by immunohistochemistry (IHC) or flow cytometry at baseline prior to bexarotene administration, after 7 days of bexarotene administration and prior to the first dose of denileukin diftitox, and then prior to each subsequent course of denileukin diftitox.

Results

Patients and treatment

Fourteen patients with CTCL were enrolled in the study. Median age was 62 years (range, 46-81 years). There were 8 women and 6 men. Seven patients had stage I-IIA disease, one had stage IIB, 3 had stage III, one had stage IVA, and 2 had stage IVB. Median number of prior therapies was 2 (range, 1-11). One patient who had received denileukin diftitox prior to this study had not achieved a partial response after 8 cycles of therapy. This was a cohort dose-escalation study with doses of bexarotene ranging from 75 mg/day to 300 mg/day. Only one patient received a dose of 75 mg, and as no upregulation of IL-2R was seen in this patient no further patients were accrued at this dose. Five patients received a dose of 150 mg daily, 4 patients received 225 mg daily, 3 patients received 300 mg daily, and one patient received a dose of 375 mg daily. The number of cycles of denileukin diftitox received by each patient varied, ranging from 1 to 12 and was determined by clinical response and toxicity. The median number of cycles was 4. Table 1 summarizes patient demographics and treatments given.

Response

Twelve of the 14 patients were evaluable for response. Patient 9 was not evaluable for clinical response because of lack of compliance with study protocol. Specifically, she did not return for any follow-up visits for several months after receiving her first denileukin diftitox treatment. Patient 13 was not evaluable because the bexarotene treatment had to be quickly discontinued after the first cycle of therapy due to the precipitous onset of hypertriglyceridemia. Of the remaining 12 patients, 4 had a complete response to treatment and 4 had a partial response, for an overall objective response rate of 67%. The one patient who had failed to respond to prior denileukin diftitox therapy had a complete response (Table 1, patient no. 8).

Tolerability of denileukin diftitox and bexarotene

The combination of bexarotene and denileukin diftitox was well tolerated with few grade 3 and 4 adverse events. One patient experienced a hypersensitivity reaction consisting of hives following denileukin diftitox infusion, which resolved with corticosteroids and antihistamine treatment. Five of the 14 patients developed peripheral edema; one of these 5 patients became hypotensive as a result of vascular leak syndrome, and the other 4 patients required diuresis for relief of symptoms.

All other adverse events were laboratory abnormalities, with no associated clinical symptoms. Grade 3 leukopenia was noted in one patient. Grade 3 and 4 lymphopenia was noted in 8 patients and resolved before the next cycle of denileukin diftitox. There were no dose reductions for hematologic toxicity. One patient developed grade 4 hyperlipidemia after the first cycle of therapy, requiring discontinuation from the study. Overall, 12 patients experienced hypertriglyceridemia that occurred even at the lowest bexarotene dosage. Hypertriglyceridemia was managed with lipid-lowering agents. Patients did not receive prophylactic antilipid agents prior to dosing with bexarotene. Nine patients experienced hypothyroidism as determined by subnormal T4 levels and received thyroid hormone replacement therapy. A summary of clinical adverse events and laboratory abnormalities observed in this study is reported in Tables 2 and 3.

Upregulation of CD25

Upregulation of CD25 expression on circulating $CD4^+$ cells was assessed in 12 of 14 patients entered on the study. Two patients were not evaluable due to the absence of blood samples at baseline

Table 1. Patient demographics and treatments

Patient no.	Age, y	Sex	Stage	No. prior systemic therapies	Bexarotene dose, mg/d	No. cycles denileukin diftitox administered
1	67	F	IIA	3	75	12
2	46	F	IVB	1	150	4
3	55	M	IIB	2	150	7
4	70	M	III	2	150	9
5	46	M	IVA	1	150	6
6	57	M	IA	1	150	9
7	72	F	III	6	225	4
8	63	F	IB	1	225	7
9	71	M	III	11	225	1
10	64	F	IIA	5	300	3
11	60	M	IB	2	300	5
12	81	F	IB	2	300	4
13	56	F	IB	1	375	4
14	61	F	IVB	1	225	4

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Table 2. Adverse events according to National Cancer Institute toxicity grading

Patient no.	HS	VLS	Hepatic enzymes	Cr	Maximum triglycerides, mM/L	Maximum total cholesterol, mM/L	Lowest T_4	WBCs	Lymphocytes	Hematocrit	Platelets
1	N	Y	Gr 2	N	861	253	2.3	Gr 3	Gr 3	Gr 1	N
2	N	Y	N	N	282	271	3.3	Gr 2	Gr 1	N	N
3	N	Y	Gr 1	N	1397	388	3.5	Gr 2	Gr 4	N	N
4	N	N	N	N	ND	ND	ND	N	Gr 3	Gr 1	Gr 1
5	N	N	N	N	382	217	7.4	N	Gr 3	N	N
6	N	Y	N	N	568	258	3.2	N	Gr 2	N	N
7	N	N	Gr 1	N	645	239	2.3	N	N	N	N
8	N	N	Gr 2	N	559	252	3.2	N	Gr 2	Gr 1	N
9	N	N	N	N	N	ND	ND	Gr 2	Gr 3	Gr 1	Gr 1
10	Y	N	N	N	619	342	ND	N	N	N	N
11	N	N	Gr 1	N	1283	407	3.6	Gr 1	Gr 3	N	N
12	N	N	N	Gr 1	233	198	2.2	N	Gr 4	N	Gr 1
13	N	N	N	N	64	298	5.8	N	Gr 3	N	N
14	N	Y	N	N	8433	806	<2.0	N	N	N	N

HS indicates hypersensitivity; VLS, vascular leak syndrome; Cr, creatinine; N, no; Y, yes; ND, not done.

and/or 7 days after treatment with bexarotene. Upregulation of CD25 expression was defined as a 50% increase in CD25 expression from baseline levels of fluorescence as detected by flow cytometry in gated populations of $CD4^+$ $CD7^-$ lymphocytes and was noted in 8 of the 12 evaluable patients (Table 4). All 4 patients who achieved a complete response and 1 of the 3 patients who achieved a partial response had evidence of CD25 upregulation after bexarotene treatment.

Discussion

Denileukin diftitox and bexarotene are both approved therapies for patients with persistent or recurrent CTCL. This is the first trial to explore the safety of these 2 agents when used in combination, and to assess CD25 upregulation on CTCL cells with bexarotene. We observed no overlapping toxicities and no maximum-tolerated dose was determined, probably because the biologic end point of upregulation of CD25 expression on circulating $CD4^+$ tumor cells was reached at a relatively low dose of bexarotene and appeared to be dose independent at doses equal to or greater than 150 mg/day.

The most significant adverse events were those already reported with bexarotene alone. Hypertriglyceridemia occurred even at the lowest doses of bexarotene and was not dose dependent in this small group of patients. Three patients (21%) experienced triglyceride elevations of more than 1000 mg/dL (grade 3-4), and 4 had cholesterol elevations of more than 300 mM/L (grade 2). The incidence of lipid anomalies in this study is comparable to that observed in larger trials of bexarotene in CTCL.[10,11] Similarly, suppression of thyroid function due to decreased TSH production occurred in 9 (64%) of 14 patients, an incidence comparable to the 54% incidence of thyroid stimulation hormone (TSH) suppression that was reported with oral bexarotene administered at a dose of 300 mg/m^2 per day.[12]

Grade 3 or 4 lymphopenia occurred in 8 (57%) of 14 patients in this trial. In the phase 3 trial of denileukin diftitox in CTCL, the incidence of grade 3 or 4 leukopenia was 16% and the incidence of grade 3 or 4 lymphopenia was 3.6%.[7] In the phase 3 studies of bexarotene in patients with advanced and refractory CTCL, the incidences of grade 2 and 3 leukopenia at a dose of 300 mg/m^2 per day, which is almost twice the highest dose administered in this study, were 7.1% and 3.6%, respectively, and there was no grade 4 leukopenia.[11] The incidence of grade 3 lymphopenia with the combination of bexarotene and denileukin diftitox was 40%. Because the relationship between bexarotene dose and lymphopenia has not been well established, it is difficult to ascertain whether

Table 3. White blood cell and lymphocyte counts before and after treatment, and NCI toxicity grading

Patient no.	Pretreatment WBC count, $10^3/\mu L$	Pretreatment absolute lymphocyte count, $10^3/\mu L$ NCI grade*	Posttreatment WBC count, $10^3/\mu L$ NCI grade*	Posttreatment lymphocyte count, $10^3/\mu L$ (NCI grade)
1	4.3	2107	1.4 (3)	574 (3)
2	7.3	1022 (2)	2.2 (2)	1846 (1)
3	6.6	330 (4)	2.9 (2)	456 (4)
4	4.7	517 (3)	5.3	890 (3)
5	10.0	900 (3)	7.3	511 (3)
6	7.9	1817 (1)	5.3	1377 (2)
7	15.0	4350	11.0	4290
8	8.7	1479 (2)	4.5	1164 (2)
9	4.0	800 (3)	2.8 (2)	742 (3)
10	7.3	1241 (2)	7.3	1660 (1)
11	6.4	1920	3.6 (1)	520 (3)
12	7.5	1125 (2)	5.1	124 (4)
13	6.1	1098 (2)	5.8	928 (3)
14	12.3	5166	7.3	3139

WBC indicates white blood cell; NCI, National Cancer Institute.
*NCI grade for abnormal values only.

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Table 4. CD25 expression change and response to treatment

Patient no.	$CD25_{pre}$	$CD25_{post}$	Clinical response
1	50	31	Partial
2	2	*23*	None
3	14	*28*	Complete
4	33	*45*	Complete
5	61	60	None
6	11	*40*	Complete
7	5	*50*	None
8	6	*18*	Complete
9	13	*18*	Partial
10	ND	ND	Not evaluable
11	14	14	Partial
12	56	ND	Partial
13	60	61	None
14	4	*16*	Not evaluable

Italicized text indicates up-regulation of IL-2R.
ND indicates specimen not available.

the incidence reported here is consistent with an RXR effect or if it may represent an interaction between bexarotene and denileukin diftitox. However, as noted in Table 3, 9 of 14 patients had grade 2 or higher lymphopenia prior to beginning therapy, and 4 of those patients had grade 3 or 4 lymphopenia. This may account, in part, for the lymphopenia observed in this study. In terms of clinical significance, the lymphopenia was short-lived, resolving within one month of cessation of therapy, and no patient developed evidence of opportunistic infection while on therapy or in the 6 months thereafter.

In this study, denileukin diftitox was administered at a dose intensity (18 mcg/kg per day $\times$ 3 days, or 54 mcg/kg per cycle) comparable to the lower dose studied in the pivotal phase 3 protocol, where the doses evaluated were 9 mcg/kg per day or 18 mcg/kg per day $\times$ 5 days, for a total of 45 mcg/kg per cycle or 90 mcg/kg per cycle. The incidence of peripheral edema attributable to low-level vascular leak was similar in both studies (28% vs 27% in the phase 3 study), but the incidence of significant hepatic transaminase elevations ($> 5\times$ normal) was significantly lower in this trial (0% vs 17%). Infusion-related events associated with denileukin diftitox occurred infrequently in this study compared with the phase 3 trial, probably due to corticosteroid premedication used in this trial.[13]

In this small series, the objective response rate was 67%, with a complete response rate and a partial response rate of 33% each. Although the number of patients treated in this study was small, the overall response rate appears to be higher than that reported with either bexarotene or denileukin diftitox administered as a single agent in comparable groups of patients with CTCL. Of interest, one patient who did not respond to denileukin diftitox alone had a complete response on this trial to the combination of denileukin diftitox and bexarotene, suggesting that this combination therapy may be useful in patients who experience a suboptimal or plateau response to denileukin diftitox.

The higher response rates observed here may be related to the effects of bexarotene on expression of IL-2R. CD25 expression was increased after 1 week of bexarotene therapy in 8 of 13 patients treated at doses of at least 150 mg/day. Of these, 6 patients with circulating Sezary cells demonstrated upregulation of CD25 expression on the tumor cells. Two of 13 patients had low numbers of circulating $CD4^+CD7^-$ cells and had evidence of upregulation of CD25 on normal populations of $CD4^+$ cells after bexarotene. Although our sample size is limited, we conclude that bexarotene likely increases CD25 expression on both Sezary cells and normal $CD4^+$ subsets. Because of the small sample size, no definite correlations can be drawn between upregulation of CD25 expression and clinical response, and likewise, the effects of bexarotene on normal immune effector cells, including immunoregulatory T cells, have not been elucidated and could potentially impact the biologic and clinical activity of this drug combination.

In conclusion, we report that the combination of denileukin diftitox and bexarotene is associated with an acceptable safety profile and a high overall response rate in patients with early and advanced refractory cutaneous T-cell lymphoma and that low doses of bexarotene are capable of modulating expression of CD25 in vivo. The impact of the upregulation of IL-2R by bexarotene on the clinical efficacy of denileukin diftitox should be further pursued in the context of a randomized clinical trial.

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